Urinary albumin excretion and atherosclerosis in essential hypertension

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1. Increased urinary albumin excretion is common in patients with essential hypertension and is at least to some extent correlated with prevailing blood pressure levels. However, the generalized vascular dysfunction present in advanced atherosclerotic disease may independently influence this parameter.

2. To evaluate this possibility, we assessed blood pressure, ultrasonographic carotid thickness, cardiac mass, minimum forearm vascular resistances, metabolic parameters and the angiotensin-converting enzyme genotype in patients with untreated essential hypertension and atherosclerotic peripheral vascular disease (n = 11). The results were compared with similar data obtained in matched groups of patients with uncomplicated hypertension and with normotensive control subjects (n = 11 per group).

3. Urinary albumin excretion was higher in hypertensive patients with atherosclerosis than in those without complications; carotid thickness was higher in atherosclerotic patients and a positive, statistically significant correlation existed between this parameter and urinary albumin excretion. In the same patient group, systolic blood pressure, fasting insulin and triacylglycerol levels were elevated and correlated with urinary albumin levels. However, differences in urinary albumin excretion persisted after taking into account the influence of those parameters by analysis of covariance. The distribution of angiotensin-converting enzyme genotype patterns and values of cardiac mass and minimum forearm vascular resistances did not differ significantly among the experimental groups.

4. The data suggest that vascular status may influence urinary albumin excretion in patients with essential hypertension, while confirming the importance of systolic blood pressure levels as a determinant of the raised urinary albumin excretion.

INTRODUCTION

Abnormal urinary albumin excretion (UAE) in a range not detectable by the usual dipstick methods for urine protein is frequently found in patients with essential hypertension [1]. This phenomenon is correlated, at least in part, with the level of blood pressure (BP) [1], but generalized vascular dysfunction may contribute to it [2, 3]. This hypothesis may apply also to the systemic microvascular damage associated with atherosclerotic disease [4]. Previous data have shown that microalbuminuria is predictive of mortality independently of hypertension in non-diabetic subjects [5]; microalbuminuric subjects at risk of early atherosclerosis manifest evidence of diffuse vascular involvement such as increased levels of von Willebrand factor [6, 7] and reduced vascular response to local nitric oxide inhibition [8]; finally, patients with advanced atherosclerotic disease show increments in UAE paralleled by abnormal neutrophilic activation [9]. Thus, both hypertension and atherosclerosis might independently contribute to albuminuria, but the overlap between the two conditions makes it difficult to understand their relative contribution to the changes in UAE. To explore this question, we compared patients with essential hypertension in whom atherosclerotic disease was fully expressed with patients without evidence of vascular pathology and with normal control subjects.

Key words: atherosclerosis, blood pressure, essential hypertension, insulin resistance, urinary albumin excretion.
Abbreviations: ACE, angiotensin-converting enzyme; ANOVA, analysis of variance; BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; FBF, forearm blood flow; FVR, forearm vascular resistance; HDL, high-density lipoprotein; IMT, intima-media thickness; IRI, immunoreactive insulin; LDL, low-density lipoprotein; LVMI, left ventricular mass index; MBP, mean blood pressure; PVD, peripheral vascular disease; SBP, systolic blood pressure; UAE, urinary albumin excretion.

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SUBJECTS AND METHODS

Subjects

Essential hypertensive patients with atherosclerotic peripheral vascular disease (PVD). Eleven essential hypertensive patients with angiographically documented aorto-iliac-femoral atherosclerotic PVD were included in the study [nine women, two men; age 58±8 years (SD), range 44–74]. They had fasting blood glucose levels less than 120 mg/dl, serum creatinine less than 1.2 mg/dl, total serum cholesterol of less than 300 mg/dl, normal urinary sediment, no urinary tract infection, body mass index (BMI) less than 30 kg/m² and no evidence or history of congestive heart failure, ischaemic heart disease, previous myocardial infarction, chronic obstructive pulmonary disease, previous amputation, pain at rest or ischaemic trophic ulcers or gangrene. Haemodynamically significant carotid stenoses were also excluded by high-resolution ultrasound. All had an ankle–brachial index [the ratio between systolic BP measured at the brachial and bilateral posterior tibial artery by Doppler sonography (Stereodop, Promelec, Bourgeais, Guer, France)] lower than 0.9 in at least one limb (0.55±0.1) and stable intermittent claudication (pain-free walking distance greater than 200 m on a treadmill). In all subjects, the angiograms had shown no evidence of renal artery stenosis, while routine clinical and haematological examinations excluded other secondary forms of hypertension. Renal ultrasound scans revealed normal-sized kidneys and no evidence of cortical scarring or obstructive uropathy in all patients. Two patients were active smokers; three were taking lipid-lowering drugs (gemfibrozil or simvastatin) and all received either ticlopidine or aspirin.

Uncomplicated essential hypertensive patients. Eleven sex-matched patients with uncomplicated essential hypertension (including two smokers) of similar age (56±9 years, range 43–73) underwent a similar diagnostic procedure (except angiography). PVD was excluded by verification of normal carotid, aortic and lower limb vessels as determined by echo Doppler scans and an ankle–brachial pressure index≥1.

