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Brief Communications

Reference Values for Aldosterone–Renin Ratios in Normotensive Individuals and Effect of Changes in Dietary Sodium Consumption

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BACKGROUND: Determination of the aldosterone-to-renin ratio (ARR) in blood is the preferred screening test for primary aldosteronism. Renin can be measured as the plasma renin activity (PRA) or the plasma renin concentration (PRC). Consequently, the ARR can be measured either based on the PRA (ARR_{pra}) or based on the PRC (ARR_{prc}). In contrast with the ARR_{pra}, the data on reference values for the ARR_{prc} are limited. Moreover, whether the ARR_{pra} or ARR_{prc} is affected by variations in salt intake is unknown.

METHODS: We measured the PRA, the PRC, and serum aldosterone in 100 normotensive individuals between 20 and 70 years of age before and after a 3-day oral sodium-loading test (SLT). Participants were stratified according to age and sex. Data are presented as the median and interquartile range (IQR).

RESULTS: Urinary sodium excretion after the SLT was ≥200 mmol/24 h in all participants. Serum aldosterone, PRA, and PRC values were significantly reduced after the SLT. PRC and PRA results were highly correlated [Spearman rank correlation r_s = 0.80 and 0.74 before and after SLT, respectively; $P <$ 0.001 for both]. The central 95% reference intervals for ARR_{pra} before and after SLT were 0.07–1.45 h^{-1} and 0.06–1.84 h^{-1}, respectively. The corresponding reference intervals for ARR_{prc} were 4.1–81.3 pmol/ng and 3.9–74.8 pmol/ng. The median ARR_{prc} decreased after the SLT from 19.5 pmol/ng (IQR, 13.0–29.4 pmol/ng) to 18.6 pmol/ng (IQR, 9.4–27.1 pmol/ng) (P = 0.005), whereas the median ARR_{pra} did not change (P = 0.12). Both the ARR_{prc} and ARR_{pra} at baseline were higher in women than in men, whereas no sex difference was observed after sodium loading.

CONCLUSIONS: We present reference values for the ARR_{prc} for healthy individuals. The ARR is affected to a variable degree by sex and sodium intake.

Primary aldosteronism is a frequent cause of secondary hypertension *(1)*. The preferred screening test is determination of the aldosterone-to-renin ratio (ARR)[3] in blood. An increased ARR is usually confirmed by sodium-loading test (SLT) results demonstrating inadequate suppression of serum or urinary aldosterone *(1)*. Reported cutoff values for these tests are affected by the characteristics of the assays used for measuring renin and aldosterone *(2–4)*. Many studies have measured renin as the plasma renin activity (PRA) *(2–4)*. More recently, direct assessment of the plasma renin concentration (PRC) has increasingly been used, because this assay is easier to perform than the PRA assay and is more suitable for automated procedures *(5, 6)*. Compared with the abundant literature on the PRA, data on reference values for the PRC and for the ARR based on the PRC (ARR_{prc}) are rather limited. Moreover, whether the PRC assay is influenced by such preanalytical factors as age, sex, and sodium intake is insufficiently known. We therefore measured plasma renin (PRA and PRC assays) and aldosterone in serum and urine in a large cohort of healthy individuals under conditions of normal and high sodium intakes.

Materials and Methods

Healthy individuals 20 to 70 years of age were stratified into 5 consecutive decade groups, with 10 males and 10 females per decade group. Participants selected for this study were normotensive (blood pressure <140/90 mmHg) and had normal results for routine laboratory measurements. Use of antihypertensives, oral contraceptives, sex hormone–replacement therapy, nonsteroidal inflammatory drugs, or glycyrrhizin-containing products was not allowed. Each participant visited the outpatient clinic before (day −1) and after (day 4) a 3-day oral SLT, during which 9 g NaCl in tablets was consumed daily in addition to the individual's usual diet (days 1–3). Blood samples were drawn between 0900 and 1100 with the participant in the sitting position after 10 min of rest. We collected 24-h urine samples on day −1 and day 3. Only participants who demonstrated a urinary sodium excretion rate af-

