

# Hypertension

JOURNAL OF THE AMERICAN HEART ASSOCIATION



*Learn and Live*<sup>SM</sup>

**Renal Damage in Primary Aldosteronism: Results of the PAPY Study**  
Gian Paolo Rossi, Giampaolo Bernini, Giovambattista Desideri, Bruno Fabris,  
Claudio Ferri, Gilberta Giacchetti, Claudio Letizia, Mauro Maccario, Massimo  
Mannelli, Mee-Jung Matterello, Domenico Montemurro, Gaetana Palumbo, Damiano  
Rizzoni, Ermanno Rossi, Achille Cesare Pessina, Franco Mantero and for the PAPY  
Study Participants

*Hypertension* 2006;48;232-238; originally published online Jun 26, 2006;

DOI: 10.1161/01.HYP.0000230444.01215.6a

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX  
72514

Copyright © 2006 American Heart Association. All rights reserved. Print ISSN: 0194-911X. Online  
ISSN: 1524-4563

The online version of this article, along with updated information and services, is  
located on the World Wide Web at:

<http://hyper.ahajournals.org/cgi/content/full/48/2/232>

Subscriptions: Information about subscribing to Hypertension is online at  
<http://hyper.ahajournals.org/subscriptions/>

Permissions: Permissions & Rights Desk, Lippincott Williams & Wilkins, a division of Wolters  
Kluwer Health, 351 West Camden Street, Baltimore, MD 21202-2436. Phone: 410-528-4050. Fax:  
410-528-8550. E-mail:  
[journalpermissions@lww.com](mailto:journalpermissions@lww.com)

Reprints: Information about reprints can be found online at  
<http://www.lww.com/reprints>

## Renal Damage in Primary Aldosteronism Results of the PAPY Study

Gian Paolo Rossi, Giampaolo Bernini, Giovambattista Desideri, Bruno Fabris, Claudio Ferri, Gilberta Giacchetti, Claudio Letizia, Mauro Maccario, Massimo Mannelli, Mee-Jung Matterello, Domenico Montemurro, Gaetana Palumbo, Damiano Rizzoni, Ermanno Rossi, Achille Cesare Pessina, Franco Mantero; for the PAPY Study Participants

**Abstract**—Primary aldosteronism (PA) has been associated with cardiovascular hypertrophy and fibrosis, in part independent of the blood pressure level, but deleterious effects on the kidneys are less clear. Likewise, it remains unknown if the kidney can be diversely involved in PA caused by aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA). Hence, in the Primary Aldosteronism Prevalence in Italy (PAPY) Study, a prospective survey of newly diagnosed consecutive patients referred to hypertension centers nationwide, we sought signs of renal damage in patients with PA and in comparable patients with primary hypertension (PH). Patients ( $n=1180$ ) underwent a predefined screening protocol followed by tests for confirming PA and identifying the underlying adrenocortical pathology. Renal damage was assessed by 24-hour urine albumin excretion (UAE) rate and glomerular filtration rate (GFR). UAE rate was measured in 490 patients; all had a normal GFR. Of them, 31 (6.4%) had APA, 33 (6.7%) had IHA, and the rest (86.9%) had PH. UAE rate was predicted ( $P<0.001$ ) by body mass index, age, urinary  $\text{Na}^+$  excretion, serum  $\text{K}^+$ , and mean blood pressure. Covariate-adjusted UAE rate was significantly higher in APA and IHA than in PH patients; there were more patients with microalbuminuria in the APA and IHA than in the PH group ( $P=0.007$ ). Among the hypertensive patients with a preserved GFR, those with APA or IHA have a higher UAE rate than comparable PH patients. Thus, hypertension because of excess autonomous aldosterone secretion features an early and more prominent renal damage than PH. (*Hypertension*. 2006;48:232-238.)

**Key Words:** hypertension, endocrine ■ aldosterone ■ mineralocorticoids ■ kidney ■ hypertrophy ■ adrenal gland

The results of large intervention trials<sup>1,2</sup> and cross-sectional studies (reviewed by Rossi et al<sup>3</sup>) have recently refueled the interest on the deleterious cardiovascular effects of excess aldosterone. Moreover, growing evidence<sup>4</sup> indicates that primary aldosteronism (PA) is a common cause of secondary hypertension: in the Primary Aldosteronism Prevalence in Italy (PAPY) study, a prospective survey of 1180 consecutive newly diagnosed hypertensive patients referred to specialized hypertension centers, aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) were found in 4.8% and 6.4% of all patients, respectively, thus leading to an overall prevalence of PA of  $\approx 11\%$ .<sup>5</sup> It has been contended that this form of secondary hypertension is relatively “benign,” that is, devoid of cardiovascular complications, because of the suppression of the renin-angiotensin system, which plays a substantial role in cardiovascular remodeling and damage.<sup>6,7</sup>

This view has, however, been challenged by recent data.<sup>3,8</sup> PA has in fact been associated with widespread tissue fibrosis,<sup>9</sup> vascular remodeling,<sup>10</sup> and excess prevalence of left ventricular hypertrophy and diastolic dysfunction<sup>11</sup> that were corrected by

adrenalectomy.<sup>12</sup> A higher incidence of cardiovascular complications, including atrial fibrillation, has also been described.<sup>13</sup> This might be attributed to the aldosterone- and hypertension-driven excess left ventricular hypertrophy, fibrosis, and hypokalemia, which all contribute to prolong the atrioventricular conduction time and thereby facilitate re-entry mechanisms.<sup>14</sup> Excess aldosterone has also been causally related to endothelial dysfunction, because the latter was corrected by blockade of the mineralocorticoid receptor.<sup>15</sup>

By contrast with the wealth of data on the adverse cardiovascular consequences of PA, information on renal damage is confined to a small series of APA patients.<sup>16,17</sup> Hence, it remains unsettled whether the patients with IHA, who usually exhibit less marked aldosterone excess than those with APA, also feature an early renal damage.

