# Pattern of Adrenal Hormonal Secretion in Patients with Adrenal Adenomas: The Relevance of Aldosterone in Arterial Hypertension

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**Context:** Approximately 10% of hypertensives are considered to exhibit autonomous aldosterone secretion (AAS). Although adrenal incidentalomas (AI) can be found in up to 19% of hypertensive individuals, data on the incidence of AAS in hypertensive patients with AI remain scarce.

**Objective:** The aim was to study adrenal aldosterone (ALD) secretory pattern in patients with adrenal adenomas with and without arterial hypertension.

**Design and Setting:** We conducted a case-control study in a tertiary general hospital.

**Patients and Main Outcome Measures:** We investigated 72 normotensive subjects with normal adrenal morphology and 191 subjects divided in three groups: 46 normotensive individuals with an AI (NAI), 89 hypertensive patients with an AI (HAI), and 56 hypertensive patients with an adrenal adenoma identified after investigation for arterial hypertension (HAA). Evaluation of autonomous cortisol secretion was based on a low-dose dexamethasone suppression test. Autonomous ALD secretion was based on a modified saline infusion test (MSI). Normal cutoff levels were obtained from the control matched population.

**Results:** Post-MSI ALD levels and the ALD/renin (REN) ratios were significantly elevated in HAI and HAA patients compared to NAI subjects. To evaluate the prevalence of AAS, we applied the combination of post-MSI ALD level and the ALD/REN ratio simultaneously (post-MSI cutoffs, ALD levels, 2.41 ng/dl; ALD/REN ratio, 0.35 ng/dl/$\mu$U/ml). Based on these cutoffs, 12% of NAI, 36.4% of HAI, and 54.2% of HAA patients had AAS. The prevalence of autonomous cortisol secretion did not differ among the three groups.

**Conclusions:** Using a MSI test, we found a remarkably increased prevalence of AAS in hypertensive patients with adrenal adenomas, even when the latter represented an incidental finding. **(*J Clin Endocrinol Metab* 97: E537–E545, 2012)**

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Abbreviations: AAS, Autonomous ALD secretion; ACS, autonomous cortisol secretion; AH, arterial hypertension; AI, adrenal incidentaloma; ALD, aldosterone; BMI, body mass index; CT, computerized tomography; DBP, diastolic blood pressure; DHEAS, dehydroepiandrosterone sulfate; HAA, hypertensive patients with adrenal adenomas identified during investigation for AH; HAI, hypertensive patients with AI; HOMA-IR, homeostatic model assessment-insulin resistance; IRMA, immunoradiometric assay; LDDST, low-dose dexamethasone suppresion test; MATSUDA, Matsuda's composite whole body insulin sensitivity index; MSI, modified saline infusion; NAI, normotensive patients with AI; QUICKI, quantitative insulin-sensitivity check index; REN, renin; SBP, systolic blood pressure.

Arterial hypertension (AH) is a well-established cardiovascular risk factor. Clinical and experimental data have revealed a close relation between cardiovascular risk and serum aldosterone (ALD) level, an emerging mediator of AH (1–6). According to cross-sectional and prospective studies, more than 10% of hypertensive patients are considered to exhibit autonomous ALD secretion (AAS) (7–11). We have recently reported an increased prevalence of AAS in a hypertensive population (up to 31%) after modification of currently used means to assess AAS. This modification consisted in performing the classical saline loading tests, after dexamethasone administration to eliminate any stimulatory ACTH effect on ALD secretion. This approach could reveal subclinical forms of AAS that could have been missed if only standard tests had been applied (12).

Adrenal incidentalomas (AI), unsuspected adrenal masses incidentally discovered on computerized tomography (CT) or magnetic resonance imaging, constitute a heterogeneous clinical entity with an estimated incidence of 8.4% in nonhypertensive populations and up to 18.9% in hypertensive populations in autopsy studies (13, 14). In a recent study from our group, the incidence of adrenal adenomas in a hypertensive population reached 24.4% (15). Cortisol hypersecretion is present in 5.3% of subjects harboring AI (16), although greater percentages (10–52%) have also been reported depending on the diagnostic criteria applied (12, 17–21). Despite previous considerations of a low prevalence of AAS ranging from 1–6% in patients with AI (22, 23), the application of newly developed criteria to document AAS has revealed a prevalence of more than 24% (12). This increased prevalence of AAS was due to the introduction of substantially lower cutoff values indicative of AAS. These cutoffs were derived from a control group with normal adrenal imaging in whom modified suppressive tests were applied after eliminating any stimulatory effect of ACTH on ALD secretion by dexamethasone administration (24, 25).

