Are Women More at Risk of False-Positive Primary Aldosteronism Screening and Unnecessary Suppression Testing than Men?

Ashraf H. Ahmed, Richard D. Gordon, Paul J. Taylor, Gregory Ward, Eduardo Pimenta, and Michael Stowasser

Endocrine Hypertension Research Centre (A.H.A., R.D.G., P.J.T., E.P., M.S.), University of Queensland School of Medicine, Greenslopes and Princess Alexandra Hospitals, Brisbane, Queensland 4102, Australia; and Sullivan and Nicolaides Pathology (G.W.), Brisbane, 4068 Queensland, Australia

Background: Because primary aldosteronism is not uncommon, specifically treatable and in some cases curable, and carries higher risks for cardiovascular morbidity and mortality than essential hypertension, screening hypertensive patients for its presence by measuring aldosterone to renin ratio (ARR) is increasingly common. A significantly higher false-positive ARR rate for women than men, resulting in unnecessary suppression tests has previously been reported.

Methods: Using a new, highly accurate aldosterone assay and both of the currently widely used renin assays, ARR was measured in 19 normal, ovulating women at three time points in the menstrual cycle and compared with single measurements in 21 normal males of similar age.

Results: ARRs in males were possibly too well down in the current normal range. Although normotensive and normokalemic, two women had raised ARRs in the luteal phase but only when direct renin concentration (DRC) was used. Their DRC levels were low at all sampling times [despite midrange plasma renin activity levels], whereas their progesterone and aldosterone levels were highest for the group. Saline suppression testing, performed in one of them, showed normal aldosterone suppressibility.

Conclusion: False-positive ARRs in normal women during the luteal phase only when DRC is used may explain the higher incidence of false-positive ARRs in hypertensive women than men and suggest the following: 1) plasma renin activity is preferable to DRC in determination of ARR and 2) new reference ranges for ARR that take into account gender and sex hormone levels are required. **(*J Clin Endocrinol Metab* 96: E340–E346, 2011)**

Primary aldosteronism (PAL) is the most common specifically treatable and potentially curable form of secondary hypertension (1–3), screened for using aldosterone to renin ratio (ARR). Anything altering aldosterone or renin levels affects ARR with the potential for false-positive or negative results (4, 5). Are there other important influences that have so far been ignored?

Fommei *et al.* (6) suggested that a rise in plasma aldosterone in the luteal phase of the menstrual cycle may have implications for screening criteria for PAL, *i.e.* if plasma aldosterone below a certain level is thought to exclude PAL (7). Pizzolo *et al.* (8) reported elevated ARR levels [using direct renin concentration (DRC)] to be more prevalent in hypertensive women than men (13.6 *vs.* 2.3%) but seldom associated with confirmed PAL. Does this high false-positive ARR rate in women demand different normal ranges for women and men? Would hormonal contraceptive and postmenopausal hormone replacement require an additional adjustment of female ranges?

False-positive ARRs in hypertensive women could lead to unnecessary and even false-positive salt-loading aldosterone suppression tests, followed by adrenal venous

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

doi: 10.1210/jc.2010-1355 Received June 15, 2010. Accepted October 4, 2010.
First Published Online October 20, 2010

Abbreviations: ARR, Aldosterone to renin ratio; DRC, direct renin concentration; LMP, last menstrual period; PAL, primary aldosteronism; PRA, plasma renin activity.

sampling. A better understanding of the effects of female hormones (natural or administered) on levels of aldosterone, renin, and ARR is required to avoid this effect. Observing the effects on aldosterone, renin, and ARR of hormone changes during normal, ovulatory menstrual cycles is a good place to start.

There is evidence that exogenous estrogen administration stimulates production of renin substrate (9), which might sequentially lead to elevated levels of angiotensin I, angiotensin II, and aldosterone and the possibility of hypertension. In practice, hypertension after the administration of an oral contraceptive containing estrogen is rare because, in a finely tuned homeostatic system, rising angiotensin levels lead, by negative feedback on the juxtaglomerular apparatus, to falls in renin release and in DRC and minimal rises in plasma renin activity (PRA) and angiotensin II (10). Falling DRC will tend to raise ARR, whereas stable PRA will not.

