Establishment of reference values for endocrine tests. III: primary aldosteronism

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ABSTRACT

Background: In our laboratory well-defined reference values for the screening test and confirmation test used in the diagnosis of primary aldosteronism were lacking. In this study we established the reference values of the plasma aldosterone concentration (PA), plasma renin activity (PRA) and PA/PRA ratio after a two-hour upright period, and of the urinary aldosterone excretion after oral sodium loading.

Methods: Fifty healthy volunteers, equally distributed according to sex and aged between 20 and 70 years, went through the screening and confirmation test of primary aldosteronism. PA, PRA and the PA/PRA ratios were measured after a two-hour upright period (screening test). Urinary aldosterone excretion was determined in two 24-hour urine samples after an oral suppletion of 6 g NaCl a day for five days (confirmation test).

Results: The following reference values were established: PA (after two-hour upright position) <0.03-1.05 nmol/l (mean: 0.47), PA/PRA ratio 0.05-0.47 (mean: 0.15) and urinary aldosterone excretion after sodium loading <3.0-47.0 nmol/24h (mean: 10.5). PRA showed a significant decrease with advancing age: median values in the 3rd to 7th decade are 3.9, 3.5, 2.5, 1.6 and 2.1 ng AI/ml/h respectively (p=0.04). PA was lower in subjects ≥50 years old. Age did not affect the PA/PRA ratio or the urinary aldosterone excretion. There were no significant differences between the sexes in any of the above-mentioned parameters.

Conclusion: In this study we established reference values for the screening and confirmation test used in the diagnosis of primary aldosteronism.

INTRODUCTION

Primary aldosteronism is a form of mineralocorticoid excess (MCE). Hypertension, an abnormally high plasma aldosterone secretion, suppressed plasma renin activity (PRA), increased urinary excretion of potassium, and hypokalaemic alkalosis are the characteristic findings in patients with primary aldosteronism.[1-3]

It is probably the most prevalent form of MCE. The most common causes of primary aldosteronism are the unilateral aldosterone-producing adenoma (APA; 64% of the cases) and bilateral idiopathic hyperaldosteronism (IHA; 32% of the cases).[4-6] It is important to diagnose primary aldosteronism in patients with hypertension because of the therapeutic implications: in APA the hypertension may be cured by surgery.[7] It has been advocated that all patients with resistant hypertension, whether normokalaemic or hypokalaemic, should be screened for primary aldosteronism.[1-3] The diagnosis of primary aldosteronism is divided into three phases according to Young *et al.* (1990): screening test, confirmation test and tests to determine the subtype of primary aldosteronism. This study concentrates on the first two phases. To distinguish patients with essential hypertension from patients with primary aldosteronism the plasma aldosterone/plasma renin ratio (PA/PRA ratio) is the screening tool of choice. This ratio has the highest accuracy according to the current literature.[1,4,8-12] In our institution a positive screening test is indicated by an increased PA/PRA ratio; a suppressed PRA; and hypokalaemia in combination with an inappropriate kaluresis. If two of the three criteria mentioned above are met, the screening test is considered positive. The diagnosis primary aldosteronism is confirmed by demonstrating an increased (unsuppressible) aldosterone excretion in a 24-hour urine sample after three

days of sodium loading. The sodium loading has to be sufficient, i.e. the urinary sodium concentration should exceed 200 mmol/24h. [6,12-14] According to Bravo *et al.* (1983) this is the single best test (sensitivity 96%, specificity 93%) to identify patients with primary aldosteronism.

As the diagnostic procedures used in the different studies of primary aldosteronism differ slightly, the resulting reference values may vary. In addition, PA and PRA can be influenced by many factors including pregnancy, age, the use of several drugs (NSAIDs, oestrogens, diuretics, spironolactone, ACE inhibitors, β-blockers and calcium antagonists), eating liquorice and race.[3,15-22] The aim of this study was to establish reference values for PA, PRA and the PA/PRA ratio after a two-hour upright period and for the urinary aldosterone excretion after oral sodium loading. The screening and the confirmation test were performed in fifty healthy subjects recruited from the general population of Amsterdam and surrounding area.