Currently, there is a relative paucity of data on the incidence of cortisol and ALD hypersecretion in patients with AH and an adrenal adenoma discovered either incidentally or during investigation for AH. Such information would be useful in the clinical setting because it could direct physicians from the diagnosis of "essential hypertension" toward a more etiologically orientated diagnosis of AH, and thus a targeted antihypertensive therapy with potentially optimal effectiveness.

In the present study, we aimed to identify the pattern of adrenal cortisol and ALD secretion in a series of patients with adrenal adenomas, with and without AH, and any potential relation that such alterations could exert on their metabolic profile. We further examined whether hypertensive patients with AI have a different secretory steroid profile compared with those where an adrenal adenoma was not an incidental finding, but was identified after investigation for AH. Furthermore, in patients in whom AAS was documented, the therapeutic effect on arterial blood pressure that ALD antagonists could exert was evaluated.

## Patients and Methods

We prospectively studied 191 patients with adrenal adenomas during a time period of 5 yr for evaluation of AI or adrenal adenomas identified during the investigation of AH. Studied subjects were categorized in three distinct groups according to the presence or absence of AH and whether the adrenal adenoma was an incidental finding or was discovered during workup for AH. Group A included 46 normotensive patients with an AI (NAI), group B included 89 hypertensive patients with an AI (HAI), and group C included 56 hypertensive patients with an adrenal adenoma identified after investigation of AH (HAA). Patients of group C were referred for exclusion of an endocrine cause of AH, such as young age of onset, difficult to control hypertension under first-line antihypertensive medication, and high suspicion of an endocrine cause of AH. Furthermore, we have included a control group of 72 normotensive subjects who had normal adrenal imaging (CT scan).

All participants underwent physical examination [body weight (kilograms), height (centimeters), and body mass index (BMI; kilograms per square meter)] and ambulatory measurements of systolic and diastolic blood pressure (SBP and DBP, respectively). AH was defined as a SBP of at least 135 mm Hg and/or a DBP of at least 85 mm Hg. All hypertensive patients receiving antihypertensive therapy known to affect the renin (REN)-ALD axis were changed to calcium channels blockers at least 3 wk before any adrenal hormonal evaluation. The patients were instructed to have free salt intake before admission to the hospital.

Biochemical investigation, including serum and 24-h urinary electrolytes, and an oral glucose tolerance test with 75 g glucose were performed in all patients. All blood sampling in basal and dynamic adrenal testing was performed in a recumbent position at 0800 h. Glucose and insulin levels were measured at time 0, 30, 60, 90, and 120 min, and the following indices of insulin resistance were calculated: sensitivity homeostatic model assessment-insulin resistance (HOMA-IR), quantitative insulin-sensitivity check index (QUICKI), and Matsuda's composite whole body insulin sensitivity index (MATSUDA). Basal adrenal hormonal investigation included serum cortisol, plasma ACTH, serum dehydroepiandrosterone sulfate (DHEAS), REN, and ALD levels, and the calculation of ALD/REN ratio. The evaluation of cortisol hypersecretion included 24-h urinary free cortisol and estimation of serum cortisol levels 6 h after the last dose of dexamethasone during a low-dose dexamethasone suppression test (LDDST), *i.e.* 0.5 mg dexamethasone administrated every 6 h for 2 consecutive days. Evaluation for AAS included a modified saline infusion (MSI) test, *i.e.* saline infusion (2 liters NaCl 0.9% within 4 h) after dexamethasone administration, where ALD

plasma levels and the ALD/REN ratio were measured before and at the end of the MSI (12).

Cutoff levels were developed using a control population of 72 subjects with normal arterial pressure (SBP and DBP <135/85 mm Hg) and normal adrenal morphology on CT imaging. These individuals had a mean age of 52.8 ± 15.5 yr, and they had an adrenal CT scan performed for reasons not related to adrenal investigation (42 for abdominal pain, 19 for fever of unknown etiology, and 11 for road traffic accident). Calculated cutoffs (mean + 2 SD) were: basal ALD/REN ratio, 2.8 ng/dl/μU/ml (78 pmol/liter/mU/liter); post-LDDST cortisol levels, 1.1 μg/dl (30 nmol/liter); post-MSI ALD levels, 2.4 ng/dl (67 pmol/liter); and post-MSI ALD/REN ratio, 0.35 ng/dl/μU/ml (9.74 pmol/mU/liter) (12).

Adrenal CT scans were performed in the same machinery and were blindly assessed by the same radiologist (C.S.). The diagnosis of an adrenal adenoma was based on the presence of a homogeneous lesion with well-defined margins and a density below 10 Hounsfield units.

Patients with a pheochromocytoma or adrenal malignancy, documented heart failure, or renal or hepatic disease were not included in this study. Patients with low serum potassium concentration and high suspicion for Conn's syndrome were excluded. The study was approved by the institutional ethics committee, and written informed consent was obtained from all study participants.

