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Increased urinary protein excretion in the “normal” range is associated with increased renin-angiotensin system activity

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Nicholl DD, Hemmelgarn BR, Turin TC, MacRae JM, Muruve DA, Sola DY, Ahmed SB. Increased urinary protein excretion in the “normal” range is associated with increased renin-angiotensin system activity. Am J Physiol Renal Physiol 302: F526–F532, 2012. First published November 16, 2011; doi:10.1152/ajprenal.00458.2011.—Increased levels of albuminuria and proteinuria, both linked to augmented renin-angiotensin system (RAS) activity, are associated with adverse kidney and cardiovascular events. However, the relationship between variations in urinary albumin excretion (UAE) and total protein excretion (UTPE) in the normal range and RAS activity is unclear. We examined the association between UAE and UTPE and the hemodynamic response to angiotensin II (ANG II) challenge, a well-accepted indirect measure of RAS activity, in healthy individuals with normal UAE and UTPE. Forty subjects (15 men, 25 women; age 38 ± 2 yr; UAE, 3.32 ± 0.55 mg/day; UTPE, 56.8 ± 3.6 mg/day) were studied in high-salt balance. Blood pressure (BP), arterial stiffness determined by applanation tonometry, and circulating RAS components were measured at baseline and in response to graded ANG II infusion. The primary outcome was the BP response to ANG II challenge at 30 and 60 min. UAE was associated with a blunted diastolic BP response to ANG II infusion (30 min, P = 0.005; 60 min, P = 0.17), a relationship which remained even after adjustment (30 min, P < 0.001; 60 min, P = 0.035). Similar results were observed with UTPE (30 min, P = 0.031; 60 min, P = 0.001), even after multivariate analysis (30 min, P = 0.008; 60 min, P = 0.001). Neither UAE nor UTPE was associated with systolic BP, circulating RAS components, or arterial stiffness responses to ANG II challenge. Among healthy individuals with UAE and UTPE in the normal range, increased levels of these measures were independently associated with a blunted diastolic BP response to ANG II, indicating increased vascular RAS activity, which is known to be deleterious to both renal and cardiac function.

albuminuria; angiotensin II; blood pressure

PROTEINURIA, EVEN WITHIN the normal range, is a powerful predictor and potential contributor to cardiovascular events (4, 11, 20, 23–25, 32, 33, 37, 53, 56, 57, 60), chronic kidney disease progression (23, 53), end-stage renal disease (23, 31, 49, 53), and all-cause mortality (20, 23, 24, 32, 33, 46, 53), although the role of albuminuria and total urinary protein excretion in the pathophysiology of these conditions remains elusive. Numerous studies have suggested that only negligible amounts of albuminuria should be considered normal (4, 56, 60). Specifically, an estimated urinary albumin excretion (UAE) >2 mg/day (15) has been associated with elevations in blood pressure, myocardial infarction, stroke, and cardiovascular mortality in both diabetic patients (20, 56) and nondiabetic subjects (20, 56, 57).

Reductions in urinary albumin and total protein excretion by means of renin-angiotensin system (RAS) antagonists have been associated with improved renal (1, 3, 12, 48) and cardiovascular (11, 25, 60) outcomes, although it remains unclear whether albuminuria and proteinuria are surrogate end points for cardiorenal disease and thus potential therapeutic targets or simply biomarkers (6). A more complete understanding of the association between albuminuria and total proteinuria, particularly at low levels, and RAS activity as it relates to the vasculature in humans would help clarify the relationship between urinary albumin and total protein excretion and cardiorenal outcomes.

We aimed to determine the relationship between urinary albumin and total protein excretion and the hemodynamic response to an angiotensin II (ANG II) challenge, a well-accepted indirect measure of RAS activity (2, 7, 8, 17, 39, 40, 44), in healthy, nondiabetic, normotensive individuals. We hypothesized that increases in measures of urinary albumin and total protein excretion occurring within the “normal” range in healthy humans would be associated with a blunted response to ANG II challenge, as measured by blood pressure, circulating RAS components, and arterial stiffness, indicating upregulation of RAS activity.