SUBJECTS AND METHODS

Subjects

Fifty subjects were recruited by advertisement in a local newspaper with a free house-to-house distribution in the Amsterdam region and by advertisements in 'Status', the biweekly information bulletin of the Academic Medical Centre of Amsterdam University. The subjects were screened for the inclusion and exclusion criteria by telephone and during the intake visit. Inclusion criteria were age between 20 and 70 years and a self-proclaimed general good health. Exclusion criteria consisted of hypertension exceeding a diastolic blood pressure of 100 mmHg, use of diuretics, spironolactone, ACE inhibitors, β-blockers, calcium antagonists, oestrogens or NSAIDs, and pregnancy. Altogether, 93 subjects satisfied the criteria mentioned above: fifty of them, selected at random, completed the two tests.

The subjects were divided into five age categories (20-29 years, 30-39 years, etc.) Each category consisted of ten persons: five men and five women. Categorising was done in order to find out if the values might be age-dependent. At the intake interview, information was given about the study and the tests, and questions were asked about race, diet (salt intake), use of liquorice, medication, smoking, use of alcohol and hypertension. The hospital's ethics committee approved the study and informed consent was obtained from all subjects.

Two subjects did not complete both tests: a 20-year-old man because of nausea and vomiting during the days of salt suppletion and a 26-year-old woman because of personal circumstances. They were excluded and replaced by subjects of the same sex, selected at random from the same age categories.

Tests

Screening test

Two consecutive 24-hour urine samples were collected separately at home. During this collection, the urine was kept refrigerated. Of the two 24-hour urine samples, total volume as well as the concentrations of sodium, potassium, creatinine and aldosterone were measured. At 9 am (t0), 15 minutes after inserting an intravenous canula, blood samples were taken in sitting position to measure the PA and PRA. At 11 am (t2), after a two-hour upright period, blood samples were taken in an upright position for the measurement of sodium, potassium, creatinine, PA and PRA. The diurnal and day-to-day variation of plasma aldosterone is high.[2,8] Therefore, the PA in all subjects was measured at the same time in the morning, when the concentration is at its peak. The values obtained after the two-hour upright period were of interest for the evaluation of the screening test. The completeness of the urine collections was assessed from the 24-hour urinary creatinine concentrations. If the creatinine excretion of the urine sample with the highest creatinine excretion was ≥150% of the creatinine excretion of the other sample, the urine collection was considered incomplete and the two samples were excluded.[23] Weight and height were recorded, and blood pressure was measured with the Dinamap® Compact monitor. The subjects were investigated after an overnight fast and smoking was not allowed.

Confirmation test

During five days before to the blood sampling the subjects took an additional 6 grams of salt: two tablets of 1 g NaCl, three times a day. During the last two days of this salt intake they collected their urine. Total volume as well as the concentrations of sodium, potassium, creatinine and aldosterone were measured per 24-hour sample. Blood sampling for the determination of plasma potassium was performed at the end of the five-day period at 9 am after an overnight fast. Smoking was not allowed. Before the blood sampling, weight and blood pressure were measured.

Analytical methods

PA was measured by a commercial RIA (radial immuno-assay) (coat-a-count, Diagnostic Products Corporation, Los Angeles, CA). The detection limit is 0.03 nmol/l, the interassay coefficient of variation (CV) 4.7 to 12.0% and the intra-assay CV 3.6 to 8.4% (at 1.53 to 0.13 nmol/l). The PRA was determined by RIA as described previously (Hollemans *et al.*, 1969). The detection limit is 0.3 ng A1/ml/h, the interassay CV 6.0 to 11.0% and the intra-assay CV 4.0 to 6.0% (at 10.6 to 1.4 ng A1/ml/h). Urinary aldosterone was determined after extraction with ethylacetate by the same RIA. The interassay coefficient of variation is less than 18%. Plasma sodium, potassium and creatinine were measured by standard clinical chemical methods and reagents

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on a Hitachi 747 analyser (Roche Diagnostics, Almere, the Netherlands). For the determination of urinary sodium, potassium and creatinine concentrations a Hitachi 911 analyser (Roche Diagnostics, Almere, the Netherlands) was used. The pregnancy test used was the ABBOT TestPack®Plus™.