Adrenal venous sampling (AVS) has been extensively used to document ALD hypersecretion. However, the success rate of catheterizing both adrenal veins was not high enough in our institution; in addition, it could have been technically laborious to evaluate all patients studied with this technique. For these reasons, adrenal venous sampling was not included in our investigational protocol. After the hormonal evaluation, targeted antihypertensive therapy with an ALD antagonist was administrated in 55 patients with adrenal adenoma and AAS, *i.e.* spironolactone or eplerenone, titrated to maintain normal serum potassium concentration, and patients were followed up for an average of 8 wk (range, 30–90 d). Spironolactone was administrated at a dose of 25–100 mg/d and eplerenone at a dose of 50–100 mg/d. Twenty-three patients belonged to the HAI group and 32 to the HAA group. Patients were asked to monitor their blood pressure at home for 7 d. The mean value of self-reported SBP and DBP values was recorded before and after the modification of the antihypertensive treatment.

### Hormone assays

Hormones were measured using the following RIA. Serum insulin was measured by the immunoradiometric assay (IRMA; DIAsource Immunoassays, Louvain-la-Neuve, Belgium). The sensitivity was 1 μIU/ml (7 pmol/liter), and intra- and interassay coefficients of variation were 2.2 and 6.5% for levels of 6.6 μU/ml (47 pmol/liter) and 14.4 μU/ml (103 pmol/liter), respectively. Serum and urinary cortisol and serum DHEAS were measured by RIA (DIAsource ImmunoAssays, Louvain-la-Neuve, Belgium). The sensitivity for cortisol was 0.08 μg/dl (2.2 nmol/liter), and intra- and interassay coefficients of variation were 6.2 and 8.7% for levels of 0.1 μg/dl (3 nmol/liter) and 0.19 μg/dl (5 nmol/liter), respectively. The assay sensitivity for DHEAS was 0.55 μg/dl (0.015 μmol/liter), and intra- and interassay coefficients of variation were 5.3 and 4.5% for levels of 10.7 μg/dl (0.3 μmol/liter) and 21.4 μg/dl (0.6 μmol/liter), respectively. Serum ACTH was measured by IRMA (CIS Bio International, Codolet, France); the sensitivity was 2 pg/ml (0.4 pmol/liter), and intra- and interassay coefficients of variation were 6.1 and 5.3% for levels of 22 pg/ml (5 pmol/liter) and 40 pg/ml (9 pmol/liter), respectively. Plasma REN concentration (REN III generation) was measured by IRMA (CIS Bio International); the sensitivity was 1.8 μU/ml (1.8 mU/liter) and intra- and interassay coefficients of variation were 3.6 and 5% for levels of 6.93 and 7.2 μU/ml (mU/liter), respectively. Serum ALD was assessed by RIA (Immunotech Assays, Marseille, France); the sensitivity was 0.6 ng/dl (17 pmol/liter) and intra- and interassay coefficients of variation were 9.5% or less and 9.9% or less, respectively.

### Statistical analysis

Statistical analysis was performed using the SPSS software package (version 17.0; SPSS Inc., Chicago, IL). The *t* test and the nonparametric Mann-Whitney test were used to analyze continuous variables with and without normal distribution, respectively. The $\chi^2$ test was used for the categorical variables of adenoma classification and cortisol and ALD secretory autonomy. One-way ANOVA and the Kruskal-Wallis test were performed for the statistical comparison of hormonal values in the adenoma classification. *P* values less than 0.05 were considered statistically significant.

## Results

The mean age of the studied population was 55.78 (±10.74) yr, the mean BMI was 32.02 (±21.23) kg/m$^2$, and the mean waist circumference was 84.58 (±18.99) cm, respectively. Seventy-two patients were male and 119 female (38 and 62%, respectively). Gender distribution was similar in all three groups (NAI, 13 males/33 females; HAI, 33 males/56 females; HAA, 26 males/30 females; $P = 0.163$). No gender difference was shown regarding patients' age, BMI, SBP, and the presence of diabetes mellitus. However, men had higher DBP (91.92 ± 15.80 *vs.* 83.79 ± 13.43 mm Hg; $P = 0.040$) and higher waist circumference values compared with females (93.51 ± 13.48 *vs.* 79.41 ± 18.95 cm; $P = 0.048$). Patients' anthropometric characteristics and comparison among the three groups are shown in Table 1, and their biochemical profile is shown in Table 2. The three groups of patients had increased BMI and waist measurement compared with the control group ($P = 0.02$ and 0.01, respectively). Furthermore, a significant difference was demonstrated regarding the serum and urinary potassium concentrations between the control group and the three patient groups ($P < 0.001$ and 0.01, respectively), with the HAA group showing the lowest serum potassium concentrations and the highest urinary potassium excretion (Table 2). There was no significant difference regarding insulin resistance and sensitivity indices (HOMA-IR, QUICKI, and MATSUDA)