A body of evidence indicates that microalbuminuria is a marker of early renal involvement.<sup>18</sup> Moreover, there is general consensus that evaluation of urine albumin excretion (UAE) rate is not only useful for the assessment of overall cardiovascular risk (reviewed by Palatini<sup>19</sup>) but also represents a cost-effective way to identify patients at higher risk of

Received March 6, 2006; first decision March 26, 2006; revision accepted May 24, 2006.

From the Department of Clinical and Experimental Medicine, Clinica Medica 4, University Hospital, Padova, Italy.

Correspondence to Gian Paolo Rossi, DMCS Clinica Medica 4, University Hospital, via Giustiniani, 2, 35126 Padova, Italy. E-mail gianpaolo.rossi@unipd.it

© 2006 American Heart Association, Inc.

*Hypertension* is available at <http://www.hypertensionaha.org>

DOI: 10.1161/01.HYP.0000230444.01215.6a

cardiovascular complication and progression to renal failure.<sup>20,21</sup> Thus, we aimed at prospectively testing the hypothesis that PA implies a more prominent renal damage, as compared with primary (essential) hypertension (PH), by measuring UAE rate in patients with a normal glomerular filtration rate (GFR).

## Methods

The protocol of the PAPY Study was approved by the Ethical Committee of the University of Padua and has been described in details elsewhere<sup>5</sup>; therefore, it will be only briefly recalled. Consecutive patients with a new diagnosis of PH, who had been referred to specialized centers for the diagnosis and treatment of PH nationwide in Italy, were enrolled to minimize the chances for any selection bias. A previous diagnosis of a secondary form of PH, the patient's refusal to participate in the study, and associated diseases (eg, diabetes) and hypertension complicated by congestive heart failure or renal insufficiency represented the exclusion criteria for this study. Serum creatinine had to be  $<115 \mu\text{mol}\times\text{L}^{-1}$  ( $1.3 \text{ mg}\times\text{dL}^{-1}$ ), and estimated creatinine clearance (abbreviated Modification of Diet in Renal Disease Study Group [MDRD] equation) had to be  $>61 \text{ mL}\times\text{min}^{-1}\times 1.73 \text{ m}^{-2}$ , which corresponds with the 95th percentile of the values observed in the entire PAPY Study population. An informed consent was obtained from each participant.

After the diagnosis of hypertension was confirmed by current guidelines,<sup>20</sup> mineralocorticoid receptor antagonists (for  $\geq 6$  weeks), diuretics,  $\beta$ -blockers, angiotensin-converting enzyme inhibitors, and angiotensin II type 1 receptor antagonists (for  $\geq 2$  weeks) were withdrawn. A long-acting calcium entry blocker and/or doxazosin were allowed if necessary for minimizing the risks of uncontrolled hypertension. On the day of the screening test, after an overnight fasting and 1 hour quiet rest in the sitting position, serum and urine  $\text{Na}^+$  and  $\text{K}^+$  concentration, plasma renin activity (PRA), aldosterone, and cortisol were measured between 7:00 AM and 9:00 AM, baseline and again 60 minutes after the oral administration of 50 mg of captopril.

The blood pressure (BP) levels were measured at baseline and after captopril with a mercury sphygmomanometer using phase V for diastolic. The aldosterone (in  $\text{ng}\times\text{dL}^{-1}$ )/PRA (in  $\text{ng}\times\text{mL}^{-1}\times\text{h}^{-1}$ ) ratio (ARR), baseline and after captopril, and a score of PA based on a validated multivariate logistic discriminant function (LDF) score,<sup>22</sup> were then calculated. This score estimates the individual probability of APA in each patient. Patients with an ARR  $\geq 40$  baseline or  $\geq 30$  after captopril and/or with an LDF score  $\geq 0.50$  underwent a saline infusion test (2 L of 0.9% NaCl solution in 4 hours), and, if necessary, an imaging test that was composed of a high-resolution computed tomography with 3-mm slices and/or magnetic resonance followed by adrenal vein sampling<sup>23</sup> or dexamethasone-suppressed adrenocortical scintigraphy.

## Biochemical Measurements

PRA was measured by radioimmunoassay using commercial kits (Ren CTK; Sorin Biomedica) in 10 centers<sup>24</sup> or Angiotensin I RIA (Radim) in the rest. Normal range sitting at rest and on a normal  $\text{Na}^+$  diet was  $0.2$  to  $2.8 \text{ ng}\times\text{mL}^{-1}\times\text{h}^{-1}$ ; intra-assay and interassay coefficients of variation (CVs) were within 8% and 10% for both kits. The assay for aldosterone was performed with the same diagnostic kit (Aldosterone Mirya, Technogenetics) in all 15 centers. Normal range was  $10$  to  $150 \text{ pg}\times\text{mL}^{-1}$  supine,  $30$  to  $320 \text{ pg}\times\text{mL}^{-1}$  upright on a normal  $\text{Na}^+$  diet; intra-assay and interassay CVs assay were both  $<5.6\%$ ; the cross-reactivity of the antibody for aldosterone for the other adrenal steroids was  $<0.001\%$ . Cortisol was measured with a commercial kit (Cortisol bridge, Adaltis); the intra-assay and interassay CVs of this assay were  $<6\%$  and  $10\%$ , respectively. The cross-reactivity of the antibody for the other adrenal steroids was  $18\%$  for 11-desoxycortisol,  $7.5\%$  for corticosterone,  $7.5\%$  for 21-desoxycortisol;  $7.3\%$  for desoxycorticosterone,  $6\%$  for  $17 \alpha$ -progesterone, and  $<0.1\%$  for aldosterone and other known steroids.