Statistical methods

Use of alcohol was defined as the intake of at least two units a day, smoking as daily smoking and liquorice use as the consumption of more than two pieces of liquorice a day. The subjects were questioned about their daily salt intake. They could choose from categories varying from a low, low/normal, normal, normal/high and high salt intake. For the body mass index (BMI) the mean value between the day of the screening test and the day of the confirmation test could be used, as there was no significant difference between the two time points. The urinary concentrations of aldosterone, creatinine, sodium and potassium were calculated as the mean of the values of the two subsequent 24-hour urine samples, as there were no significant differences between the two samples. Values below the detection limit of the assays were included in the analyses as having a value of 50% of the detection limit. Differences between groups were analysed with the Wilcoxon signed-rank test, with the exception of the plasma potassium concentration and the systolic and diastolic blood pressure, for which the paired t-test was used. Sex differences, influence of smoking, alcohol, race, liquorice and history for hypertension were analysed with the Mann-Whitney U test. Effects of age were tested by the Kruskal-Wallis test, possible correlations with the Spearman's rank correlation. We used SPSS 10.1 for the statistical analysis. In all tests, p values below 0.05 were considered statistically significant. Reference values are given as mean ±2 SD. PA, PA/PRA ratio and urinary aldosterone excretion showed a non-parametric distribution. After logarithmic transformation the distribution appeared to be normal, allowing for the assessment of mean ±2 SD of these parameters. Age-dependent reference values are given as the observed range in view of the small number of observations.

RESULTS

Table 1 gives some characteristics of the subjects. During the screening test three subjects had a diastolic blood pressure between 90 and 100 mmHg. A similar blood pressure was found in two subjects during the confirmation test.

Screening test

The urine samples of four subjects were excluded because of incomplete/incorrect urine collection. In addition, one PA assay could not be included, because of a preanalytical mistake.
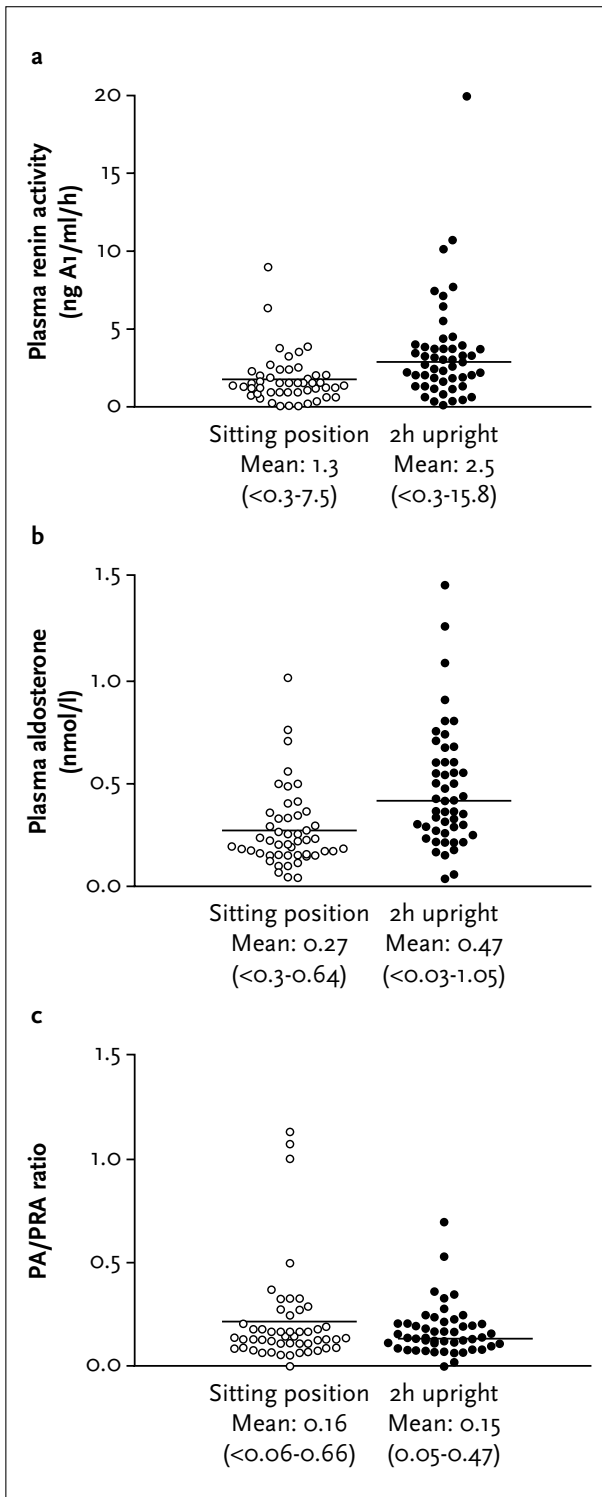
PRA, PA and PA/PRA