[3] Nonstandard abbreviations: ARR, aldosterone-to-renin ratio; SLT, salt-loading test; PRA, plasma renin activity; PRC, plasma renin concentration; ARR_{prc}, ARR based on the PRC; IQR, interquartile range; r_s, Spearman rank correlation coefficient; ARR_{pra}, ARR based on the PRA.

ter the SLT of ≥200 mmol/24 h were included in the final analysis. Each blood sample was prepared within 1 h after venipuncture, and all samples were stored at −20 °C until assayed. Liver function tests and creatinine, electrolyte, and nonfasting plasma glucose measurements were made with an automated clinical chemistry analyzer (Roche Hitachi Modular; Roche Diagnostics, http://www.roche.com). Renin and aldosterone were measured with commercial assays exactly according to each manufacturer's instructions. The PRC was measured with an immunoradiometric renin assay (Renin III Generation®; Cisbio, http://www.htrf.com). The limit-of-detection and the functional-sensitivity thresholds (i.e., the lowest concentration with an interassay CV ≤20%) were 0.7 ng/L and 1.0 ng/L, respectively. The intraassay imprecision (expressed as the CV) was 5.5%, 4.7%, and 1.6% at 6.0 ng/L, 19.9 ng/L, and 52.2 ng/L, respectively. The interassay CV was 14%, 9.2%, and 4.0% at 5.1 ng/L, 19.8 ng/L, and 54.7 ng/L, respectively. The PRA was measured after incubating the sample at 4 °C (blank) and at 37 °C for 90 min and by subsequent measurement of angiotensin I generation by RIA (REN-CT2®; Cisbio, http://www.htrf.com). The limit-of-detection and functional-sensitivity thresholds were 0.45 and 0.70 $nmol \cdot L^{-1} \cdot h^{-1}$, respectively. The intraassay CV was 20%, 5.3%, and 4.9% at 0.70, 3.53, and 7.02 $nmol \cdot L^{-1} \cdot h^{-1}$, respectively. The interassay CV was 12%, 9.4%, and 15% at 1.61, 2.79, and 5.68 $nmol \cdot L^{-1} \cdot h^{-1}$, respectively. Aldosterone in serum and urine was measured with a competitive fixed-time solid-phase RIA (Coat-a-Count®; Siemens Medical Solutions Diagnostics, http://www.siemens.com). The limit-of-detection and functional-sensitivity thresholds were 30 and 50 pmol/L, respectively. The intraassay CV was 11%, 7.8%, and 3.9% at 120, 140, and 710 pmol/L, respectively. The interassay CV was 14%, 5.2%, and 7.3% at 110, 710, and 1510 pmol/L, respectively. Assay validations were performed according to the EP5 and EP9 protocols of the CLSI (http://www.clsi.org/). The protocol was approved by the local medical ethics committee, and all participants provided written informed consent.

STATISTICS

Data are presented as the median and interquartile range (IQR). Within-group changes and between-group differences in variables were evaluated with the Wilcoxon signed rank test and the Mann–Whitney *U*-test, respectively. Relationships between parameters were evaluated by Spearman rank correlation (r_s) analysis. Reference intervals were defined as the central 95% of the population (i.e., the 2.5th and 97.5th percentiles). The results for the 2 different renin assays were compared by Passing–Bablok regression analysis with Analyse-it (version 2.20; Analyse-it Software). All other calculations were performed with SPSS (version 16.0; IBM/SPSS). Two-sided *P* values <0.05 were considered statistically significant.