## GFR and UAE Rate

GFR was measured by using the so called "abbreviated equation," which takes into consideration serum creatinine, age, gender, and race (is available at [www.kdoqi.org](http://www.kdoqi.org)) for easy computation: estimated GFR ( $\text{mL}\times\text{min}^{-1}\times 1.73 \text{ m}^{-2}$ ) =  $186 \times (\text{serum creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$ .<sup>25</sup>

On the day of the screening test, patients came to the ward with 24-hour urine collections for the determination of creatinine (to assess the adequateness of urine collection), sodium (to estimate sodium intake), and potassium. Urinary albumin and creatinine excretion was measured with commercially available radioimmunoassay (H ALB kit-double antibody; Sclavo SpA) or immunoturbidimetry assay kit (Sera-Pak, Bayer). The mean intra-assay and interassay CVs are within 4.5% and 11.0%, respectively.<sup>26</sup> Urinary creatinine was measured by Jaffe's reaction (CV = 7.2%).

UAE rate was analyzed as milligrams  $\times 24 \text{ hours}\times^{-1}$  and also after normalization for milligrams of urinary creatinine. The normal range of UAE rate was  $30$  to  $300 \text{ mg}\times\text{g}^{-1}$  of creatinine (or  $30$  to  $300 \text{ mg}\times 24 \text{ hours}^{-1}$ ). According to the timed 24-hour UAE rate, samples were divided into 3 groups: normoalbuminuric (UAE  $<28.8 \text{ mg}\times 24 \text{ hours}^{-1}$ , eg,  $<20 \mu\text{g}\times\text{min}^{-1}$ ), microalbuminuric (UAE  $28.8$  to  $288 \text{ mg}\times 24 \text{ hours}^{-1}$ , eg,  $20$  to  $200 \mu\text{g}\times\text{min}^{-1}$ ), and macroalbuminuric (UAE  $>288 \text{ mg}\times 24 \text{ hours}^{-1}$ , eg,  $>200 \mu\text{g}\times\text{min}^{-1}$ ).

## Conclusive Diagnosis

On completion of the diagnostic workup, the presence or absence of PA and its underlying cause were conclusively diagnosed by an adjudication committee (G.P.R. and F.M.). In patients with an ARR  $>40$  and/or an LDF score  $>0.80$ , APA was initially diagnosed based on imaging tests, evidence of lateralized aldosterone secretion at adrenal vein sampling, or adrenocortical dexamethasone-suppressed scintigraphy. The diagnosis had to be thereafter retrospectively confirmed at surgery, pathology, and overall by the observation of cure or improvement of hypertension at follow-up after adrenalectomy. To this end, the American Heart Association guidelines<sup>27</sup> were used: cure was defined as a systolic BP  $<140 \text{ mm Hg}$  and diastolic BP  $<90 \text{ mm Hg}$  without medications; and improvement was a systolic and diastolic BP  $<140/90 \text{ mm Hg}$ , respectively, on the same and/or reduced number of defined daily doses of medications, as described by the World Health Organization.<sup>28</sup>

## Statistical Analysis

GFR values were proportioned to  $1.73 \text{ m}^2$  of body surface area. Quantitative variables were tested beforehand for normal distribution by graphical plot and Shapiro-Welch test; appropriate transformations were undertaken for skewed variables until a normal distribution was attained. PRA, plasma aldosterone, cortisol, and serum creatinine values required natural logarithm transformation to attain a gaussian distribution before use for statistical analysis. UAE rate values required square-root transformation to achieve a normal distribution. Univariate and multivariate outliers were identified with the procedure described by Tabachnick<sup>29</sup> and excluded from the analysis. Univariate outliers were identified as those with  $z$  scores  $>3.29$  that corresponded with  $P < 0.001$ . Mahalanobis distances were assessed by regression analysis to identify multivariate outliers; cases with  $\chi^2 > 4.5$  (at  $\alpha = .001$ ) were considered outliers. Data are presented as mean and SEM. One-way ANOVA followed by Bonferroni's test was then used to compare normally distributed quantitative variables between groups. The frequency of categorical variables was investigated by  $\chi^2$  analysis. Multiple linear regression analysis (backward, Wald criterion) was used to identify the determinants of GFR and UAE rate with inclusion and exclusion cutoffs of 0.05 and 0.10, respectively. GFR and UAE rate were then analyzed after adjustment for the effect of their significant determinants. Significance was set at  $P < 0.05$ ; SPSS 13.0 for Windows (SPSS Italy Inc) was used for these analyses.

## Results

### Anthropometric Characteristics

On locking the database on September 30, 2005, of the 1180 patients that had been enrolled, 490 satisfied the inclusion/

**TABLE 1. Anthropometric and Biochemical Characteristics of the Patients With PH and PA Caused by an APA and IHA**

Variable	PH (n=426)	P	APA (n=31)	P	IHA (n=33)	P (IHA vs PH)
Age, y	45±12	P<0.016	51.5±12	NS	49±12	NS
BMI, kg×m <sup>-2</sup>	26.8±4.8	NS	28.3±4.8	NS	27.0±4.5	NS
Systolic BP, mm Hg	147±18	P<0.05	158±19	NS	155±16	P<0.04
Diastolic BP, mm Hg	95±11	NS	96±9.7	NS	100±10	P<0.016
Serum K <sup>+</sup> , mEq×L <sup>-1</sup>	4.1±0.4	P<0.0001	3.5±0.5	P<0.003	3.8±0.3	P<0.001
Na <sup>+</sup> <sub>u</sub> V, mEq×day <sup>-1</sup>	154±4.5	NS	132±12	NS	137±8	NS
Serum creatinine, μmol×L <sup>-1</sup>	79±18	NS	83±17	NS	83±18	NS
PRA, ng×mL <sup>-1</sup> ×h <sup>-1</sup>	1.62±0.13	P<0.001	0.65±0.19	NS	0.5±0.13	P<0.001
Plasma aldosterone, pmol×L <sup>-1</sup>	148±5	P<0.0001	332±41	NS	303±31	P<0.001
ARR, (ng×dL <sup>-1</sup> )×(ng×mL <sup>-1</sup> ×h <sup>-1</sup> ) <sup>-1</sup>	24±3	P<0.0001	103±25	NS	72±10	P<0.002
Plasma cortisol, nmol×L <sup>-1</sup>	140±3	NS	126±6	NS	137±8	NS

Na<sup>+</sup><sub>u</sub>V indicates sodium urinary excretion; NS, not significant.

exclusion criteria for this study and had complete UAE rate and GFR data. Their baseline clinical features, shown in Table 1, did not differ from those of the whole PAPY study.