**TABLE 1.** Anthropometric characteristics of the controls and the three patient groups and *P* values

| | | | | | *P* value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control group | Group A (NAI) | Group B (HAI) | Group C (HAA) | Within patient groups | Control –group A | Control –group B | Control – group C | Group A–B | Group A–C | Group B–C |
| Age (yr) | 53.24 (12.15) | 53.74 (10.13) | 58.01 (10.27) | 53.98 (11.44) | **0.01** | **0.02** | **<0.001** | **0.01** | **0.02** | 0.91 | 0.91 |
| SBP (mm Hg) | 120.41 (9.63) | 120.18 (12.19) | 150.08 (22.71) | 160.76 (19.24) | **<0.001** | 0.22 | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| DBP (mm Hg) | 76.38 (6.36) | 74.04 (7.96) | 91.27 (15.02) | 94.67 (12.26) | **<0.001** | **0.04** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| BMI (kg/m$^2$) | 28.33 (3.98) | 27.78 (5.29) | 34.73 (29.66) | 31.01 (5.84) | **0.02** | 0.15 | 0.46 | 0.40 | 0.18 | **0.02** | **0.02** |
| Waist measurement (cm) | 77.13 (14.29) | 76.38 (15.14) | 87.71 (20.93) | 86.55 (16.52) | **0.01** | 0.15 | 0.28 | 0.34 | **0.01** | **0.01** | **0.01** |

Statistically significant results appear in *bold*. Continuous variables are shown as mean (SD).

among the controls and the three patient groups, respectively.

Hormonal levels in the basal state and after suppressive testing are shown in Table 3. No differences were observed between basal ACTH, cortisol, and REN levels in the three groups. All three groups had significantly higher ALD and ALD/REN ratio in basal testing and after MSI compared with the control group. However, ALD levels and the ALD/REN ratio were significantly different in both the basal state and after MSI among the three groups, with the highest levels obtained in patients with HAA ($P < 0.01$). ALD levels in both basal state and after the MSI in the three groups are shown in Fig. 1. There was no correlation between ALD levels and patients' age in either of the studied groups.

To document AAS, we employed the combination of post-MSI ALD level and MSI ALD/REN ratio simultaneously [ALD, 2.4 ng/dl (67 pmol/liter); ALD/REN ratio, 0.35 ng/dl/$\mu$U/ml (9.74 pmol/mU/liter)]. When applying the above cutoffs, 12% of NAI patients, 36% of HAI patients, and 54% of HAA patients were considered to have AAS. In contrast to AAS, the prevalence of autonomous cortisol secretion (ACS) did not differ among the three groups (50% in NAI, 51% in HAI, and 30% in HAA; $P = 0.094$). Furthermore, combined cortisol and ALD secretion was present in 11% of NAI, 19% of HAI, and 13% of HAA patients. Overall, the prevalence of ACS was similar between male and female patients; however, the prevalence of AAS was significantly higher in males compared with female patients (48 *vs.* 32%; $P = 0.04$). Using only the basal ALD/REN ratio to screen for AAS, we found an elevated basal ALD/REN ratio in 5% of NAI, 24% of HAI, and 33% of HAA patients. These results are markedly lower compared with the prevalence of AAS based on the MSI cutoffs.

Although no correlation between plasma ACTH, serum cortisol levels, urinary free cortisol, SBP, and DBP was found, a significant correlation between SBP and DBP and serum ALD and ALD/REN ratio after the MSI ($P < 0.01$ in all correlations) was documented. This relation remained significant even when the seven highest values for ALD (>30 ng/dl) and ALD/REN ratio (3.7 ng/dl/$\mu$U/ml) were excluded (Fig. 2). In addition, a significant negative correlation between SBP and DBP and serum potassium levels was found in all groups ($r = -0.217$ and $-0.232$; $P = 0.03$ and 0.01, respectively). Overall, an inverse association was found between serum potassium concentration and ALD levels both in basal testing and after the MSI ($r = -0.207$, $P = 0.021$; and $r = -0.288$, $P < 0.001$, respectively). In addition, urinary potassium excretion was positively correlated with basal ALD levels ($r = 0.189$; $P = 0.050$).