Results

One hundred healthy Caucasian participants completed the study. The median age was 45 years (IQR, 31–57 years), and the median body mass index was 24.2 kg/m^2 (IQR, 22.3–26.5 kg/m^2). The body weights of the participants increased after the SLT, whereas blood pressure and creatinine clearance values remained unchanged (Table 1). Urinary sodium excretion increased and urinary aldosterone-18-glucuronide excretion decreased significantly after the SLT (Table 1). Urinary sodium excretion was higher in men than in women, both before and after the SLT (Table 1); however, the difference between the sexes in urinary sodium excretion was not significantly different after correcting for body weight (data not shown). The median serum potassium concentration decreased slightly, from 4.1 mmol/L (IQR, 4.0–4.3 mmol/L) at baseline to 4.0 mmol/L (IQR, 3.8–4.2 mmol/L) after the SLT ($P < 0.001$). Serum aldosterone, PRA, and PRC values were significantly reduced after the SLT (Table 1). The ARR based on the PRA (ARR_{pra}), was not influenced significantly by the SLT ($P > 0.10$, Table 1). The ARR_{prc} decreased significantly after the SLT ($P = 0.005$, Table 1). The following central 95% reference intervals (before and after SLT, respectively) were established for the entire group: urinary aldosterone-18-glucuronide, 6.7–84.3 nmol/24 h and 6.4–37.6 nmol/24 h; serum aldosterone, 35–827 pmol/L and 15–408 pmol/L; PRA, 0.10–2.35 $nmol \cdot L^{-1} \cdot h^{-1}$ and 0.09–1.84 $nmol \cdot L^{-1} \cdot h^{-1}$; PRC, 3.4–29.6 ng/L and 1.8–20.0 ng/L; ARR_{pra}, 0.07–1.45 h^{-1} and 0.06–1.84 h^{-1}; ARR_{prc}, 4.1–81.3 pmol/ng and 3.9–74.8 pmol/ng.

Several differences between men and women were observed for renin and aldosterone. Serum aldosterone values before the SLT were somewhat higher in women than in men ($P = 0.07$), whereas the reverse was found after the SLT ($P < 0.05$, Table 1). PRA values at baseline were not different, but they were lower in women after the SLT. PRC values were lower in women than in men both before and after the SLT. The median relative decrease in the PRA after the SLT was −61% (IQR, −77% to −31%) and −43% (IQR, −68% to −8%) in women and men, respectively ($P < 0.05$). The respective values for the median decrease in PRC values were −42% (IQR, −62% to −21%) and −28% (IQR, −46% to −11%) ($P < 0.05$). Both ARR_{pra} and ARR_{prc} values were higher in women at baseline, but these ratios were not different from those of men after the SLT. There

Table 1. Baseline characteristics and effect of oral sodium loading in the entire group (n = 100), in men (n = 50), and in women (n = 50).[a]

Characteristic	Before SLT	After SLT
Weight, kg	74.0 (66.3–82.5)	74.8 (67.1–83.4)[b]
Men	81.8 (75.0–89.0)	81.3 (75.0–89.9)[b]
Women	67.5 (62.4–73.3)[c]	67.8 (63.0–74.1)[b,c]
Blood pressure, mmHg	125/80	123/80
Men	125/82	127/83[d]
Women	125/80	120/79[e]
Creatinine clearance, $mL \cdot min^{-1} \cdot (1.73\ m^2)^{-1}$	108 (95–122)	110 (97–125)
Men	109 (102–124)	113 (104–127)
Women	106 (90–114)	103 (88–122)
Urinary sodium, mmol/24 h	164 (124–208)	322 (267–367)[b]
Men	194 (157–225)	344 (304–372)[b]
Women	130 (110–178)[c]	295 (253–356)[b,e]
Urinary aldosterone-18-glucuronide, nmol/24 h	29.6 (23.6–37.0)	18.4 (14.9–23.9)[b]
Men	30.0 (24.6–36.8)	18.8 (15.3–24.7)[b]
Women	27.4 (23.1–38.1)[e]	18.2 (13.6–22.9)[b]
Serum aldosterone, pmol/L	215 (163–330)	120 (80–180)[b]
Men	210 (160–280)	140 (98–190)[b]
Women	255 (168–363)	105 (60–163)[b,e]
PRA, $nmol \cdot L^{-1} \cdot h^{-1}$	0.90 (0.50–1.30)	0.40 (0.20–0.70)[b]
Men	1.00 (0.60–1.35)	0.50 (0.30–0.90)[b]
Women	0.80 (0.40–1.20)	0.30 (0.20–0.43)[b,e]
PRC, ng/L	11.5 (8.0–15.7)	7.2 (4.6–9.9)[b]
Men	13.1 (8.8–18.3)	8.6 (6.4–13.3)[b]
Women	9.4 (7.2–13.6)[e]	5.4 (3.5–8.3)[b,c]
ARR_{pra}, h^{-1}	0.28 (0.17–0.46)	0.31 (0.17–0.54)
Men	0.24 (0.15–0.33)	0.29 (0.16–0.50)[d]
Women	0.39 (0.18–0.50)[e]	0.33 (0.17–0.63)
ARR_{prc}, pmol/ng	19.5 (13.0–29.4)	18.6 (9.4–27.1)[d]
Men	16.9 (10.2–23.7)	18.0 (9.3–26.6)
Women	24.8 (15.7–41.9)[e]	19.8 (11.0–27.6)[d]