### Conclusive Diagnosis

At the end of the thorough diagnostic workup, a conclusive diagnosis was attained in all 490 of the patients. Of them, 64 patients were held to have PA, thus leading to a PA prevalence in this cohort of 13.1%, with no differences between genders. Of these patients, 33 had IHA, and 31 had APA. On average, the APA patients had lower serum K<sup>+</sup> than those with IHA, although they did not differ for PRA and plasma aldosterone (Table 1). As anticipated by definition, the APA and IHA patients had lower PRA and serum K<sup>+</sup> and higher aldosterone than PH patients. They were also older and had higher systolic BP than PH patients, whereas they were similar for the remaining variables, including known duration of hypertension. At the time of the renal function tests, 40% of the patients were untreated, 41% were on a calcium entry blocker or doxazosin, and 19% were on both agents. With the exception of BP values, there were no significant differences of any variables, including UAE rate and hormone values, across treatment groups in either the PA or the PH patients, thus excluding a systematic effect of these treatments on the outcome variables.<sup>30,31</sup> Likewise, there were no differences in UAE rate, PRA, aldosterone, and cortisol across centers, thus making unlikely a center effect on results of these measurements.

### Predictors of GFR and UAE Rate

With stepwise regression analyses, we found that plasma aldosterone and body mass index (BMI) significantly predicted GFR, other than age. These 3 variables explained 16% of GFR variance (adjusted R<sup>2</sup>=0.161; P<0.001). Likewise, we could identify that BMI, age, urinary Na<sup>+</sup> excretion, serum K<sup>+</sup>, and mean BP were significant predictors of UAE rate (Table 2). By contrast, no significant effects of GFR, PRA, aldosterone, and cortisol on UAE rate could be identified. Overall, the 5 variables that remained in the model accounted for only 5.2% of UAE rate (adjusted R<sup>2</sup>=0.052; P<0.0001). Hence, both GFR and UAE rate were adjusted

for the effects of its significant predictors for the comparisons between PA and PH patients.

### UAE Rate and Macroalbuminuria and Microalbuminuria by Conclusive Diagnosis

UAE rate was significantly (P<0.001) higher in the patients with PA (28.1±4.3 μg×mL<sup>-1</sup>) than with PH (18.8±1.2). The results of the comparison of PH patients with PA patients divided into APA and IHA are shown in Figure 1 and Table 3. Although we found no patients with macroalbuminuria in this cohort of hypertensive subjects with normal GFR, overall there were more patients with microalbuminuria and less patients with normoalbuminuria in the PA rather than in the PH group (χ<sup>2</sup>=9.92; P=0.002). The percentage of patients with microalbuminuria by conclusive diagnosis is shown in Figure 2.

### UAE Rate by Serum K<sup>+</sup>

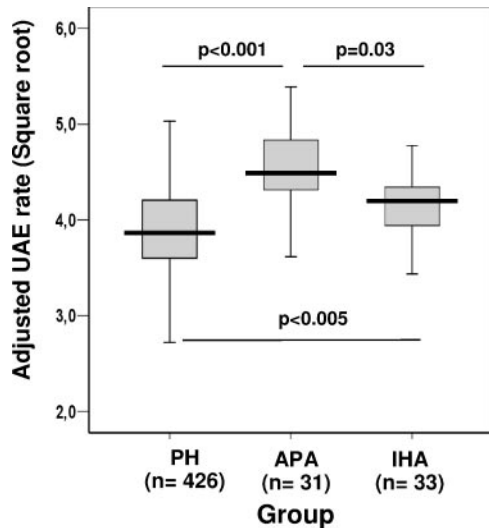
About half of the patients with APA and 83% of those with IHA had normal serum K<sup>+</sup> at the time of the screening test. We could, therefore, test the hypothesis that hypokalemia was

**TABLE 2. Results of a Stepwise Regression Analysis (Backward Method) to Identify Predictors of UAE**

Variables	β	(95% CI)	t	P
Variables in the model				
BMI	0.115	(0.011 to 0.085)	2.56	0.011
Age	0.101	(0.002 to 0.032)	2.28	0.023
Na <sup>+</sup> <sub>u</sub> V	0.082	(0.0001 to 0.004)	1.81	0.071
Serum K <sup>+</sup>	-0.136	(-1.05 to -0.206)	-2.2	0.004
MBP	0.078	(-0.002 to 0.027)	1.71	0.088
Variables that did not enter in the model				
Log (PRA)			0.010	NS
Log (plasma aldosterone)			-0.090	NS
K <sup>+</sup> <sub>u</sub> V			-0.559	NS
Log (plasma cortisol)			-0.748	NS
GFR			-1.479	NS

Adjusted R<sup>2</sup>=0.052; F=6.49; P<0.0001; Na<sup>+</sup><sub>u</sub>V indicates sodium urinary excretion; NS, not significant.