PRA increased significantly between t0 (sitting position) and t2 (after a two-hour upright period) (*figure 1a* and *table 2*). The increase in the PRA between t0 and t2 varied from 0 to 11.0 ng A1/ml/h with a median of 1.1 ng A1/ml/h. The PRA at time t2, used for the formal assessment of the screening test, declined (p=0.04) with advancing age (*table 3*). Subjects of 50 years and older had significantly lower reference values (range: <0.3-7.5 ng A1/ml/h, median: 1.8 ng A1/ml/h) than subjects under 50 years (range: 0.3 to 20 ng A1/ml/h, median: 3.4 ng A1/ml/h) (p<0.01).

PA also increased significantly in upright posture (*table 2* and *figure 1b*). The increase varied from -0.10 to 0.73 nmol/l (median: 0.15 nmol/l). Especially after the two-hour upright period the range was rather wide. PA tended to decline with advancing age (p=0.05) (*table 3*). Subjects of 50 years and older showed a significantly lower PA value while upright as compared with younger subjects (p=0.03). *Figure 2* shows the strong correlation between PA and PRA (p=0.72, p<0.01) on time t2.

Values for the PA/PRA ratio are given in *figure 1c* and *table 2*. Although the mean values of the PA/PRA ratio were quite similar at time t0 and t2, the range was smaller at t2.

Table 1
Subject characteristics (n=50) (median; range)

Age (years)		44	(20-69)
Body mass index	Women (n=25)	25.2	(19.7-39.6)
	Men (n=25)	24.9	(18.5-33.0)
Race	Caucasian	41	(82%)
	Non-Caucasian	9	(18%)
Blood pressure (mean)	Screening test	127/72	mmHg
	Confirmation test	122/68	mmHg
Smoking		13	(26%)
Alcohol use		8	(16%)
Liquorice use		5	(10%)

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Figure 1

The plasma aldosterone concentration (PA), plasma renin activity (PRA) and PA/PRA ratio during the screening test in sitting position (t0) and after a two-hour upright period (t2) (mean values indicated by horizontal lines)

The PRA is significantly lower (p<0.01) at t0 (n=50) in comparison with t2 (n=50) (a). The PA is significantly higher on t2 (n=50) in comparison with t0 (n=49) (p<0.01) (b). The mean of the plasma aldosterone/ plasma renin activity ratio at t0 (n=49) is not significantly different from to the ratio on t2 (n=49) (c).
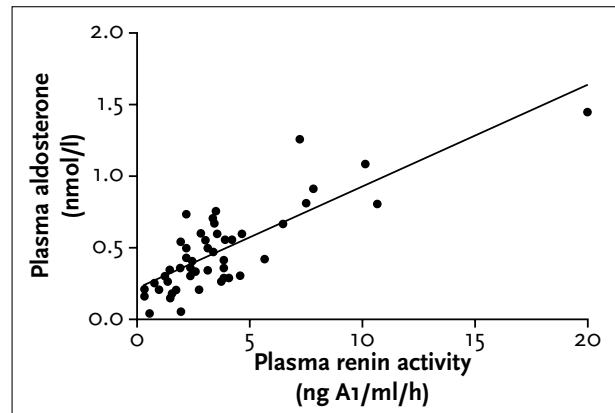
Figure 2