All 55 patients with documented AAS after the introduction of the newly established cutoffs who received treatment with an ALD antagonist exhibited a marked reduction in both minimum and maximum SBP and DBP

**TABLE 2.** Serum and urine biochemical data and insulin sensitivity indices in controls and the three patient groups

| | | | | | *P* value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control group | Group A (NAI) | Group B (HAI) | Group C (HAA) | Within patient groups | Control –group A | Control –group B | Control – group C | Group A–B | Group A–C | Group B–C |
| Glucose (mg/dl) | 82.23 (13.47) | 86.65 (15.06) | 86.29 (19.99) | 93.38 (13.18) | 0.59 | 0.79 | 0.73 | 0.29 | 0.92 | 0.29 | 0.29 |
| K (mEq/liter) | 4.21 (0.36) | 4.12 (0.34) | 4.04 (0.47) | 3.78 (0.57) | **<0.001** | 0.60 | 0.19 | **0.001** | 0.33 | **0.001** | **0.001** |
| Na (mEq/liter) | 141.80 (2.61) | 141.84 (2.45) | 140.03 (14.77) | 139.66 (18.18) | 0.95 | 0.96 | 0.50 | 0.51 | 0.42 | 0.42 | 0.43 |
| 24-h urine K (mEq/24 h) | 58.23 (24.10) | 59.4 (23.18) | 57.86 (23.28) | 73.46 (31.5) | **0.01** | 0.69 | 0.47 | 0.1 | 0.74 | **0.02** | **0.02** |
| 24-h urine Na (mEq/24 h) | 148.36 (71.34) | 123.82 (54.01) | 121.07 (58.72) | 124.14 (59.12) | 0.29 | 0.11 | **0.05** | 0.13 | 0.81 | 0.98 | 0.98 |
| HOMA-IR | 2.22 (1.30) | 2.13 (1.18) | 2.36 (1.48) | 2.38 (2.28) | 0.76 | **0.02** | **0.01** | 0.06 | 0.42 | 0.55 | 0.55 |
| QUICKI | 0.15 (0.01) | 0.15 (0.02) | 0.15 (0.02) | 0.14 (0.01) | 0.88 | **0.05** | **0.05** | **0.03** | 0.79 | 0.59 | 0.59 |
| MATSUDA | 4.62 (3.38) | 4.71 (3.28) | 4.56 (3.46) | 4.12 (2.20) | 0.65 | 0.07 | 0.09 | 0.14 | 0.83 | 0.34 | 0.34 |

Continuous variables are shown as mean (SD). Statistically significant results appear in *bold*. To convert glucose concentrations to mmol/liter, multiply by 0.05551.

**TABLE 3.** Hormonal levels in basal and dynamic adrenal testing among the controls and the three patient groups and *P* values

| | | | | | *P* value | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control group | Group A (NAI) | Group B (HAI) | Group C (HAA) | Within patient groups | Control –group A | Control –Group B | Control – Group C | Group A–B | Group A–C | Group B–C |
| Cortisol basal ($\mu$g/dl) | 15.38 (6.54) | 19.26 (8.20) | 16.97 (7.04) | 17.05 (7.60) | 0.51 | 0.28 | 0.868 | 0.98 | 0.19 | 0.28 | 0.77 |
| ACTH basal (pg/ml) | 23.35 (19.96) | 22.59 (21.99) | 16.09 (12.88) | 19.56 (12.29) | 0.13 | 0.23 | **0.01** | 0.44 | 0.20 | 0.59 | **0.03** |
| DHEAS (ng/ml) | 767 (742) | 771 (757) | 769 (654) | 1180 (1095) | **0.01** | **0.001** | **<0.001** | 0.08 | 0.86 | 0.13 | 0.06 |
| ALD basal (ng/dl) | 5.75 (4.92) | 12.49 (10.46) | 12.51 (8.80) | 23.52 (17.53) | **<0.001** | 0.22 | 0.43 | **0.03** | 0.69 | **0.004** | **0.003** |
| REN basal ($\mu$U/ml) | 20.95 (21.24) | 18.37 (17.98) | 20.73 (27.62) | 9.92 (7.24) | **<0.001** | 0.73 | 0.45 | **0.001** | 0.58 | **<0.001** | **0.001** |
| ALD/REN basal (ng/dl/$\mu$U/ml) | 0.41 (0.40) | 1.29 (2.21) | 1.77 (2.88) | 5.16 (1.02) | **<0.001** | 0.47 | 0.86 | 0.66 | 0.56 | 0.71 | 0.75 |
| Urinary free cortisol ($\mu$g/24 h) | 52.70 (26.45) | 80.20 (78.77) | 77.73 (84.71) | 77.49 (69.55) | 0.18 | **<0.001** | **0.001** | 0.12 | 0.67 | **0.03** | **0.05** |
| Cortisol after LDDST ($\mu$g/dl) | 0.84 (0.18) | 4.44 (7.88) | 4.14 (6.89) | 2.91 (5.35) | **<0.001** | **0.03** | **0.05** | **<0.001** | 0.65 | **0.001** | **0.001** |
| ALD after MSI (ng/dl) | 1.44 (0.44) | 2.52 (1.81) | 3.80 (5.33) | 8.09 (10.86) | **<0.001** | **0.002** | **<0.001** | **<0.001** | 0.18 | **<0.001** | **0.01** |
| ALD/REN after MSI (ng/dl/$\mu$U/ml) | 0.16 (0.09) | 0.37 (0.39) | 0.69 (1.09) | 1.58 (0.37) | **<0.001** | **0.002** | **<0.001** | **<0.001** | 0.18 | **<0.001** | **0.01** |