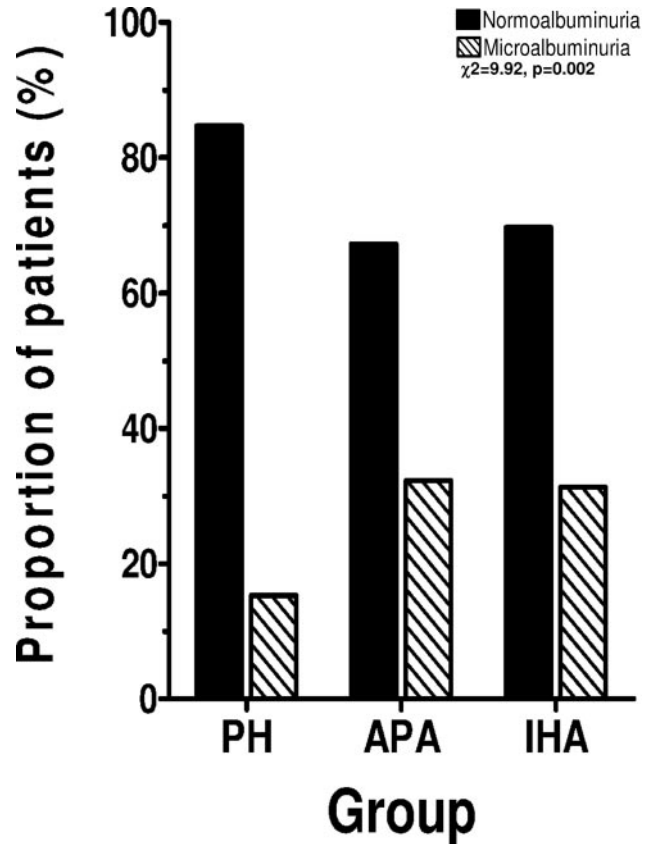


**Figure 1.** The bar graph shows the covariates-adjusted UAE rate in the patients with PH and primary aldosteronism because of APA and IHA. (median, interquartile range, and extreme values). In both the APA and the IHA group, the UAE rate was significantly higher than in the PH group.

associated with increased UAE rate. We found an inverse relation between serum  $K^+$  and UAE rate ( $r=-0.136$ ;  $P=0.002$ ); however, when splitting patients into those with and without hypokalemia, defined with a cutoff of  $3.5 \text{ mEq}\times\text{L}^{-1}$ , no higher UAE rate and no higher rate of microalbuminuria in the hypokalemic than in the normokalemic patients could be found.

**Discussion**

Compelling evidence indicates that hyperaldosteronism implies detrimental consequences on the cardiovascular system<sup>32</sup> (reviewed by Ross et al<sup>3</sup>), but only 2 studies on renal involvement are available.<sup>16,17</sup> According to their results, UAE rate would be higher in PA than in PH patients, but only patients with APA were studied, and no information on patients with IHA were given. Furthermore, both studies came from the same center,<sup>16,17</sup> and, thus, the possibility of a selection bias could not be excluded. Based on these data, it was contended that even despite the suppression of the renin-angiotensin system, a renal damage occurs early on in the course of PA.<sup>16</sup> To further investigate this hypothesis, we prospectively measured GFR and UAE rate and urinary creatinine excretion in the PAPY study. At the participating



**Figure 2.** The bar graph shows the prevalence of microalbuminuria in the patients with PH and primary aldosteronism because of APA and IHA.

centers, state-of-the-art tests for diagnosing PA were available, thus allowing us to conclusively establish the presence or absence of PA and to accurately identify its underlying cause. Hence, we had a unique opportunity to examine UAE rate in the context of an unequivocal diagnosis of PA subtype and of exhaustive information on hormonal data regarding PRA, aldosterone, and cortisol levels. The impact of these and other variables on UAE rate could, therefore, be examined with unprecedented statistical power.

**Determinants of UAE Rate and Prevalence of Microalbuminuria**

Several factors can affect the UAE rate and thereby the prevalence of microalbuminuria in hypertension. The main

**TABLE 3. GFR, UAE, and Urinary Creatinine Excretion in Patients With PH and Primary Aldosteronism Caused by an APA and IHA**

Group	P±H (n=426)	P	APA (n=31)	P	IHA (n=33)	P (IHA vs PH)
Variable						
GFR, $\text{mL}\times\text{min}^{-1}\times 1.73 \text{ m}^{-2}$	92±1	$P<0.016$	85±3	$P<0.016$	86±3	NS
Adjusted GFR, $\text{mL}\times\text{min}^{-1}\times 1.73\text{m}^{-2}$	92±1	$P<0.001$	85±2	$P=0.011$	88±1	NS
UAE, $\mu\text{g}\times\text{mL}^{-1}$	18.30±1.1	NS	25.1±3.8	NS	31.0±7.5	$P=0.024$
UCE, $\text{g}\times\text{day}^{-1}$	1.51±0.05	NS	1.58±0.2	NS	1.35±0.14	NS
UAE/UCE, $\mu\text{g}\times\text{mL}^{-1}/\text{g}\times\text{day}^{-1}$	13.5±0.1	NS	19.5±4.8	NS	311.1±13.4	$P<0.005$
Adjusted UAE, $\text{mL}\times\text{min}^{-1}$	18.1±0.01	$P<0.001$	23.0±0.01	$P=0.03$	20.8±0.02	$P<0.005$

Adjusted GFR indicates GFR adjusted for the BMI, age, and plasma aldosterone; Adjusted UAE, UAE adjusted for the BMI, age,  $\text{Na}^+_{\text{e}}$ , serum  $K^+$ , and mean BP; UCE, urinary creatinine excretion.

determinant of UAE rate in subjects with mild hypertension and no cardiovascular complications seems to be the hemodynamic load, although activation of the renin–angiotensin–aldosterone system can accelerate the onset of early renal changes.<sup>33</sup> Moreover, in subjects with more severe hypertension and associated target organ damage, the augmented urinary albumin leak is held to be the consequence of endothelial dysfunction and glomerular damage. Hence, adjustment for the effect of significant predictors of UAE rate is mandatory when undertaking comparisons of groups that may have an unbalanced distribution of these confounders. By using regression analysis, we could examine the impact of renin, aldosterone, and cortisol, both baseline and after captopril, and several other variables on UAE rate. Although we could not detect any significant impact of the hormones and GFR, we found that UAE rate was predicted by BMI, age, urinary Na<sup>+</sup> excretion, serum K<sup>+</sup>, and mean BP (Table 2).