MATERIALS AND METHODS

Subjects. Forty healthy, normotensive (blood pressure <140/90 mmHg and not on any antihypertensive medications) nondiabetic subjects were enrolled in the study (15 men and 25 women). All were nonobese and nonsmokers and on no prescription medications (including oral contraceptives). Subjects underwent a medical history, physical examination, and laboratory screening. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (36). The study protocol was approved by the Conjoint Health Research Ethics Board at the University of Calgary. Written informed consent was obtained from all study subjects in accordance with the Declaration of Helsinki.

Protocol. Subjects were instructed to consume >200 mmol sodium/day for 3 days before the study. A 24-h urine collection (n = 9) or a morning void spot urine (n = 31) was used to determine urinary sodium, creatinine, albumin, and total protein excretion (30). Twenty-four-hour urinary albumin excretion (UAU) was calculated by dividing urine albumin by urine creatinine and multiplying by 8.89 μmol/mg (21), a well-accepted and reliable method (35). Twenty-four
hour urinary total protein excretion (UTPE) was estimated using the same method. Subjects were studied in the supine position in a warm, quiet room after an 8-h fast. All female subjects were studied during the late-follicular phase of the menstrual cycle (14 days after the first day of the last menstrual period), determined by counting days and measuring 17β-estradiol levels (7).

At 8 a.m., an 18-gauge peripheral venous cannula was inserted into the antecubital vein of each arm (1 for infusion and 1 for blood sampling). After a 90-min equilibration period, blood pressure was measured at baseline and in response to two doses of ANG II (3 ng·kg⁻¹·min⁻¹ × 30 min and 6 ng·kg⁻¹·min⁻¹ × 30 min) as an index of RAS activity (2, 7, 8, 17, 39, 40, 44). Blood samples were collected at baseline, after each ANG II infusion, and after a 30-min recovery period.

Blood pressure was recorded every 15 min by an automatic recording device (Dinamap; Critikon). Subjects were studied in the supine position using a standard cuff placed on the right arm. The mean of two readings taken by the same registered nurse is reported. Plasma renin activity (PRA) and aldosterone were measured at baseline and in response to ANG II. Measures of arterial stiffness were determined at baseline and in response to a graded ANG II challenge, as outlined above. The aortic augmentation index (Alx) was determined by applanation tonometry of the right radial artery using a Millar piezoresistive pressure transducer (Miller SPT 301, Millar Instruments) coupled to a Sphygmoconor device (PWV Medical). Alx was calculated from the aortic pressure waveform obtained by applying a transfer function to the radial pressure waveform. Pulse-wave velocity (PWV_r) was determined by sequential acquisition of pressure waveforms from the carotid and the radial arteries by use of the same tonometer. The timing of these waveforms was compared with that of the R wave on a simultaneously recorded ECG. PWV was determined by calculation of the difference in carotid to radial path length divided by the difference in R wave to waveform foot times. The distance from the sternal notch to the radial artery was used to calculate PWV_r.

Laboratory measurements. Urinary sodium was determined by an indirect potentiometry assay using an ion-selective electrode (Roche Cobras Integra Sodium, Roche). Urinary albumin excretion was determined using an immunoturbidimetric assay for quantification of albumin in human urine (Integra 800). Urinary protein excretion was determined by a turbidimetric endpoint assay using benzethonium chloride (Roche Total Protein Urine/CSF Gen. 3, Roche). An activity assay was utilized for PRA (DiaSorin Clinical Assays, Stillwater, MN). In brief, ANG I, the primary product of PRA, was generated at 37°C from endogenous renin and renin substrate at pH 6.0. The integrity of the generated ANG I was maintained by inhibition of proteolytic activity using EDTA and phenylmethylsulfon fluoride in the generation system. The accumulated ANG I reflects PRA under these controlled conditions. The ANG I generated was determined by RIA using competitive binding principles, where the antibody was immobilized onto the lower inner wall of coated tubes. Aldosterone was also measured using an RIA assay. 25°OH vitamin D was measured using a Liaison 25 OH vitamin D Total assay which employs chemiluminescent immunoassay technology for the quantitation determination of 25-hydroxyvitamin D and other hydroxylas.