Continuous variables are shown as mean (SD). Statistically significant results appear in *bold*. To convert cortisol to nmol/liter, multiply by 27.59. To convert ACTH to pmol/liter, multiply by 0.22. To convert DHEAS to $\mu$mol/liter, multiply by 0.0027. To convert aldosterone to pmol/liter, multiply with 27.74.

values ($P < 0.001$) (Fig. 3). The majority of these patients were previously receiving combined antihypertensive medication with inadequate blood pressure control, as shown in Table 1. The mean reductions ($\pm$SD) in the maximum SBP and DBP values were 40 $\pm$ 22 and 20 $\pm$ 11 mm Hg, respectively. All patients had SBP values below 150 mm Hg, and only 10 of 55 had SBP above 140 mm Hg, whereas DBP values increased to above 85 mm Hg in four patients.

## Discussion

The present study assessed normotensive and hypertensive patients with AI (NAI and HAI, respectively), as well as hypertensive patients in whom imaging evaluation identified adrenal adenomas (HAA). We revealed a graded prevalence of AAS in patients with adrenal adenomas with and without AH. We have previously shown an increased prevalence of AAS in patients harboring an adrenal adenoma (12). An interesting finding of the present study is the impressively high prevalence of AAS in hypertensive patients, even when their adenomas were an incidental finding. This high prevalence was shown only after applying cutoffs based on an aged-matched control population with normal arterial pressure and negative adrenal CT scan in whom the saline infusion test was performed under dexamethasone suppression.

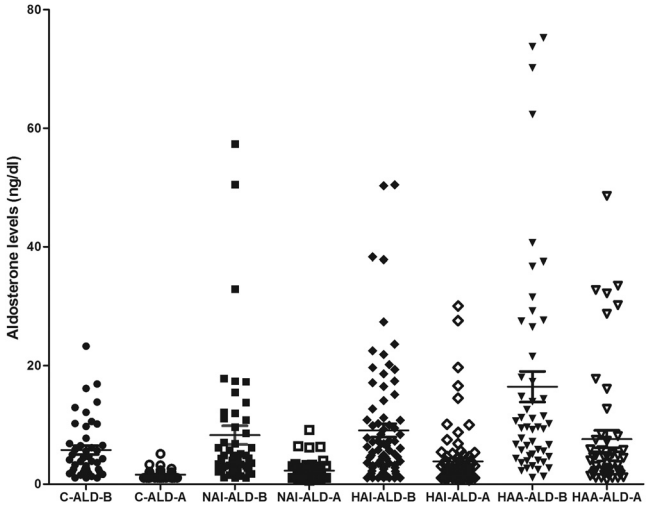


**FIG. 1.** Comparison of ALD levels among the controls and the three patient groups. ALD-B, Basal ALD levels; ALD-A, ALD levels after MSI test; C, control group.

The high prevalence of AH among patients with AI (89 of 135 patients, or 66%) is in the same direction with the findings of other studies evaluating the metabolic profile of AI patients (26–29).

In accordance with the graded increase of AAS prevalence among the three groups, ALD levels and ALD/REN ratios showed a similar profile, as shown in Fig. 1. HAI and HAA patients had higher ALD levels and ALD/REN ratios either basally or after testing, and among them, HAA subjects had the highest levels. The study provides data for an increased prevalence of AAS also in hypertensive patients with an AI, prevalence much higher than the one so far reported (22). Although a previous study has found a 5.5% prevalence of AAS in hypertensive patients harboring an AI, ALD secretory autonomy was based on the basal ALD/plasma REN activity ratio, whereas only two hypertensive patients with an AI underwent confirmatory testing with saline loading and captopril test (22). Furthermore, in that