Progesterone, secreted during the second half of an ovulatory menstrual cycle, has mineralocorticoid-antagonist activity (11) and can cause natriuresis, lower plasma volume, and a compensatory increase in plasma renin and aldosterone. Fluctuations in estrogen and progesterone during the menstrual cycle thus have the potential to complicate interpretation of both ARR and aldosterone suppression tests.

Might different laboratory methodologies for measuring renin be equally likely to lead to false-positive or -negative ARR? Plasma renin was once measured almost exclusively as PRA, which tests the ability of all the components of plasma, including substrate, to generate angiotensin I. Today it is measured as either PRA or DRC (12). In the present study, we decided to use both methods, to permit comparison, while measuring ARR in normal men and while evaluating the effects of hormonal changes during the phases of the menstrual cycle on ARR in normal women.

Aldosterone has until recently been measured almost exclusively by immunoassay methods, despite concerns over their accuracy (13). Substantially different aldosterone concentrations have been reported when four different immunoassay methods were used (14). Because recently developed HPLC-tandem mass spectrometry methods measure plasma aldosterone accurately and specifically (15–18), we used a method (16) developed in our center.

Subjects and Methods

Subjects

This study was approved by the Institutional Human Ethics Review Committee. Informed, written consent was obtained from 21 healthy males without evidence of renal, liver, or cardiovascular diseases, not hypertensive, and not receiving any medications within the previous 2 months and from 23 healthy premenopausal women with no evidence of renal, liver, or cardiovascular disease, regular menstrual cycles of approximately 28 d, no history of pregnancy within the preceding 2 yr, not hypertensive, and not receiving any medications (including contraceptives) within the previous 2 months. Instructions were given to maintain their usual sodium intake during the period of study, and compliance was assessed by measuring urinary sodium excretion at each visit. The results in 21 men were compared with those in 19 women who had an ovulatory cycle.

Sampling

Blood and spot morning urine samples were collected once from each male participant and from each female participant during menses and at 10 ± 1 and 20 ± 1 d (SD) after the first day of the last menstrual period (LMP). Samples were collected in all subjects for measurement of plasma aldosterone, DRC, PRA, cortisol, sodium, potassium and creatinine and urinary sodium, potassium, creatinine, cortisol, and aldosterone, and in females only for serum estradiol, progesterone, LH, and FSH. Blood samples were collected between 0900 and 1000 h after sitting for 5–15 min and centrifuged immediately at 2500 rpm for 10 min. Plasma was snap frozen and stored at −20 C. Blood pressure (mean of two readings after sitting for 10–15 min) and heart rate were recorded.

Analytic methods

Plasma aldosterone and cortisol were measured by HPLC-tandem mass spectrometry (16). For aldosterone, the interassay coefficient of variation was 9.3% at 242 pmol/liter and 6.0% at 1321 pmol/liter. The intraassay coefficient of variation was 7.3% at 238 pmol/liter and 4.3% at 1344 pmol/liter. The interassay coefficient of variation for cortisol was 2.1% at 55 and 1.8% at 462 ng/ml. The intraassay coefficient of variation was 3.7% at 56 ng/ml and 1.6% at 472 ng/ml. DRC was assayed by chemiluminescent immunoassay (DiaSorin, Liaison, Italy). The interassay coefficient of variation was 7.4% at 26 mU/liter and 6.0% at 106 mU/liter. The intrassay coefficient of variation at 15, 33, 82, and 258 mU/liter was 3.7, 2.8, 2.0, and 1.2%, respectively. PRA was assayed by GammaCoat RIA (DiaSorin, Stillwater, MN). The interassay coefficient of variation was 5.6, 7.6, and 6.8% at 1.6, 10.7, and 15.2 ng/ml · h, respectively. The intraassay coefficient of variation was 10.0% at 1.6 ng/ml · h, 4.6% at 6.2 ng/ml · h, and 9.4% at 17.9 ng/ml · h. LH, FSH, estradiol, and progesterone were measured by immunoassay (Abbott Diagnostics-Abbott Architect, Sydney, Australia). Urinary cortisol was measured by HPLC and urinary aldosterone by RIA (Siemens Diagnostic Products Corp., Los Angeles, CA).