Analysis. Data are reported as means ± SE or median (range). Our primary outcome was the association between measures of urinary albumin and protein excretion and the change in blood pressure at 30 and 60 min in response to ANG II as a measure of RAS activity. Secondary study outcomes were the changes in circulating RAS components and arterial stiffness in response to ANG II. Associations were analyzed by univariate regression analysis Pearson coefficients. Multivariate linear regression analysis was employed to evaluate the relative contributions of covariates to changes in blood pressure, circulating RAS components, and arterial stiffness in response to ANG II challenge as a function of UAE or UTPE. The following variables were included in the multivariate models in addition to UAE or UTPE: age, gender, body mass index, 25°OH vitamin D, and baseline PRA. Baseline systolic blood pressure (SBP) or diastolic blood pressure (DBP) were included in the analysis for SBP and DBP responses to ANG II infusion. An identical approach was employed in analyzing measures of arterial stiffness in response to ANG II challenge, although baseline measures of arterial stiffness were also included in the analysis. Baseline heart rate was included for Alx. Baseline MAP was included for the analysis of circulating RAS components and measures of arterial stiffness. Gender comparisons were conducted with either an unpaired t-test or the Mann-Whitney U-Test. To ensure an appropriate vasoconstrictor response to ANG II was achieved, we conducted either a paired t-test for outcomes with two measurements (measures of arterial stiffness) or repeated measures ANOVA with a Bonferroni correction for outcomes with multiple measurements (blood pressure and circulating RAS components). All model assumptions were tested and met. All statistical analyses were performed with the statistical software package SPSS V.17.0 (SPSS, Chicago, IL) and were two-tailed with a significance level of 0.05.

RESULTS

Baseline characteristics. Subject characteristics are presented in Table 1. Subjects were normotensive, nonobese, nondiabetic, and in high-salt balance, a state of maximal RAS suppression, as indicated by urine sodium excretion. All subjects had normal urinary albumin (<30 mg/day) and total protein (<200 mg/day) excretion as defined by National Kidney Foundation guidelines (35).

Systemic responses to ANG II challenge. As anticipated, all subjects demonstrated significant changes in all indices of blood pressure, circulating RAS components, and arterial stiffness in response to ANG II challenge compared with baseline values (Table 2).

Association between urinary albumin and total protein excretion and blood pressure, circulating RAS components, and arterial stiffness responses to ANG II challenge. There was a significant inverse relationship between UAE and DBP response to ANG II infusion at 30 min [r = –0.435, β = –0.648 (–1.089, –0.208), P = 0.005; Fig. 1A], a relationship which remained after adjustment for covariates [β = –0.726 (–1.075, –0.376), P < 0.001]. UAE was not linearly correlated to the DBP response to ANG II infusion at 60 min [r = –0.223, β = –0.471 (–1.149, 0.206), P = 0.167; Fig. 2A], although after multivariate adjustment there was a significant association between UAE and change in DBP: every 10 mg/day increase in UAE was associated with a 7.26-mmHg blunting in the diastolic response to ANG II challenge [β = –0.752 (–1.449, –0.055), P = 0.035]. However, removal of the outlier rendered the association between UAE and DBP statistically nonsignificant (P = 0.1). UAE was not correlated with the SBP response to ANG II challenge univariate or multivariate analysis.

As observed with UAE, UTPE was also inversely related to the DBP response to ANG II infusion at 30 min [r = –0.341, β = –0.077 (–0.147, –0.007), P = 0.031; Fig. 1B] on univariate analysis, an association that remained after multivariate analysis [β = –0.088 (–0.151, –0.024), P = 0.008]. UTPE was also inversely related to the DBP response to ANG II infusion at 60 min [r = –0.529, β = –0.170 (–0.261, –0.055), P = 0.001; Fig. 2B] and remained a significant covariate after multivariate analysis [β = –0.182 (–0.285,
Responsiveness to ANG II challenge for all subjects to mortality worldwide (38), although its determinants remain poorly understood. Urinary albumin excretion >6 mg/day is independently associated with the development of hypertension (5, 16, 28, 57) and mortality (54). As DBP was the strongest BP predictor of coronary artery disease in patients <50 yr of age in the Framingham Heart Study (18), it is perhaps not unexpected that the most significant associations observed in our young healthy study population were between urinary albumin and total protein excretion and the DBP response to ANG II challenge. Furthermore, in a study of patients with essential hypertension, greater levels of albuminuria were independently related to increased DBP, rather than SBP (43), providing further evidence of a link between urinary protein excretion and DBP. While further studies are required to completely elucidate the pathophysiology linking these two entities, the current investigation provides evidence of a dose-response relationship between the level of UAE and UTPE, which is ultimately detrimental to cardiorenal outcomes (4, 11, 20, 23–25, 31–33, 37, 49, 53, 56, 57, 60) and mortality (20, 23, 24, 32, 33, 46, 53).