### UAE Rate in PA and PH Patients

An unbalanced distribution of these covariates across groups might confound the detection of differences of UAE rate between PA and PH patients, given the marked differences observed for some of the variables (Table 1). Hence, to circumvent this possibility, we compared the covariates-adjusted UAE rate across diagnosis groups. This analysis showed that PH patients had significantly lower UAE rate than patients with PA, either because of APA or because of IHA, even despite slightly higher GFR (Table 3 and Figure 1). These differences could not be accounted for by differences of age, BMI, BP, or serum K<sup>+</sup>, or urinary Na<sup>+</sup> excretion between PA and PH patients. Of interest, there were no significant differences of either raw or adjusted UAE rates between the PA patients with APA and IHA (Figure 1). Hence, it would seem that PA, per se, rather than its underlying cause, implies an early renal involvement. Thus, our present findings confirm and extend to IHA patients previous reports in patients with APA.<sup>16,17</sup> Of note, even despite the increased UAE rate of PA, we could observe no significant impact of aldosterone on the UAE rate. Whether this lack of correlation depends on the intrinsic inaccuracy of plasma aldosterone values, as assessed at a single time point, to reflect the chronic aldosterone excess and/or on the fact that multiple factors concur with aldosterone excess to increase the UAE rate remains to be clarified.

PA patients were described previously to have relative hyperfiltration that would be related to the increased UAE rate and could be unveiled by adrenalectomy.<sup>17</sup> Furthermore, it was shown that the infusion of aldosterone for 1 week to normotensive dogs determined a 20% to 24% increase of GFR, which was associated with an increase of renal perfusion pressure.<sup>34</sup> However, in the present study, GFR was slightly higher in PH than in PA patients with APA and IHA, both when raw and when covariate-adjusted GFR values were compared. Furthermore, we found a weak inverse significant relation ( $r = -0.135$ ;  $P < 0.001$ ) of GFR with UAE rate at univariate correlation, although this was not significant at multivariate analysis. Thus, the results of this cross-sectional study do not seem to support the contention that glomerular hyperfiltration is a hallmark of chronic PA and accounts for the observed excess UAE rate. The hyperaldosteronism- and

hypertension-induced nephron loss can be, in our view, a plausible explanation for the lack of glomerular hyperfiltration of PA patients in this study. Hence, it would be of interest to determine whether heterogeneity of GFR across nephrons exists.

Based on available information and on the present results, we would like to propose another mechanism. UAE rate is held to reflect endothelial damage or dysfunction<sup>35,36</sup>; moreover, excess aldosterone has also been causally related to endothelial dysfunction in experimental and clinical studies.<sup>37,38</sup> Thus, the more marked early renal damage found in PA patients, as compared with PH without hyperaldosteronism, might reflect endothelial dysfunction. Of note, with excess aldosterone this may occur even despite the suppression of the renin–angiotensin system, which was held to minimize the detrimental effects of high BP on the cardiovascular system. Because we could obtain no information on oxidant stress and endothelial function in this study, this hypothesis remains contentious. However, it is consistent with the fact that removal of APA, with ensuing correction of hyperaldosteronism, was followed by a decrease in the UAE rate.<sup>16</sup>

### UAE Rate by Serum K<sup>+</sup>

K<sup>+</sup> supplementations have been shown to protect against stroke and endothelial dysfunction in hypertensive rats.<sup>39,40</sup> Moreover, K<sup>+</sup> prevents nephron loss by exerting a protective action on renal tubules, arteries, and glomeruli.<sup>41</sup> Hence, hypokalemic nephropathy has been contended to be responsible for the increased UAE rate in PA patients,<sup>15</sup> but this hypothesis has never been formally tested. Hypokalemia is held to be a hallmark of PA, although normokalemia is currently detected more often than hypokalemia in PA cases.<sup>42,43</sup> In accord with this view, the majority of the patients not only with PH, but also with PA, had normal serum K<sup>+</sup> in the PAPY at the time of the screening test. We could, therefore, test the hypothesis that hypokalemia was associated with the UAE rate. Interestingly, we found an inverse relation (Figure 3) between UAE rate and serum K<sup>+</sup>. The latter remained among predictors of UAE rate in the regression model (Table 2), thus suggesting a role for hypokalemia in raising the UAE rate. However, the

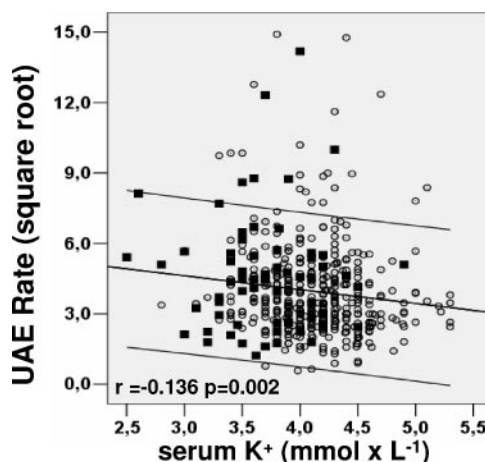


Figure 3. The scatter plots illustrate the relationship between UAE rate and serum K<sup>+</sup>. ■, PA; ○, PH patients.