[a] Data are presented as the median (IQR).
[b] $P < 0.001$, after the SLT vs baseline (before the SLT).
[c] $P < 0.001$, male vs female participants.
[d] $P < 0.05$, after the SLT vs baseline (before the SLT).
[e] $P < 0.05$, male vs female participants.

was no relationship between age and any of the hormonal parameters after the SLT. Before the SLT, age and serum aldosterone were negatively correlated ($r_s = -0.28$; $P = 0.005$), as were age and urinary aldosterone ($r_s = -0.19$; $P = 0.06$), and age and the PRA ($r_s = -0.19$; $P = 0.07$). Baseline aldosterone values were higher in women younger than 50 years than in women older than 50 years (presumed to be premenopausal and postmenopausal, respectively), both in serum [325 pmol/L (IQR, 215–460 pmol/L) vs 210 pmol/L (IQR, 143–238 pmol/L), $P < 0.005$] and in urine [33.1 nmol/24 h (IQR, 23.9–47.0 nmol/24 h) vs 24.9 nmol/24 h (IQR, 22.9–29.4 nmol/24 h), $P < 0.01$]. No differences in hormonal parameters were found between men younger and older than 50 years. Fig. 1 shows the relationship between the PRA and PRC before ($r_s = 0.80$) and after ($r_s = 0.74$) the SLT according to a Passing–Bablok analysis.

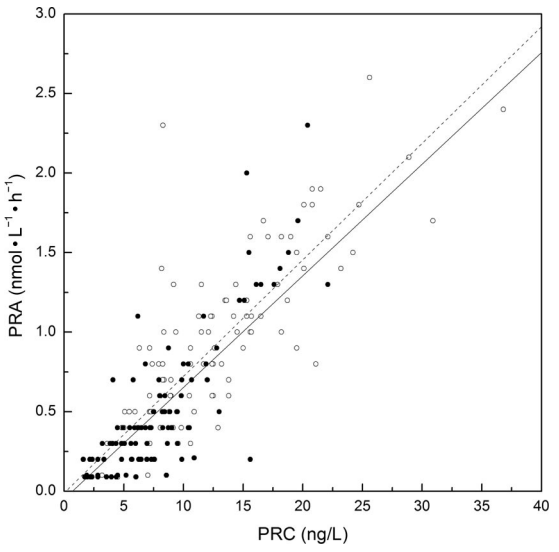

Fig. 1. Passing–Bablok analysis comparing PRA and PRC results before (dashed line) and after (continuous line) the SLT (r_s = 0.80 and 0.74, respectively; $P < 0.001$ for both).

Indicated are data before the SLT (○) ($y = -0.073x - 0.011$ nmol · L^{-1} · h^{-1}) and after the SLT (●) ($y = -0.070x - 0.049$ nmol · L^{-1} · h^{-1}).

Discussion

To the best of our knowledge, this study is the first to present reference PRC and ARR_{prc} values for a well-defined and relatively large group of healthy individuals before and after their consumption of high amounts of sodium. In general, the results of the PRC assay before and after sodium loading (SLT) correlated well with those of the PRA assay. In contrast to the ARR_{pra}, the ARR_{prc} for the entire group was affected by the amount of sodium consumption. Both the ARR_{prc} and the ARR_{pra} were higher in women than in men at baseline, but not after sodium loading. We found additional relationships between the sex of the participants and their PRA, PRC, and aldosterone values in serum and urine. Age was inversely related with serum aldosterone and tended to be negatively correlated with the PRA and urinary aldosterone.