Statistical analysis

SPSS 17 for Windows (SPSS, Chicago, IL) was used to analyze the data. Data are presented as median (range in parentheses) unless otherwise stated. Nonparametric testing (Friedman test) was used for comparisons between the three samples collected during the menstrual cycle. Pairwise comparisons were performed by the Wilcoxon test. The Mann-Whitney *U* test was used to compare results with the male group. Spearman's test was used to seek correlations between measured parameters. A $P < 0.05$ was considered statistically significant.

TABLE 1. ARR and other measured parameters according to phase of menstrual cycle in 19 healthy, nonhypertensive women and 21 men

	Women				Men			
	Menses	Follicular phase (10 ± 1 SD d after LMP)	Luteal phase (20 ± 1 SD d after LMP)	Friedman test (*P* value)		Male *vs.* menses Mann-Whitney *U* test (*P* value)	Male *vs.* follicular Mann-Whitney *U* test (*P* value)	Male *vs.* luteal Mann-Whitney *U* test (*P* value)
Aldosterone								
(pmol/liter)	153 (107–389)	170 (133–524)	454 (181–1141)	<0.001	189 (107–449)	NS	NS	<0.001
(ng/dl)	5.52 (3.84–13.97)	6.09 (4.76–18.8)	16.3 (6.48–40.9)	<0.001	6.78 (3.84–16.1)	NS	NS	<0.001
DRC (mU/liter)	25 (12–50)	28 (10–58)	38 (15–78)	<0.001	40 (20–53)	<0.001	<0.01	NS
ARR using DRC	7.9 (2.6–27.8)	8.3 (2.3–49.9)	14.2 (2.3–75.7)	<0.001	4.8 (3.8–9.9)	<0.05	<0.05	<0.001
PRA (ng/ml · h)	1.7 (1.0–4.4)	2.1 (1.0–5.7)	3.8 (1.6–9.2)	<0.001	4.6 (1.2–6.8)	<0.001	<0.01	NS
ARR using PRA								
(pmol/liter)/(ng/ml · h)	107 (32–223)	109 (24–227)	133 (30–300)	NS	61 (21–161)	<0.01	<0.01	<0.01
(ng/dl)/(ng/ml · h)	3.85 (1.13–8.02)	3.91 (0.87–8.15)	4.78 (1.07–10.7)	NS	2.18 (0.75–5.74)	<0.01	<0.01	<0.01
Plasma cortisol (ng/ml)	54.7 (35.6–121)	90.2 (42.5–174)	56.6 (41.3–131)	<0.001	92 (68–100)	<0.01	NS	<0.05
FSH (IU/liter)	5.1 (3.1–10.3)	4.9 (3.4–35.9)	2.6 (1.3–15.2)	<0.001				
LH (IU/liter)	3.2 (1.1–4.5)	5.4 (3.4–10.6)	1.3 (0.8–11.7)	<0.01				
Estradiol (pmol/liter)	144 (80–313)	389 (202–820)	263 (104–777)	<0.001				
Progesterone (nmol/liter)	0.6 (0.3–2.1)	0.5 (0.3–7.6)	39.8 (12.1–71.5)	<0.001				
Plasma Na^+ (mmol/liter)	138 (137–141)	138 (136–142)	137 (136–140)	NS	139 (137–145)	NS	NS	NS
Plasma K^+ (mmol/liter)	4.0 (3.8–4.2)	4.0 (3.8–4.4)	3.9 (3.8–4.2)	NS	4.1 (3.7–4.5)	NS	NS	NS
Plasma creatinine (μmol/liter)	59 (48–73)	61 (51–76)	61 (50–77)	NS	70 (60–88)	<0.001	<0.001	<0.01
Systolic blood pressure (mm Hg)	120 (102–132)	120 (100–130)	122 (100–132)	NS	128 (120–138)	<0.01	<0.01	<0.01
Diastolic blood pressure (mm Hg)	80 (72–86)	78 (70–84)	80 (70–84)	NS	88 (80–90)	<0.01	<0.001	<0.001
Heart rate (beats/min)	75 (62–85)	75 (65–90)	79 (65–90)	NS	87 (78–92)	<0.001	<0.001	<0.001

Values are presented as medians (range). Friedman test was used to perform multiple comparisons, and the *P* value indicates the degree of significance of changes occurring during the cycle. See Fig. 1 for pairwise comparisons between the three collection time points in women. The Mann-Whitney *U* test was used to compare male *vs.* female data. NS, Not significant.