**TABLE 4. List of Participating Centers and PAPY Study Investigators**

Center	Investigators
1 Padova DMCS Internal Medicine 4	GP Rossi, A. Semplicini, C Ganzaroli, AC Pessina
2 Padova Endocrinology	Franco Mantero, Mee-Yung Mattarello
3 Ancona Endocrinology Internal Medicine	Gilberta Giacchetti, Vanessa Ronconi, Marco Boscaro
4 Reggio Emilia	Ermanno Rossi
5 Pisa Internal Medicine	Giampaolo Bernini, Angelica Moretti
6 L'Aquila, Department of Internal Medicine and Public Health	Claudio Ferri, Giovambattista Desideri
7 Palermo Medical Clinical General	Giuseppe Andronico
8 Brescia Medical Clinical General	Damiano Rizzoni, Enzo Porteri
9 Legnano Internal Medicine	Gaetana Palumbo
10 Roma Internal Medicine	Claudio Letizia, Chiara Caliumi
11 Trieste	Bruno Fabris
14 Firenze Endocrinology	Massimo Mannelli, Gabriele Parenti
15 Torino Endocrinology	Mauro Maccario, Ezio Ghigo
17 Reggio Calabria Nephrology	Francesca Mallamaci, Carmine Zoccali
18 Bari Internal Medicine	Anna Belfiore

correlation was a weak one ( $r = -0.136$ ). Moreover, when splitting groups into those with and without hypokalemia, UAE rate and the rate of microalbuminuria were no higher in the patients with than in those without hypokalemia. Thus, it would seem that a diagnosis of PA, rather the presence of hypokalemia, per se, implies an increased risk of microalbuminuria. Nonetheless, our present results do support the hypothesis that hypokalemia is a determinant of early renal damage, as revealed by microalbuminuria, in hypertensive patients with PA, albeit not a major one. Whether correction of hypokalemia with  $K^+$  supplementations can correct microalbuminuria even despite leaving BP unaffected in PA patients remains to be investigated.

In summary, we showed that in newly diagnosed hypertensive patients referred to specialized centers for arterial hypertension, PA implies an early and more prominent renal involvement, as compared with PH. This renal damage precedes the overt decrease of GFR and is unrelated to the suppression of the renin-angiotensin system and to the plasma level of aldosterone. Because excess aldosterone implies enhanced oxidant stress,<sup>44,45</sup> which is a major causative factor for endothelial dysfunction, we propose that the excess microalbuminuria might reflect widespread endothelial dysfunction.

### Perspectives

Considering the very high prevalence of arterial hypertension in the general population and the high proportion of cases because of excess aldosterone and given the reported association of plasma aldosterone with the risk of developing high BP,<sup>8</sup> research should be aimed at further identifying the mechanisms by which hyperaldosteronism exerts its deleterious effects on the kidney. Follow-up studies with assessment of markers of oxidant stress and endothelial dysfunction and with direct measurement

of renal blood flow and GFR would be important in PA patients treated by adrenalectomy or mineralocorticoid receptor antagonists to clarify these mechanisms.

### Appendix

A list of all PAPY study participants is provided in Table 4.

### Sources of Funding

This study was carried out under the auspices of the Società Italiana dell'Iperensione Arteriosa.

### Disclosures

None.

### References

- Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med*. 1999;341:709–717.
- Pitt B, Remme W, Zannad F, Neaton J, Martinez F, Roniker B, Bittman R, Hurlley S, Kleiman J, Gatlin M. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. *N Engl J Med*. 2003;348:1309–1321.
- Rossi G, Boscaro M, Ronconi V, Funder JW. Aldosterone as a cardiovascular risk factor. *Trends Endocrinol Metab*. 2005;16:104–107.
- Rossi GP. Primary aldosteronism: a needle in a haystack or a yellow cab on fifth avenue? *Curr Hypertens Rep*. 2004;6:1–4.
- 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. A prospective study of the prevalence of primary aldosteronism in hypertensive patients. *J Am Coll Cardiol*. 2006 In press.
- Alderman MH, Ooi WL, Cohen H, Madhavan S, Sealey JE, Laragh JH. Plasma renin activity: a risk factor for myocardial infarction in hypertensive patients. *Am J Hypertens*. 1997;10:1–8.
- Rossi G, Rossi A, Sacchetto A, Pavan E, Pessina AC. Hypertensive cerebrovascular disease and the renin-angiotensin system. *Stroke*. 1995;26:1700–1706.
- Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, Benjamin EJ, Levy D. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med*. 2004;351:33–41.
- Campbell SE, Diaz-Arias AA, Weber KT. Fibrosis of the human heart and systemic organs in adrenal adenoma. *Blood Press*. 1992;1:149–156.
- Rizzoni D, Porteri E, Castellano M, Bettoni G, Muiesan ML, Tiberio G, Giulini SM, Rossi GP, Bernini G, Agabiti-Rosei E. Endothelial dysfunction in hypertension is independent from the etiology and from vascular structure. *Hypertension*. 1998;31:335–341.
- Rossi GP, Sacchetto A, Visentin P, Canali C, Graniero GR, Palatini P, Pessina AC. Changes in left ventricular anatomy and function in hypertension and primary aldosteronism. *Hypertension*. 1996;27:1039–1045.
- Rossi GP, Sacchetto A, Pavan E, Palatini P, Graniero GR, Canali C, Pessina AC. Remodeling of the left ventricle in primary aldosteronism due to Conn's adenoma. *Circulation*. 1997;95:1471–1478.
- Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol*. 2005;45:1243–1248.
- Rossi GP. Cardiac consequences of aldosterone excess in human hypertension. *Am J Hypertens*. 2006;19:10–12.
- Farquharson CAJ, Struthers AD. Spironolactone increases nitric oxide bioactivity, improves endothelial vasodilator dysfunction, and suppresses vascular angiotensin I/angiotensin II conversion in patients with chronic heart failure. *Circulation*. 2000;101:594–597.
- Halimi JM, Mimran A. Albuminuria in untreated patients with primary aldosteronism or essential hypertension. *J Hypertens*. 1995;13:1801–1802.
- Ribstein J, du CG, Fesler P, Mimran A. Relative glomerular hyperfiltration in primary aldosteronism. *J Am Soc Nephrol*. 2005;16:1320–1325.
- Zelmanovitz T, Gross JL, Oliveira J, de Azevedo MJ. Proteinuria is still useful for the screening and diagnosis of overt diabetic nephropathy. *Diabetes Care*. 1998;21:1076–1079.
- Palatini P. Microalbuminuria in hypertension. *Curr Hypertens Rep*. 2003;5:208–214.

20. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens*. 2003;21:1011–1053.
21. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*. 2003;42:1206–1252.
22. Rossi GP, Rossi E, Pavan E, Rosati N, Zecchel R, Semplicini A, Perazzoli F, Pessina AC. Screening for primary aldosteronism with a logistic multivariate discriminant analysis. *Clin Endocrinol (Oxf)*. 1998;49:713–723.
23. Rossi GP, Sacchetto A, Chiesa-Corona M, De Toni R, Gallina M, Feltrin GP, Pessina AC. Identification of the etiology of primary aldosteronism with adrenal vein sampling in patients with equivocal computed tomography and magnetic resonance findings: results in 104 consecutive cases. *J Clin Endocrinol Metab*. 2001;86:1083–1090.
24. Morganti A, Pelizzola D, Mantero F, Gazzano G, Opocher G, Piffanelli A. Immunoradiometric versus enzymatic renin assay: results of the Italian Multicenter Comparative Study. Italian Multicenter Study for Standardization of Renin Measurement. *J Hypertens*. 1995;13:19–26.
25. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130:461–470.
26. Palatini P, Mormino P, Mos L, Mazzer A, Dorigatti F, Zanata G, Longo D, Garbelotto R, De Toni R, Graniero G, Pessina AC. Microalbuminuria, renal function and development of sustained hypertension: a longitudinal study in the early stage of hypertension. *J Hypertens*. 2005;23:175–182.
27. Rundback JH, Sacks D, Kent KC, Cooper C, Jones D, Murphy T, Rosenfield K, White C, Bettmann M, Cortell S, Puschett J, Clair D, Cole P. Guidelines for the reporting of renal artery revascularization in clinical trials. Am Heart Association. *Circulation*. 2002;106:1572–1585.
28. 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *J Hypertens*. 1999;17:151–183.
29. Tabachnick BG. *Using Multivariate Statistics*, 4th ed. Needham Heights, MA: Allyn & Bacon; 2001.
30. Fiad TM, Cunningham SK, Hayes FJ, McKenna TJ. Effects of nifedipine treatment on the renin-angiotensin-aldosterone axis. *J Clin Endocrinol Metab*. 1997;82:457–460.
31. Mulatero P, Rabbia F, Milan A, Paglieri C, Morello F, Chiandussi L, Veglio F. Drug effects on aldosterone/plasma renin activity ratio in primary aldosteronism. *Hypertension*. 2002;40:897–902.
32. Young MJ, Funder JW. Mineralocorticoids, salt, hypertension: effects on the heart. *Steroids*. 1996;61:233–235.
33. Baldoncini R, Desideri G, Bellini C, Valenti M, De Mattia G, Santucci A, Ferri C. High plasma renin activity is combined with elevated urinary albumin excretion in essential hypertensive patients. *Kidney Int*. 1999;56:1499–1504.
34. Hall JE, Granger JP, Smith MJ Jr, Premen AJ. Role of renal hemodynamics and arterial pressure in aldosterone “escape.” *Hypertension*. 1984;6:1183–1192.
35. Pedrinelli R, Giampietro O, Carmassi F, Melillo E, Dell’Omo G, Catapano G, Matteucci E, Talarico L, Morale M, De Negri F. Microalbuminuria and endothelial dysfunction in essential hypertension. *Lancet*. 1994;344:14–18.
36. Fioretto P, Stehouwer CD, Mauer M, Chiesa-Corona M, Brocco E, Carraro A, Bortoloso E, van Hinsbergh VW, Crepaldi G, Nosadini R. Heterogeneous nature of microalbuminuria in NIDDM: studies of endothelial function and renal structure. *Diabetologia*. 1998;41:233–236.
37. Nishizaka MK, Zaman MA, Green SA, Renfro KY, Calhoun DA. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. *Circulation*. 2004;109:2857–2861.
38. Farquharson CA, Struthers AD. Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. *Clin Sci (Lond)*. 2002;103:425–431.
39. Tobian L, Lange J, Ulm K, Wold L, Iwai J. Potassium reduces cerebral hemorrhage and death rate in hypertensive rats, even when blood pressure is not lowered. *Hypertension*. 1985;7:1110–1114.
40. Sugimoto T, Tobian L, Ganguli MC. High potassium diets protect against dysfunction of endothelial cells in stroke-prone spontaneously hypertensive rats. *Hypertension*. 1988;11:579–585.
41. Tobian L, MacNeill D, Johnson MA, Ganguli MC, Iwai J. Potassium protection against lesions of the renal tubules, arteries, and glomeruli and nephron loss in salt-loaded hypertensive Dahl S rats. *Hypertension*. 1984;6:1170–1176.
42. Mulatero P, Dluhy RG, Giacchetti G, Boscaro M, Veglio F, Stewart PM. Diagnosis of primary aldosteronism: from screening to subtype differentiation. *Trends Endocrinol Metab*. 2005;16:114–119.
43. Mulatero P, Stowasser M, Loh KC, Fardella CE, Gordon RD, Mosso L, Gomez-Sanchez CE, Veglio F, Young WF Jr. Increased diagnosis of primary aldosteronism, including surgically correctable forms, in centers from five continents. *J Clin Endocrinol Metab*. 2004;89:1045–1050.
44. Pu Q, Neves MF, Virdis A, Touyz RM, Schiffrin EL. Endothelin antagonism on aldosterone-induced oxidative stress and vascular remodeling. *Hypertension*. 2003;42:49–55.
45. Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. *Hypertension*. 2002;40:504–510.