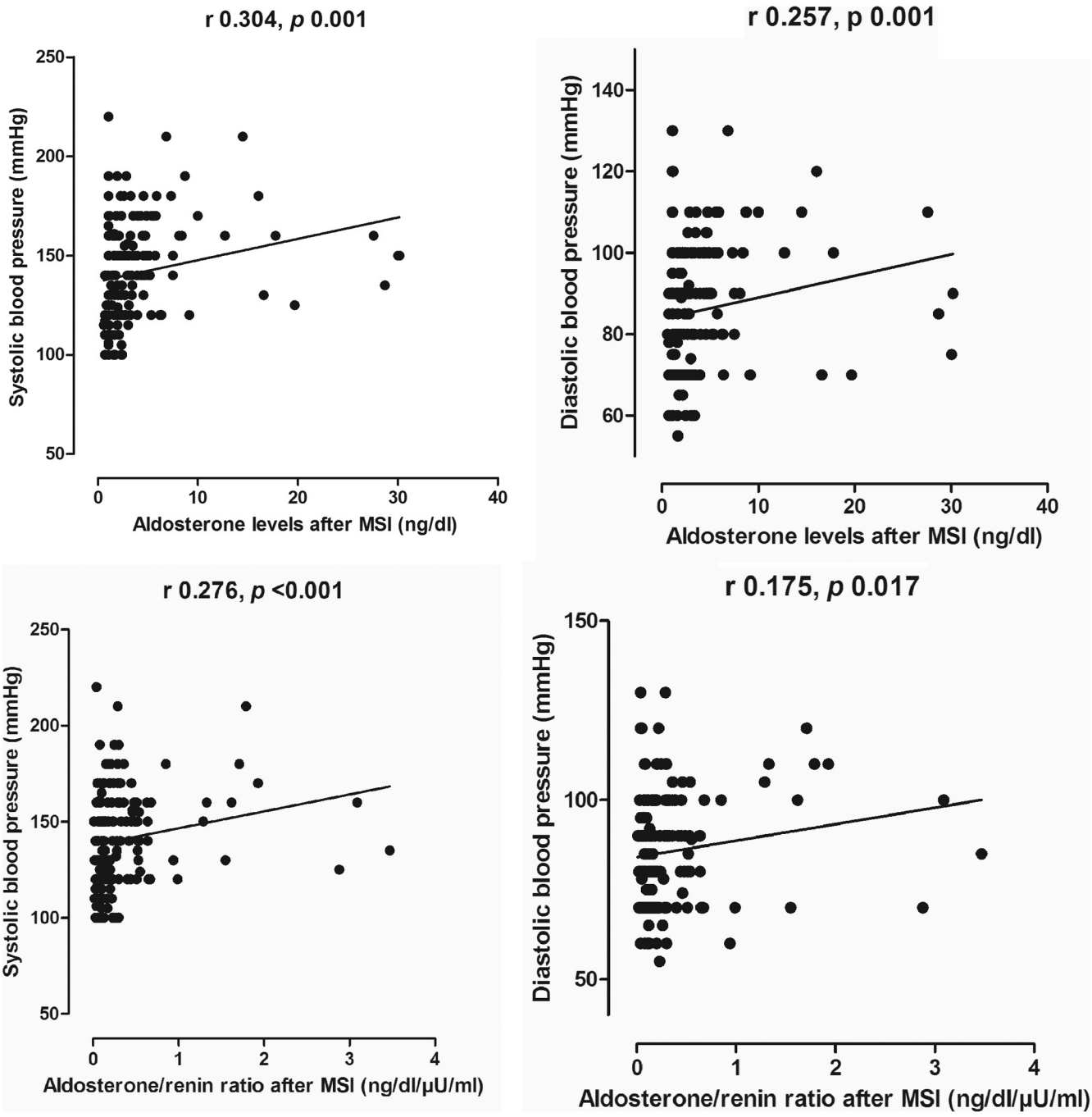


**FIG. 2.** Correlation of SBP and DBP with ALD levels and ALD/REN ratio after MSI.

study the documentation of an adrenal lesion in the majority of patients with essential hypertension was based on abdominal ultrasonography, a method that is less sensitive compared with CT scan.

Another interesting finding of the present study was that even NAI individuals exhibited a 12% prevalence of AAS. Although there are currently no data regarding the natural history of such individuals, they could be considered as a group that requires close long-term follow-up for the development of AH. This finding is also in agreement with the observation derived from the Framingham Offspring Study, where normotensive subjects with ALD levels in the upper normal range are at an increased risk for the development of hypertension later in life (30). In alignment with the former, Ito *et al.* (31) recently reported an appreciable prevalence of AAS in prehypertensive subjects.