Results

Group data

Table 1 includes one set of data for males and three sets for females and the significance of variability during the cycle in females (Friedman test). Male data were compared with female data for each of the three sampling points. ARR values were much higher in women than men during all three phases of the cycle, with the least difference during menses. In the females, aldosterone, PRA, DRC, ARR calculated using DRC (but not using PRA), cortisol, FSH, LH, estrogen, and progesterone all varied significantly during the cycle. There were no significant changes in plasma sodium, potassium, creatinine, blood pressure, or heart rate. In Table 1, the results of aldosterone and ARR calculated by PRA are shown in both picomoles per liter and nanograms per deciliter.

Pairwise (Wilcoxon) comparisons between each of the three collection time points for women are shown in Fig. 1, and were performed only for the parameters showing statistically significant changes by Friedman testing except for ARR calculated using PRA.

Follicular phase levels were significantly ($P < 0.01$) higher than menstrual for aldosterone, DRC, and PRA, and ($P < 0.001$) for estrogen, cortisol, and LH. There were no significant differences between menstrual and follicular phases for ARR (calculated by either DRC or PRA), progesterone, or FSH.

Progesterone levels were higher in the luteal phase than the follicular phase, consistent with ovulation, as were levels for plasma aldosterone, PRA, and ARR calculated using DRC, with two women exceeding the upper limit of normal (70) for ARR calculated using DRC. The upper limit of normal (600) was not exceeded by any women when ARR was calculated using PRA, which did not rise significantly. Because only two women had elevated luteal ARR (aldosterone/DRC) values, comparisons were also made without them, but the difference between luteal and follicular ARR calculated by DRC remained significant [14.0 (2.3–18.2) *vs.* 7.8 (2.3–11.4), $P < 0.01$].

FSH and cortisol levels were significantly lower in the luteal than either the menstrual or follicular phases, whereas aldosterone, progesterone, PRA, DRC ($P < 0.01$), and ARR calculated by DRC were significantly ($P < 0.001$) higher (Fig. 1). Luteal levels of estrogen were higher than menstrual but lower than follicular.