It has recently come into question whether current definitions of “normal” protein excretion (as defined by urinary albumin excretion) accurately reflect cardiovascular risk (4), as

### Table 1. Baseline characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All Subjects</th>
<th>Men</th>
<th>Women</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>40</td>
<td>15</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>38 ± 2</td>
<td>34 ± 2</td>
<td>40 ± 3</td>
<td>0.2</td>
</tr>
<tr>
<td>Race, % Caucasian</td>
<td>85</td>
<td>87</td>
<td>84</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26 ± 1</td>
<td>26 ± 1</td>
<td>25 ± 1</td>
<td>0.4</td>
</tr>
<tr>
<td>UAE, mg/day†</td>
<td>2.42 (0.58, 21.48)</td>
<td>1.77 (0.81, 3.40)</td>
<td>3.14 (0.58, 21.48)</td>
<td>0.002</td>
</tr>
<tr>
<td>UTPE, mg/day†</td>
<td>49.2 (24.4, 108.0)</td>
<td>41.1 (24.4, 107.2)</td>
<td>60.0 (34.2, 108.0)</td>
<td>0.010</td>
</tr>
<tr>
<td>Urine Na, mmol/day</td>
<td>351 ± 20</td>
<td>375 ± 35</td>
<td>336 ± 19</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum creatinine, μmol/l</td>
<td>73 ± 2</td>
<td>85 ± 3</td>
<td>67 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>eGFR, ml·min⁻¹·1.73 m⁻²</td>
<td>101 ± 3</td>
<td>102 ± 4</td>
<td>101 ± 4</td>
<td>0.9</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.4 ± 0.04</td>
<td>5.3 ± 0.06</td>
<td>5.5 ± 0.05</td>
<td>0.022</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.7 ± 0.08</td>
<td>4.8 ± 0.12</td>
<td>4.6 ± 0.10</td>
<td>0.2</td>
</tr>
<tr>
<td>25′OH vitamin D, nmol/l</td>
<td>69 ± 4</td>
<td>78 ± 8</td>
<td>64 ± 4</td>
<td>0.2</td>
</tr>
<tr>
<td>SBP, mmHg*</td>
<td>117 ± 2</td>
<td>120 ± 3</td>
<td>115 ± 3</td>
<td>0.3</td>
</tr>
<tr>
<td>DBP, mmHg*</td>
<td>69 ± 1</td>
<td>69 ± 2</td>
<td>69 ± 2</td>
<td>0.8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>63 ± 1</td>
<td>62 ± 2</td>
<td>63 ± 2</td>
<td>0.9</td>
</tr>
<tr>
<td>PRA, ng·l⁻¹·s⁻¹⁻¹</td>
<td>0.25 ± 0.02</td>
<td>0.32 ± 0.04</td>
<td>0.20 ± 0.02</td>
<td>0.010</td>
</tr>
<tr>
<td>ANG II, ng/l</td>
<td>17.3 ± 12</td>
<td>17.5 ± 14</td>
<td>17.2 ± 19</td>
<td>0.3</td>
</tr>
<tr>
<td>Aldosterone, pmol/l</td>
<td>142 ± 13</td>
<td>179 ± 23</td>
<td>119 ± 13</td>
<td>0.019</td>
</tr>
<tr>
<td>Alx, %*</td>
<td>10.33 ± 2.68</td>
<td>−1.07 ± 4.04</td>
<td>17.16 ± 2.78</td>
<td>0.001</td>
</tr>
<tr>
<td>PWVcr*, m/s*</td>
<td>8.20 ± 0.41</td>
<td>8.12 ± 0.23</td>
<td>8.25 ± 0.68</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE unless otherwise specified. BMI, body mass index; UAE, urinary albumin excretion; UTPE, urinary total protein excretion; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure; PRA, plasma renin activity; Alx, aortic augmentation index; PWVcr, pulse-wave velocity carotid radial. *Mean of 2 readings. †Expressed as median (range).