We have identified several preanalytical factors that may influence the renin and/or aldosterone assays, such as the participant's position during sampling, the timing and storage conditions of samples, medication, sodium intake, sex, and age *(1)*. All of these factors were well controlled in the present study. Reference values for aldosterone, the PRC, and the ARR_{prc} have recently been described for a large, healthy German population with the same assays that we used *(7)*. The results of that study are confounded by the lack of control for sodium intake, the allowance of estrogen use by the female participants, the variable timing of blood sampling, and nonstratification by age.

Our results show that circulating aldosterone, PRA, and PRC values are influenced by sex. This finding suggests that sex-specific reference intervals are needed. The present study was not powered to yield sex-specific reference values. Sex-specific differences in the renin–angiotensin–aldosterone system have been well described *(8)*. In general, estrogens increase angiotensinogen concentrations and decrease renin concentrations, angiotensin-converting enzyme activity, the density of angiotensin II receptor type 1, and aldosterone production. Of note is that renin measured as the PRA is usually less affected by estrogens than the PRC *(9)*. The significantly lower PRC values at baseline in women and the absence of a sex effect with respect to the PRA are compatible with this observation. We found that both the ARR_{prc} and ARR_{pra} at baseline were higher in women than in men. This result is in agreement with the results of a recent study that compared the ARR_{prc} and ARR_{pra} in a group of healthy men and women *(10)*. The same study also demonstrated that the ARR_{prc} is higher during the luteal phase than in the follicular phase, whereas the ARR_{pra} remained unchanged during the menstrual cycle *(10)*. Concerns have been raised that the ARR_{prc} is more likely to produce a false-positive test result in women than the ARR_{pra} *(10, 11)*. Currently, however, comparative studies that have used both the ARR_{prc} and ARR_{pra} as a screening test in hypertensive individuals are not available. In addition, our results show that the sex differences in either ratio are sodium dependent, because they disappear after sodium loading. The ARR value is determined not only by the characteristics of the renin assay but also by the method of aldosterone measurement, because significant variation between several commercially available aldosterone immunoassays has been demonstrated *(4)*. An important development for circumventing this variation has been the recent introduction of HPLC–tandem mass spectrometry, which measures aldosterone accurately and specifically *(12)*.

We also demonstrate that serum and urinary aldosterone concentrations at baseline are substantially higher in premenopausal women than in postmenopausal women. This finding is most likely due to the higher progesterone concentrations in premenopausal women, given that in vitro studies have shown that progesterone increases aldosterone production in zona glomerulosa cells *(13)*. In addition, progesterone acts as an antagonist for the mineralocorticoid receptor, which may produce a compensatory increase in aldosterone production *(8)*.

An age-dependent decrease in serum aldosterone has been described previously, although the effect of

high sodium intake was not examined *(14)*. Again, this inverse relationship was absent after sodium loading. The negative trend between PRA and age in our study also has been previously reported *(14–16)*.

Determination of the ARR is widely advocated as the preferred screening test for detecting primary aldosteronism *(1)*. A limitation of the present study is that we examined only normotensive individuals. ARR_{prc} values in patients with essential hypertension or primary aldosteronism have been shown to be considerably higher than those in our participants *(1, 17–19)*. Consequently, primary aldosteronism is highly unlikely in a hypertensive patient with an ARR_{prc} value within the reference interval we have described.

In conclusion, we found the ARR to be affected to a variable degree by sex and sodium intake.