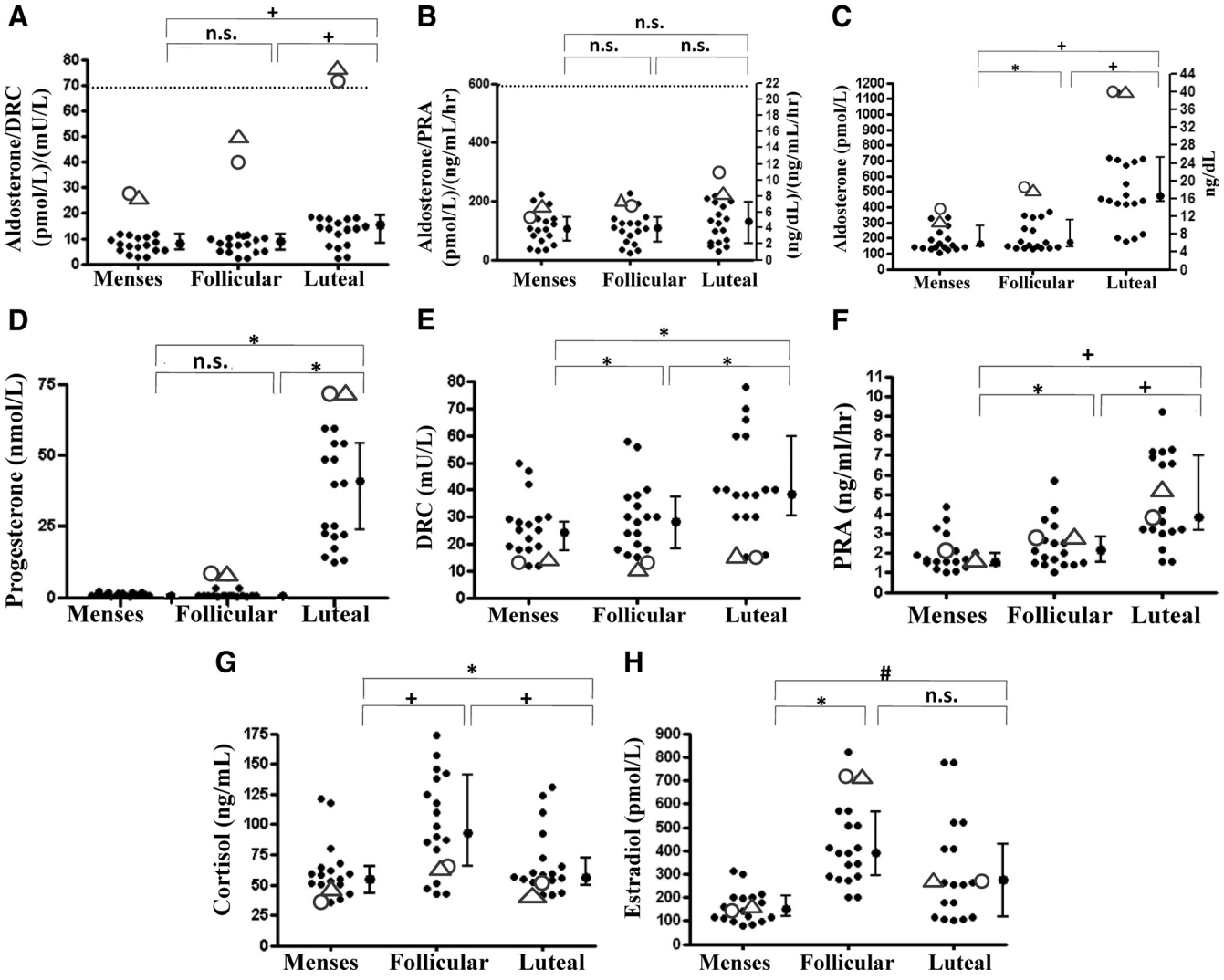

FIG. 1. Median ARR using DRC (A), ARR using PRA (B), aldosterone (C), progesterone (D), DRC (E), PRA (F), cortisol (G), and estradiol (H) during menses and follicular and luteal phases. *Error bars* indicate interquartile ranges. #, $P < 0.05$, *, $P < 0.01$, and +, $P < 0.001$ for pairwise comparisons (Wilcoxon). n.s., Not significant. The *horizontal dotted lines* in A and B indicate the upper limit of the normal range for ARR. *Open symbols* denote the two women with positive luteal ARR using DRC.

A significant negative correlation was found between luteal progesterone and DRC [($r = -0.58$, $P < 0.05$) but not between luteal progesterone and PRA ($r = 0.11$, $P > 0.05$). Significant positive correlations were found between luteal progesterone and luteal ARR calculated using DRC ($r = 0.60$, $P < 0.01$) but not using PRA ($r = 0.41$, $P > 0.05$). There was also a significant positive correlation between luteal aldosterone and progesterone ($r = 0.51$, $P < 0.05$).

Urinary parameters (corrected for creatinine) are presented in Fig. 2. Median urinary aldosterone was significantly higher in the luteal [6.9 (2.7–94) pmol/mmol] than follicular phase [3.2 (1.1–23.8) pmol/mmol, $P < 0.05$] or during menses [2.2 (1.2–7.1) pmol/mmol, $P < 0.001$].

Individual data for women with positive ARR

If we focus on the data for the two women with elevated ARR (using DRC), they were distinguished in several ways (*open symbols*, Fig. 1). Their progesterone and aldosterone levels were highest for the group in the luteal phase and even in the follicular phase. Their DRC levels were among the lowest for the group in all three phases, guaranteeing the highest aldosterone/DRC ARRs in all three phases so that they were distinguishable, even during menses. In contrast, their PRAs were in the midrange at each phase, ensuring that ARR using PRA, although among the highest values, was unremarkable. Their estradiol levels were among the highest in the follicular phase but in the midrange during menses and luteal phases. Their cortisol levels were among the lowest throughout.