Correlation between plasma aldosterone concentration (PA) and plasma renin activity (PRA) after a two-hour upright period (11.00 h, t2) (r=0.72, p<0.01)

Age did not affect the ratio. All other factors, i.e. race, BMI, smoking, use of alcohol, use of liquorice, history for hypertension, salt intake and sex, did not affect PA, PRA and PA/PRA ratio.

Potassium, sodium and creatinine in plasma and urine
Men had a higher urinary potassium excretion than women (p<0.01), but plasma potassium did not differ between sexes (*table 2*). During the screening test as well as during the confirmation test the urinary and plasma concentrations of creatinine were higher for men than for women (p<0.01). The values for the plasma and urine sodium concentrations were not affected by age, BMI, race, smoking, use of alcohol, use of liquorice, salt intake and a history of hypertension.

Confirmation test
Urine samples of eight persons were excluded because of incomplete/incorrect urine collection. The mean 24-hour volume of the collected urine after oral salt loading increased significantly as compared with those collected in the screening test: the mean increased from 1569 ml to 1900 ml (p<0.01). The urinary aldosterone concentration was significantly lower during the confirmation test (p<0.01) (*table 2* and *figure 3a*). The difference varied from -58 to +7 nmol/24h with a median -6 nmol/24h. Neither sex nor age effected the urinary aldosterone. The mean urinary sodium excretion was normally distributed and increased significantly after salt suppletion (p<0.01) (*figure 3b*). The mean difference of the sodium excretion between the screening test and the confirmation test was +79.5 mmol/24h (range: -18.4 to +226.9 mmol/24h). The plasma potassium concentration decreased significantly after administration of NaCl (p<0.01) (*table 2*). The urinary potassium excretion differed slightly with sex; men showed a higher urinary potassium excretion than women (p=0.04) (*table 2*).

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Table 2

Results of the screening and confirmation tests for primary aldosteronism

			SCREENING TEST			CONFIRMATION TEST	
PA (nmol/l) (total)	Sitting position (t0)	0.27	<0.03-0.64	n=49			
	2 hours upright (t2)	0.47	<0.03-1.05[*]	n=49			
PRA (ng AI/ml/h)	Sitting position (t0)	1.31	<0.3-7.49	n=50			
	2 hours upright (t2)	2.47	<0.3-15.84[*]	n=50			
PA/PRA	Sitting position (t0)	0.16	0.04-0.66	n=49			
	2 hours upright (t2)	0.15	0.05-0.47	n=49			
Plasma potassium (mmol/l)		4.1	3.6-4.7	n=50	3.9	3.5-4.3[†]	n=50
Plasma creatinine (mmol/l)	Men	71	49-94	n=25			
	Women	59	43-76[‡]	n=25			
Urinary aldosterone (nmol/24h)		18.8	4.6-76.8	n=46	10.5	<3.0-47.0[†]	n=42
Urinary sodium (mmol/24h)		149.7	47.3-251.1	n=46	225.6	106.3-344.9[†]	n=42
Urinary potassium (mmol/24h)	Men	87.5	32.7-142.3	n=22	82	34.4-129.6	n=23
	Women	64.9	27.2-102.6[‡]	n=24	82	29.8-104.4[§]	n=19
Urinary creatinine (mmol/24h)	Men	14.3	8.4-20.2	n=22	13.9	8.6-19.2	n=23
	Women	9.8	6.9-12,7[‡]	n=24	9.4	5.8-13.0[‡]	n=19