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References

1. Funder JW, Carey RM, Fardella C, Gomez-Sanchez CE, Mantero F, Stowasser M, et al. Case detection, diagnosis, and treatment of patients with primary aldosteronism: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2008;93:3266–81.
2. Montori VM, Young WF Jr. Use of plasma aldosterone concentration-to-plasma renin activity ratio as a screening test for primary aldosteronism. A systematic review of the literature. Endocrinol Metab Clin North Am 2002;31:619–32.
3. Sealey JE, Gordon RD, Mantero F. Plasma renin and aldosterone measurements in low renin hypertensive states. Trends Endocrinol Metab 2005; 16:86–91.
4. Schirpenbach C, Seiler L, Maser-Gluth C, Beuschlein F, Reincke M, Bidlingmaier M. Automated chemiluminescence-immunoassay for aldosterone during dynamic testing: comparison to radioimmunoassays with and without extraction steps. Clin Chem 2006;52:1749–55.
5. Derkx FHM, de Bruin RJA, van Gool JMG, van den Hoek MJ, Beerendonk CCM, Rosmalen F, et al. Clinical validation of renin monoclonal antibody-based sandwich assays of renin and prorenin, and use of renin inhibitor to enhance prorenin immunoreactivity. Clin Chem 1996;42:1051–63.
6. de Bruin RA, Bouhuizen A, Diederich S, Perschel FH, Boomsma F, Deinum J. Validation of a new automated renin assay. Clin Chem 2004;50: 2111–6.
7. Hannemann A, Friedrich N, Ludemann J, Volzke H, Rettig R, Peters J, et al. 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 2010;42: 392–9.
8. Komukai K, Mochizuki S, Yoshimura M. Gender and the renin-angiotensin-aldosterone system. Fundam Clin Pharmacol 2010;24:687–98.
9. Campbell DJ, Nussberger J, Stowasser M, Danser AH, Morganti A, Frandsen E, et al. Activity assays and immunoassays for plasma renin and prorenin: information provided and precautions necessary for accurate measurement. Clin Chem 2009;55:867–77.
10. Ahmed AH, Gordon RD, Taylor PJ, Ward G, Pimenta E, Stowasser M. Are women more at risk of false-positive primary aldosteronism screening and unnecessary suppression testing than men? J Clin Endocrinol Metab 2011;96:E340–6.
11. Pizzolo F, Raffaelli R, Memmo A, Chiecchi L, Pavan C, Guarini P, et al. Effects of female sex hormones and contraceptive pill on the diagnostic work-up for primary aldosteronism. J Hypertens 2010;28:135–42.
12. Taylor PJ, Cooper DP, Gordon RD, Stowasser M. Measurement of aldosterone in human plasma by semiautomated HPLC–tandem mass spectrometry. Clin Chem 2009;55:1155–62.
13. Szmuilowicz ED, Adler GK, Williams JS, Green DE, Yao TM, Hopkins PN, et al. Relationship between aldosterone and progesterone in the human menstrual cycle. J Clin Endocrinol Metab 2006;91: 3981–7.
14. Hegstad R, Brown RD, Jiang NS, Kao P, Weinshilboum RM, Strong C, et al. Aging and aldosterone. Am J Med 1983;74:442–8.
15. Crane MG, Harris JJ. Effect of aging on renin activity and aldosterone excretion. J Lab Clin Med 1976;87:947–59.
16. Tsunoda K, Abe K, Goto T, Yasujima M, Sato M, Omata K, et al. Effect of age on the renin-angiotensin-aldosterone system in normal subjects: simultaneous measurement of active and inactive renin, renin substrate, and aldosterone in plasma. J Clin Endocrinol Metab 1986;62: 384–9.
17. Trenkel S, Seifarth C, Schobel H, Hahn EG, Hensen J. Ratio of serum aldosterone to plasma renin concentration in essential hypertension and primary aldosteronism. Exp Clin Endocrinol Diabetes 2002;110:80–5.
18. Perschel FH, Schemer R, Seiler L, Reincke M, Deinum J, Maser-Gluth C, et al. Rapid screening test for primary hyperaldosteronism: ratio of plasma aldosterone to renin concentration determined by fully automated chemiluminescence immunoassays. Clin Chem 2004;50:1650–5.
19. Westerdahl C, Bergenfelz A, Isaksson A, Wihl A, Nerbrand C, Valdemarsson S. High frequency of primary hyperaldosteronism among hypertensive patients from a primary care area in Sweden. Scand J Prim Health Care 2006;24:154–9.

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