Saline suppression testing (during which plasma aldosterone was measured basally and at the conclusion of an iv infusion of 2 liters of 0.9% normal saline given over a 4 h period between 0800 h and 1200 h) has so far been performed in one of the two women and revealed normal

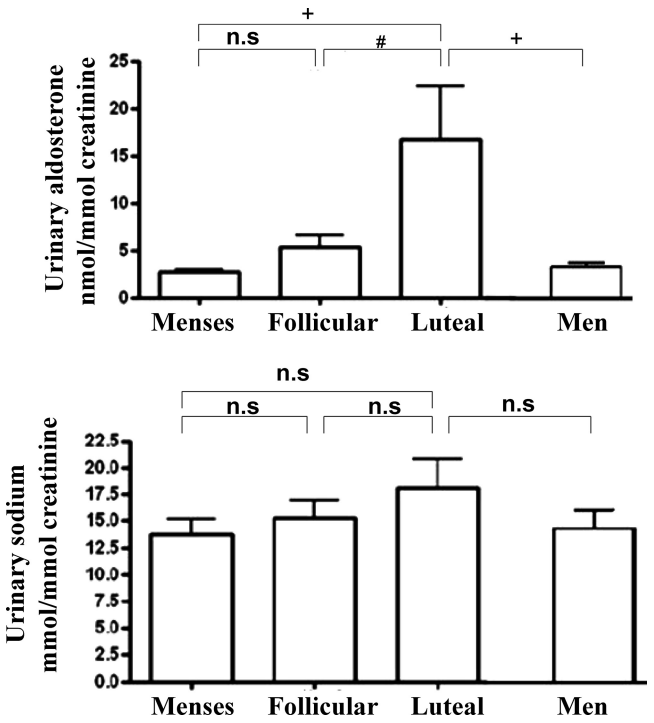

FIG. 2. Mean urinary aldosterone and sodium, corrected for creatinine, in males and females during menses and follicular and luteal phases. *Error bars* indicate SEM. #, $P < 0.05$; +, $P < 0.001$. n.s., Not significant. The Friedman test was used to perform multiple comparisons within women and the Wilcoxon test was used for pairwise comparisons. The Mann-Whitney *U* test was used to compare male *vs.* female data.

suppressibility of plasma aldosterone (basal 303 pmol/liter, 4 h < 60 pmol/liter).

Discussion

The main findings of this study were that: (1) plasma aldosterone, DRC, PRA, and the ARR measured using DRC (but not by PRA) are all higher in the luteal *vs.* the follicular phase in ovulating normotensive women; 2) false-positive ARR (using DRC) were encountered during the luteal phase in two of the 19 women; 3) women had much higher ARR values than men; and 4) ARR values in men were well down into the current reference range.

Both aldosterone and renin (as PRA) have already been reported to be higher, as here, in the luteal than the follicular phase of the normal menstrual cycle (19, 20) but not necessarily proportionately increased and hence a potential to alter the ARR. Progesterone is a natriuretic hormone by occupying the aldosterone receptor in the distal nephron and acting as a competitive inhibitor (21, 22), leading to natriuresis, and provoking a compensatory increase in renin and aldosterone to counteract any consequent hypovolemia. In addition, progesterone is thought to possibly stimulate aldosterone production directly (21).

Obvious limitations of the present study include a relatively small number of participants and measurement of ARR in normal healthy volunteers and not in hypertensive patients. However, we found apparently false-positive ARRs (confirmed in one subject by normal aldosterone suppression during saline infusion) in healthy, ovulating, normotensive women, when aldosterone/DRC ARR values exceeded the upper limit of the normal range. This was associated with statistically significantly higher luteal ARR levels for the cohort (calculated using DRC but not PRA) than follicular or menstrual. A sustained increase in angiotensinogen after the estrogen surge in the late follicular phase could have favored the relatively greater increase in luteal PRA than DRC. The fact that the two women with elevated luteal ARR were also distinguished from the other subjects by having the highest luteal progesterone and aldosterone levels, and near-highest mid-follicular estrogen levels, would be in keeping with a role for sex hormones in modifying aldosterone and renin and the ARR (using DRC).

Of interest was the observed alteration in cortisol concentrations during the menstrual cycle. A likely explanation for the higher cortisol (measured here as total, rather than free, cortisol) during the follicular phase would be higher estrogen concentrations causing increased production of cortisol binding globulin (23). Similarly, an effect of high follicular phase estrogen in the liver to increase the level of renin substrate, with a half-life long enough to carry the increase on into the luteal phase, could explain the unexpected negative correlation between progesterone and DRC in the luteal phase. When increased renin substrate results in higher angiotensin I and angiotensin II, these will feed back negatively on renin secretion and DRC will fall.