Values are mean ±2 SD. [*] Significant difference from sitting posture, p<0.01; [†] significant difference from the screening test, p<0.01; [‡] significant difference from the men, p<0.01; [§] significant difference from the men, p=0.04.
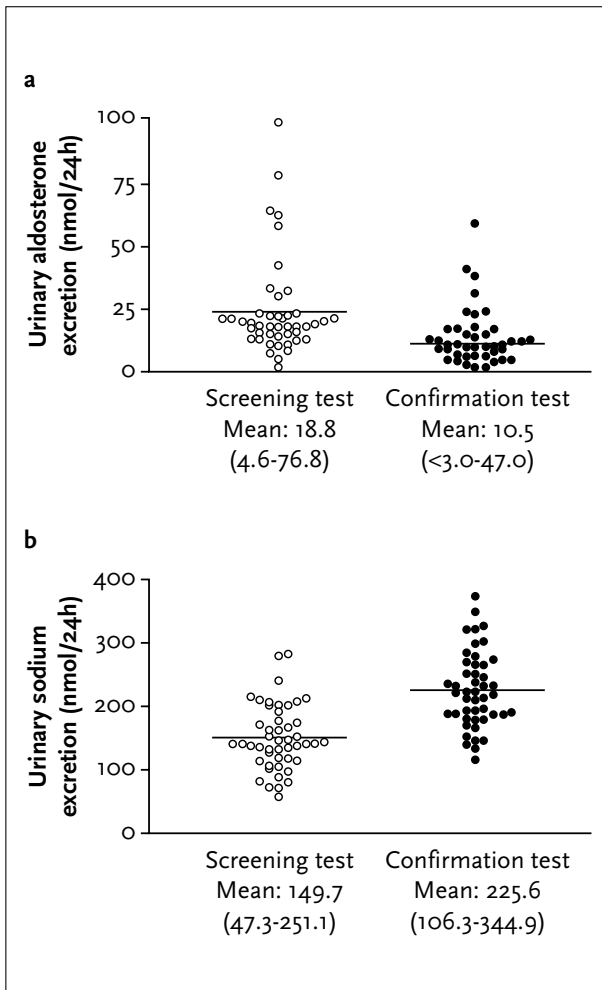
Table 3

Reference values for tests in the evaluation of primary aldosteronism

			REFERENCE VALUES	
Screening test	PRA	20-29 years	0.3-2.0	ng A l/ml/h
(after a two-hour upright period)		30-39 years	1.9-7.8	ng A l/ml/h
		40-49 years	0.5-7.2	ng A l/ml/h
		50-59 years	<0.3-4.5	ng A l/ml/h
		60-69 years	0.3-7.5	ng A l/ml/h
	PA	20-29 years	0.21-1.45	nmol/l
		30-39 years	0.26-0.90	nmol/l
		40-49 years	0.04-1.25	nmol/l
		50-59 years	0.15-0.55	nmol/l
		60-69 years	0.16-0.80	nmol/l
	PA/PRA		0.05-0.47	
Confirmation test	Urinary aldosterone after sodium loading		<3.0-47.0	nmol/24h

DISCUSSION

For the evaluation of the screening test, PA and PRA measurements are essential. As could be expected, the upright posture causes an increase in PRA through stimulation of the renin-angiotensin-aldosterone system, compared with time t0 (in sitting position). We observed a decrease in PRA with advancing age, in accordance with the literature. In a study by Hegstad (1983), PRA of older subjects (upright, normal salt intake) was lower than that of younger subjects.[17] PRA of persons over 50 years fell by 50%.[2] In our study one outlying value of 20 ng AI/ml/h in the age category 20-29 years occurred in a subject with a normal blood pressure (103/78 mmHg);

this PRA value thus still belongs to the normal spectrum. PA in our study increased after a two-hour upright period as well and declined with advancing age. According to the literature PA concentrations in subjects older than 50 years are lower than those in subjects younger than 30 years.[17] This was also evident in our study: the subjects of 50 years and older had a lower PA than those younger than 50 years.