The increased prevalence of AAS in our study of patients with adrenal adenomas compared with previous reports (22, 23) is attributed to the lower cutoffs applied that were derived from a control group consisting of normotensive patients with established normal adrenal anatomy,

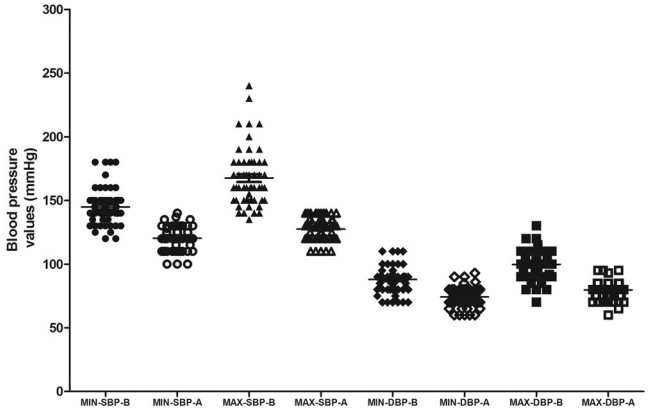


**FIG. 3.** Minimum and maximum SBP and DBP values before and on ALD antagonist treatment in 55 patients with AAS. MIN, Minimum; MAX, maximum; -B, before ALD antagonist treatment; -A, on ALD antagonist treatment.

in contrast to previous studies that included apparently "normal" individuals lacking any imaging adrenal studies (17, 18, 23). In addition, the administration of dexamethasone before the saline infusion suppresses any ACTH effect on ALD secretion and thus minimizes the range of ALD levels (25). The use of the combination of post-MSI ALD levels and ALD/REN ratio as the most reliable confirmatory test for AAS takes into account REN levels after MSI. This helps eliminate false-positive results, *i.e.* falsely elevated post-MSI ALD levels in cases of inadequate suppression of REN after MSI, for example in subjects with a low-sodium diet (15).

Apart from AAS, we found an increased prevalence of ACS that did not differ among the three groups. Interestingly, neither cortisol nor ACTH levels were statistically different among the patient groups. The increased prevalence of ACS in our study subjects is in accordance with previous studies using control groups with normal adrenal morphology (12, 21). Additionally, as recently reported, the combined cortisol and ALD secretion is not an uncommon finding and should not be ignored, especially when evaluating a hypertensive patient with an adrenal adenoma (12, 32).

ALD is an emerging hormonal factor that is linked with cardiovascular disease (2, 33–36) and also exerts a significant role in potassium handling (37). In our study, we showed a positive association between SBP and DBP and ALD levels after MSI testing. Furthermore, a significant inverse association was found between serum potassium concentrations and SBP and DBP. This observation strengthens our initial notion that the role of ALD on AH is probably greater than previously recognized. In our follow-up of 55 patients with AAS, we demonstrated a significant improvement of SBP and DBP to almost normotensive values after switching to ALD antagonist, an observation requiring validation with further clinical studies. This finding strengthens the validity of our MSI cutoffs and is in the same direction with our previously reported data in AAS patients undergoing unilateral adrenalectomy. Over one third of surgically treated AAS subjects became normotensive after adrenalectomy (12). Although spironolactone has been successfully administrated in patients with essential hypertension, our patients without AAS did not receive ALD antagonists as first-line treatment because this form of treatment is not considered the first choice in patients with essential hypertension. Spironolactone or eplerenone is recommended whenever resistant hypertension is present and combination therapy with antihypertensive drugs is required. It should be noted that patients who are considered to have essential hypertension and are successfully treated with ALD antagonists might belong to the group of patients showing AAS according to our proposed cutoffs, and they would otherwise not be identified using the classical methodology (38).

Certain points should be taken into consideration when interpreting the results of this study. We acknowledge the limited number of control subjects (n = 72), although we feel that the careful recruitment of our control group (with normal arterial pressure and adrenal anatomy established with CT scan) counteracts the limitation of group size. Furthermore, the sensitivity of the basal ALD/REN ratio might be affected by the supine position of the patients during the blood sampling. The high prevalence of AAS could be partially overestimated due to selected patient samples because SBP and DBP levels of HAA patients were higher than those of NAI and HAI subjects. It is therefore possible that the selection of HAA patients was skewed and included patients with less well-controlled AH, lower serum potassium concentration, and thus a higher probability of primary aldosteronism. This may have led to adrenal imaging with CT and the identification of an adrenal adenoma, a bias in the estimation of AAS prevalence in the HAA group. Another point that should be addressed is that we did not perform the classical saline infusion without prior dexamethasone suppression to directly compare the results of the two tests. A direct comparison of the results of the classical saline infusion with the MSI would have been methodologically more appropriate. However, this is a very demanding protocol, which is extremely difficult to be applied in a large number of patients. In conclusion, we have shown a high prevalence of AAS in hypertensive patients with AI (36%), as well as in patients with adrenal adenomas found during the evaluation of AH (54%). Targeted treatment with an ALD antagonist in patients with AAS resulted in a marked improvement of blood pressure control. These findings could alert physicians for the increased probability of AAS when

evaluating hypertensive patients in the presence of an adrenal adenoma. In accordance with existing evidence and in the prospect of well-designed clinical trials, tailored therapy should not only adequately control AH in subjects with AAS, but also reduce the adverse ALD-mediated cardiovascular action.

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