The normal range for ARR currently does not distinguish between men and women or between women at various phases of the cycle or receiving hormonal contraception or hormone replacement therapy. In the current study, however, the ARR in females across the menstrual cycle was consistently higher than in males (2–3 times higher in the case of luteal ARR values). This study also found that hormonal changes during the menstrual cycle significantly affect ARR, especially if DRC is used to measure the renin component of the ratio. These observations suggest the need for revised normal ranges and support the use of the aldosterone to PRA ratio, and not the aldosterone to DRC ratio, in women who are ovulating and possibly also those who are receiving contraceptive agents or hormone replacement therapy.

Because ARR values appear to be significantly higher in young females than young males, different normal ranges for each gender should be developed, as has been sug-

gested by others (24). Otherwise, there is the probability of some young females inappropriately going on to more complex, unnecessary investigations for PAL. Current criteria for normal suppressibility of aldosterone below an arbitrary level by oral salt loading (7) or saline infusion (25) should also be revised for young women in the luteal phase of the cycle. Because of raised ARR using DRC, Pizzolo *et al.* (8) went on to perform suppression tests (saline infusion) in 54 hypertensive women, and 33 (61.1%) turned out not to have PAL (*i.e.* they had false positive ARR). In contrast, only four of 27 men (14.8%) with positive ARR suppressed normally with saline infusion. They found elevated ARR (using DRC) in eight of 59 normotensive, premenopausal women (13.6%) (mean age 28 yr) compared with only one in 44 normotensive young men (2.3%). They found no differences between aldosterone, renin (DRC), or ARR (DRC) between women and men and no differences in ARR (DRC) across the menstrual cycle, raising the possibility of false-positives ARR at all points in the cycle and not only in the luteal phase. As we have found using PRA in normotensive young women, Fommei *et al.* (6) found no effect of phases of the menstrual cycle on ARR (calculated using 30 min recumbent PRA, normal range < 0.65 ng/ml · h) in 26 mildly hypertensive premenopausal women aged 26–55 yr with normal PRA. Supine PRA and aldosterone were highest in the luteal phase. In 12 of 26 women, plasma aldosterone was below a threshold (of 15 ng/dL for suspicion of primary aldosteronism) at d 7 of the menstrual cycle but above it at d 21. The percentage of positive ARRs went up from 7 of 26 in the follicular phase (27%)to 16 of 26 in the luteal phase (64%), leading them to suggest that the normal range for ARR in menstruating women be redefined. We agree and would suggest, in addition, separate ranges for males, postmenopausal women, and women receiving oral contraceptives or hormone replacement therapy. It will be helpful if, in that redefinition, highly accurate methods for measurement of aldosterone, such as that used in the present study, are used, and, ideally, mass spectrometry measurement of generated angiotensin in a PRA assay.