### Table 2. Responses to ANG II challenge for all subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg*</td>
<td>117 ± 2</td>
<td>128 ± 3†</td>
<td>134 ± 3†‡</td>
</tr>
<tr>
<td>DBP, mmHg*</td>
<td>69 ± 1</td>
<td>80 ± 1†</td>
<td>81 ± 1†</td>
</tr>
<tr>
<td>Circulating RAS components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA, ng·l⁻¹·s⁻¹⁻¹</td>
<td>0.25 ± 0.02</td>
<td>0.15 ± 0.02†</td>
<td>0.12 ± 0.01†‡</td>
</tr>
<tr>
<td>Aldosterone, pmol/l</td>
<td>142 ± 13</td>
<td>234 ± 20†</td>
<td>362 ± 26‡‡</td>
</tr>
<tr>
<td>Arterial stiffness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alx, %*</td>
<td>10.33 ± 2.68</td>
<td>28.82 ± 3.20†</td>
<td>10.68 ± 0.64‡‡</td>
</tr>
<tr>
<td>PWVcr*, m/s*</td>
<td>8.20 ± 0.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 40. RAS, renin-angiotensin system. *Mean of 2 readings. †P < 0.001, compared with baseline. ‡P < 0.001, compared with 30 min.
In a healthy human, the amount of urinary albumin excreted normally represents <1% of the albumin filtered at the glomerular level (47). ANG II, the effector molecule of the RAS, has proinflammatory effects (59). This increased inflammation, coupled with the augmented intraglomerular pressure and resulting increased local leakage of albumin (54) attributed to upregulated RAS activity, may contribute to widespread vascular permeability even in healthy subjects (10, 13, 27), underscoring the plausibility of this pathophysiological relationship. Furthermore, the ANG II type 1 (AT₁) receptor has been implicated in pathways known to cause endothelial damage such as synthesis of interleukin-6 (51) and generation of reactive oxygen species (58), which damage the glomerular filtration barrier. Conversely, urinary albumin may also stimulate the RAS in proximal tubular cells (22). In humans with chronic kidney disease, proteinuria is a good marker of kidney disease progression (23, 53). However, in kidney disease, urinary total protein loss usually results locally from a specific increase in cardiovascular disease risk has been reported with albuminuria levels of >2 mg/day (15). In the population-based Prevention of Renal and Vascular End stage Disease (PREVEND) study (24), urinary albumin in the normal range was associated with increased mortality. The Framingham Heart Study (4) found that low levels of UAE, well below the microalbuminuria range, predicted cardiovascular events in a community-based nonhypertensive, nondiabetic population. Similarly, the Prevention of Events With ACE Inhibition (PEACE) trial (37, 52) found that increased urinary albumin, even within the normal range, was associated with increased risk for all-cause mortality and cardiovascular death in relatively healthy patients with stable coronary disease. While the precise pathophysiology underlying albuminuria and its relationship to the RAS remain unknown, we have demonstrated that increased urinary albumin and total protein excretion were associated with increased vascular RAS activity, although it is difficult to discern from our study which is the causative factor.
lesion within the kidney and therefore does not necessarily reflect the health status of the overall vasculature (26). Further reinforcing the pathophysiological relationship between proteinuria and upregulated RAS activity, reductions in urinary albumin and protein excretion by means of RAS blockade have been associated with improved renal (1, 3, 12, 48) and cardiovascular (11, 25, 60) outcomes at a clinical level. The increased cardiovascular risk associated with albuminuria in the PEACE trial was attenuated by ACE inhibition (52). The Survival And Ventricular Enlargement (SAVE) trial (29) of patients with left ventricular dysfunction found that patients with trace or higher proteinuria were most likely to benefit from ACE-inhibition treatment. Finally, the Reduction of Endpoint in NIDDM with Angiotensin II Antagonist Losartan (RENAAL) study (11) found that suppression of albuminuria was the strongest predictor of long-term protection from cardiovascular events. Further underscoring the relationship between urinary protein excretion and the RAS, proteinuria remained a strong predictor for cardiovascular morbidity despite effective blood pressure lowering by non-RAS-blocking therapies (50).