Factors other than posture and age may affect PA and PRA. The production of renin increases under the influence of oestrogens and reference values for PA can vary per sex.[2,3] In our study, however, we did not find sex differences for PA, PRA and the PA/PRA ratio. PA and PRA were not dependent on sodium intake, which corresponds with the

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Figure 3

The urinary aldosterone and the urinary sodium excretion during the screening and confirmation test (mean values indicated by horizontal lines)

The urinary aldosterone excretion during the screening test (salt intake ad lib) (n=46) is significantly higher than during the confirmation test (after administration of 6 g NaCl per day for five days) (n=42) (p<0.01) (a). The urinary sodium excretion increased during the confirmation test (after administration of 6 g NaCl a day for five days) (n=46) compared with the screening test (salt intake ad lib) (n=42) (p<0.01) (b).

findings of Hiramatsu (1981) and Loh (2000). Although PA and PRA are suppressed by the active components of liquorice (glycyrrhizic acid and glycyrrhitic acid), liquorice did not emerge in our study as a factor influencing PA and PRA, probably due to the small number of subjects (10%) eating liquorice regularly.[19-21] PA and PRA in blacks are lower than in whites.[22] The non-Caucasian subjects in our study, however, were of Asian origin, and race had no effect on the reference values.

The PA/PRA ratio can be calculated on basis of the PA and PRA in the sitting position or after a two-hour upright period. For evaluation of the screening test the PA/PRA ratio after a two-hour upright period is preferred in view of its smaller range as compared with the sitting position. Likewise, the urinary aldosterone excretion before oral salt loading seems to be less useful for the confirmation of the diagnosis of primary aldosteronism, because the range is much wider than after salt loading.

As expected, the urinary aldosterone excretion decreased after salt suppletion as a result of the inhibition of the renin-angiotensin-aldosterone system. The sodium loading has to be sufficient for a proper evaluation of the confirmation test. The loading is considered sufficient if the sodium concentration exceeds 200 mmol/24h urine.[6] The mean concentration was indeed higher than 200 mmol/24h, but in 18 subjects (43%) it remained below this value. This cut-off value may be too high. It does not seem appropriate either to use the difference between the sodium concentration before and after salt loading as a cut-off value, because the sodium excretion in some of our subjects declined after salt suppletion (mean change: +79.5 mmol/24h, range: -18.4 to +226.9 mmol/24h). A decrease in sodium excretion could be caused by reduction of dietary sodium intake during the confirmation test, when subjects believe that their total salt intake is too high. The sodium excretion should at least be 103 mmol, if the subjects take all the NaCl tablets (6 g NaCl corresponds with 103 mmol NaCl).

The plasma potassium concentration before oral salt loading was higher than after salt loading. A possible explanation is the increased availability of sodium after salt loading. Because of the sodium suppletion the reabsorption of sodium diminishes in the proximal tubules. Therefore, the sodium availability increases in the distal tubules, in which the exchange of sodium and potassium takes place. As a result, more potassium can be exchanged for sodium, decreasing the plasma potassium concentration. This also occurs in normokalaemic patients with primary aldosteronism who develop hypokalaemia after salt loading.[6] The decreasing potassium plasma after salt loading does indicate that it is essential for patients with a suspicion of primary aldosteronism to be supplemented with potassium. The urinary potassium excretion of men was higher than that of women, which is in accordance with the study by Watenpaugh.[24]

In summary, we have established reference values for both the screening and the confirmation test in the diagnosis of primary aldosteronism. These values are presented in *table 3*. However, the most appropriate cut-off values should be ascertained in a study of patients suspected of having primary aldosteronism, who pass the same diagnostic protocol and finally can be labelled as having primary aldosteronism or not. In this respect, the cut-off value of urinary aldosterone excretion in the confirmation test might well be 39 nmol/24h as indicated in the literature:[5] in fact, only one of our healthy volunteers had a value above 39 nmol/24h (*figure 3a*).

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