Acknowledgments

Address all correspondence and requests for reprints to: Professor Michael Stowasser, Endocrine Hypertension Research Centre, University of Queensland School of Medicine, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane 4102, Australia. E-mail: m.stowasser@uq.edu.au.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Gordon RD, Stowasser M, Tunny TJ, Klemm SA, Rutherford JC** 1994 High incidence of primary aldosteronism in 199 patients referred with hypertension. Clin Exp Pharmacol Physiol 21:315–318
2. **Rossi GP, Bernini G, Caliumi C, Desideri G, Fabris B, Ferri C, Ganzaroli C, Giacchetti G, Letizia C, Maccario M, Mallamaci F, Mannelli M, Mattarello MJ, Moretti A, Palumbo G, Parenti G, Porteri E, Semplicini A, Rizzoni D, Rossi E, Boscaro M, Pessina AC, Mantero F** 2006 A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. J Am Coll Cardiol 48:2293–2300
3. **Young Jr WF** 1999 Primary aldosteronism: a common and curable form of hypertension. Cardiol Rev 7:207–214
4. **Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, Veglio F** 2002 Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. Hypertension 40:897–902
5. **Stowasser M, Gordon RD** 2004 The aldosterone-renin ratio in screening for primary aldosteronism. Endocrinologist 14:267–276
6. **Fommei E, Ghione S, Ripoli A, Maffei S, Di Cecco P, Iervasi A, Turchi S** 2009 The ovarian cycle as a factor of variability in the laboratory screening for primary aldosteronism in women. J Hum Hypertens 23:130–135
7. **Young WF** 2007 Primary aldosteronism: renaissance of a syndrome. Clin Endocrinol (Oxf) 66:607–618
8. **Pizzolo F, Raffaelli R, Memmo A, Chiecchi L, Pavan C, Guarini P, Guidi GC, Franchi M, Corrocher R, Olivieri O** 2010 Effects of female sex hormones and contraceptive pill on the diagnostic work-up for primary aldosteronism. J Hypertens 28:135–142
9. **Gordon MS, Chin WW, Shupnik MA** 1992 Regulation of angiotensinogen gene expression by estrogen. J Hypertens 10:361–366
10. **Fischer M, Baessler A, Schunkert H** 2002 Renin angiotensin system and gender differences in the cardiovascular system. Cardiovasc Res 53:672–677
11. **Oelkers WK** 1996 Effects of estrogens and progestogens on the renin-aldosterone system and blood pressure. Steroids 61:166–171
12. **Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, Young Jr WF, Montori VM** 2008 Case detection, diagnosis, and treatment of patients with primary aldosteronism: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 93:3266–3281
13. **Gordon RD** 2004 The challenge of more robust and reproducible methodology in screening for primary aldosteronism. J Hypertens 22:251–255
14. **Schirpenbach C, Seiler L, Maser-Gluth C, Beuschlein F, Reincke M, Bidlingmaier M** 2006 Automated chemiluminescence-immunoassay for aldosterone during dynamic testing: comparison to RIAs with and without extraction steps. Clin Chem 52:1749–1755
15. **Turpeinen U, Hämäläinen E, Stenman UH** 2008 Determination of aldosterone in serum by liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 862:113–118
16. **Taylor PJ, Cooper DP, Gordon RD, Stowasser M** 2009 Measurement of aldosterone in human plasma by semiautomated HPLC-tandem mass spectrometry. Clin Chem 55:1155–1162
17. **Guo T, Taylor RL, Singh RJ, Soldin SJ** 2006 Simultaneous determination of 12 steroids by isotope dilution liquid chromatography-photospray ionization tandem mass spectrometry. Clin Chim Acta 372:76–82
18. **Vogeser M, Parhofer KG** 2007 Liquid chromatography tandem-mass spectrometry (LC-MS/MS)—technique and applications in endocrinology. Exp Clin Endocrinol Diabetes 115:559–570
19. **Gray MJ, Strausfeld KS, Watanabe M, Sims EA, Solomon S** 1968 Aldosterone secretory rates in the normal menstrual cycle. J Clin Endocrinol Metab 28:1269–1275

20. **Katz FH, Romfh P** 1972 Plasma aldosterone and renin activity during the menstrual cycle. J Clin Endocrinol Metab 34:819–821
21. **Szmuilowicz ED, Adler GK, Williams JS, Green DE, Yao TM, Hopkins PN, Seely EW** 2006 Relationship between aldosterone and progesterone in the human menstrual cycle. J Clin Endocrinol Metab 91:3981–3987
22. **Elger W, Beier S, Pollow K, Garfield R, Shi SQ, Hillisch A** 2003 Conception and pharmacodynamic profile of drospirenone. Steroids 68:891–905
23. **Qureshi AC, Bahri A, Breen LA, Barnes SC, Powrie JK, Thomas SM, Carroll PV** 2007 The influence of the route of estrogen administration on serum levels of cortisol-binding globulin and total cortisol. Clin Endocrinol (Oxf) 66:632–635
24. **Hannemann A, Friedrich N, Lüdemann J, Völzke H, Rettig R, Peters J, Reincke M, Döring A, Nauck M, Wallaschofski H** 2010 Reference intervals for aldosterone, renin, and the aldosterone-to-renin ratio in the population-based Study of Health in Pomerania (SHIP-1). Horm Metab Res 42:392–399
25. **Rossi GP, Pessina AC, Heagerty AM** 2008 Primary aldosteronism: an update on screening, diagnosis and treatment. J Hypertens 26:613–621