Proteinuria is the cardinal sign of kidney disease. However, there is no clear agreement among published guidelines as to the definition of clinically significant proteinuria or whether proteinuria should be defined in terms of albumin or total protein loss (34). Accordingly, the discrepancies in the response to ANG II challenge associated with UAE and UTPE in our study deserve mention. UAE was associated with a negative DBP response at 30 min, but not at 60 min, suggesting that the AT receptors may be saturated at the lower ANG II dose (40). It must be noted, however, that this association was mainly driven by the subject with the greatest UAE, although the value was still within the normal range. Conversely, the association between UTPE and the DBP response to ANG II was greatest at the higher doses of exogenous ANG II. This suggests that a nonalbumin component of total protein may also play a role in control of the RAS. Certainly, previous investigations have suggested that β2-macroglobulin, β2-glycoprotein-1, retinol-binding protein, α1-microglobulin, β2-microglobulin, and N-acetyl-β-D-glucosaminidase have all been associated with renal disease (14, 42). However, this is purely speculative and is an area that merits further research.

Urinary albumin excretion may be overestimated in women due to decreased muscle mass and urinary creatinine excretion (9), so it is possible that our UAE and UTPE results represent an overestimation of the true value. However, as female gender predicts an increased sensitivity to ANG II, likely reflecting lower baseline RAS activity (8, 19, 39–41, 45, 55), this suggests that the blunted DBP response to ANG II associated with increased UAE and UTPE observed in our study may actually be an underestimate of the true effect, as our results would be biased toward the null. Furthermore, the associations observed between UAE and UTPE and the response to ANG II challenge remained significant with the inclusion of gender as a covariate on multivariate analysis.

Our study has limitations. First, our study sample was limited to healthy subjects with normal blood pressure, renal function, urinalysis, and absence of comorbid disease, limiting the generalizability of study results. However, by studying a healthy population, we aimed to examine the impact of measures of urinary albumin and total protein excretion on blood pressure and measures of arterial stiffness without any confounding factors. Second, we attempted to minimize the effect of sample size and intradividual variability by utilizing a homogenous study group and careful prestudy design. We ensured that all participants were ingesting similar amounts of salt to ensure maximum RAS suppression, that no female participant was ingesting oral contraceptives, and that all female subjects were studied during the same stage of the menstrual cycle, during the follicular (low estrogen) stage to control for the effect of estrogen on blood pressure and the RAS. In addition, all subjects were studied at the same time of the day, while resting in the supine position in a warm, quiet room after an 8-h fast. We also enrolled healthy subjects free of diabetes, hypertension, and cardiovascular disease, as these conditions can influence UAE and UTPE. Third, as it is not possible to measure vascular RAS activity directly in humans, we utilized the well-accepted method of indirectly measuring RAS in humans by observing the response to ANG II challenge (2, 7, 8, 17, 39, 40, 44). A spot urine was used for determination of creatinine, albumin, and protein excretion in place of the gold standard 24-h urine sample; however, even in the most careful of research settings, 24-h urine collections are prone to error and there is excellent correlation between spot morning urine samples and 24-h urine collection for estimation of albumin and protein excretion (21). We utilized the carotid radial method for PWV in place of the gold standard carotid femoral pulse wave velocity although this would not affect calculation of the AIx. Finally, due to the cross-sectional nature of our study design, we cannot demonstrate directionality of the association nor comment on causality, and it is possible that increased RAS activity caused an increase in UAE and UTPE. Nevertheless, our study suggests that in the population with increased blood pressure who excrete urinary albumin and total protein excretion is at the upper end of normal, antihypertensive agents that interrupt the RAS may ultimately provide the greatest benefit in terms of reducing overall cardiovascular risk.

In conclusion, in our young healthy population with normal urinary excretion of albumin and protein, we found that increased levels of albuminuria and total proteinuria were associated with increased vascular RAS activity. It is possible that patients with hypertension should be routinely screened for both albuminuria and total proteinuria as these entities appear to modulate ANG II-dependent control of DBP, the primary vascular predictor of cardiovascular disease risk in young healthy individuals (18). Larger, prospective studies are required to confirm our findings and to determine whether proteinuria has a causal role in the progression of cardiovascular and kidney disease.

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AUTHOR CONTRIBUTIONS


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