Normotensive control subjects. Eleven normal, non-obese, sedentary, sex-matched, untreated normotensive subjects (including two smokers) of similar age (57±9 years, range 46–70) with normal physical examination, routine blood and urinary tests, BP, ECG, abdominal echograms and ankle–brachial index were enrolled into the study as controls. Hypertensive patients of both groups (four of whom had never been treated while calcium channel blockers or angiotensin-converting enzyme inhibitors or both were stopped for at least 2 weeks before the study) had BP values consistently greater than 140/90 mmHg as outpatients. Lipid-lowering drugs were withdrawn for the same period. At the end of the washout period, experimental evaluations were completed in a 2-week period. According to institutional guidelines, subjects were aware of the investigational nature of the study and agreed to participate. The study was carried out in accordance with the Declaration of Helsinki and the protocol was approved by the local ethics committee.

Experimental procedures

Casual systolic (SBP) and diastolic (DBP) (Korotkoff V phase) blood pressure were measured repeatedly in the morning in the supine position using a mercury sphygmomanometer. Anthropometric measurements (height and weight) were made after each participant had removed his or her shoes and upper garments. Blood samples were obtained between 08.00 and 09.00 hours after an overnight fast and 15 min of supine rest.

Urinary albumin excretion. To minimize the confounding influence of daily physical activity and to facilitate the collection procedure, urine was collected from 20.00 to 08.00 hours for 3 consecutive days as described in detail elsewhere [7]. Urinary albumin was measured by nephelometry (Istituto Behring, Scoppito, Italy; limit of detection 0.6 mg/dl, interassay coefficient of variation 3.5%).

Carotid wall thickness. The sonographer demonstrated as clearly as possible the interfaces required for the measurements of the arterial wall thickness [10], searching for the thickest interface at 4–5 sites in the far wall of the right and left common carotid artery (i.e. the arterial segment extending from 8 to 16 mm below the tip of the flow divider into the common carotid artery) and the carotid bifurcation (bulb) using anterolateral, lateral and posterolateral fixed angles of examination. Patients who had tortuous or calcified arteries or without identifiable references were excluded. The ultrasound system was an AU 590 asynchronous system (Ansaldo, Genova, Italy) with a linear 7.5-MHz probe and an axial resolution of 0.1 mm. Scanning and measurements were obtained by the same observer unaware of the clinical status of the subject under examination. All measurements were made with the image at the maximum depth of focus. Gains and image pre- and post-processing options were set up by the operator for each patient and for each artery to obtain the best possible image. During scanning, distance measurements were recorded using software-driven cursors with a digital display expressing distances in mm. Complete scanning of each subject took about 20 min plus 20 min for the measurements. The intraindividual correlation of carotid scanning values at the common carotid and the bulb repeated at 1-month intervals was 0.84 and 0.78 (P<0.001) respectively.

Echocardiographic and plethysmographic parameters. Wall thickness and chamber volumes were measured by mono- and bidimensional echocardiograms (Hewlett Packard Sonos 1000, Andover, MA,
U.S.A.) with 2.5- and 3.5-MHz transducers [11]. Forearm blood flow (FBF) was measured by strain gauge venous plethysmography (DE Hokanson EC 5R, Issaquah, WA, U.S.A.). For the evaluation of post-ischaemic reactive hyperaemia, maximal hyperaemia was induced by occlusion of the left forearm by inflation of a plethysmographic cuff to 300 mmHg for 13 min with dynamic exercise (10–20 hand contractions) during the last minute of ischaemia. Peak FBF was defined as the highest FBF during the 3 min after ischaemic release, and BP was measured indirectly at the contralateral forearm [11].

Metabolic parameters. Blood glucose was measured by the glucooxidase method. Immunoreactive insulin (IRI) concentrations were measured by radioimmunoassay (Sorin, Saluggia, Italy); total and high-density lipoprotein (HDL)-cholesterol and triacylglycerols were assessed by enzymic colorimetric techniques (Boehringer-Mannheim, Mannheim, Germany). Low-density lipoprotein (LDL)-cholesterol was calculated as total cholesterol—(HDL-cholesterol + triacylglycerol/5).

Angiotensin-converting enzyme (ACE) D/I polymorphism. As ACE D/I gene polymorphism may influence UAE [12] and predict cardiovascular disease [13], the parameter was determined in each subject from 1 μl of EDTA-anticoagulated blood using a previously described procedure [13]. Assays were performed blindly with regard to study group or UAE status.

Data analysis

Data processing. UAE (μg/min) was determined as the average of three consecutive collections. The projection showing the greatest distance between the lumen—intima and the media—adventitia interface at the bulb level was defined as maximum intima—media thickness (IMT max); wall thickenings ≥1.6 mm were defined as plaques [14]. The average of the right and left far-wall intima—media determinations at the common carotid segment was defined as IMT. Reported values for casual systolic and diastolic BP were the average of multiple record-ings. Minimal forearm vascular resistances (Rmin) were the ratio of preischaemic mean BP (MBP, diastolic + 1/3 pulse pressure) and maximum postischaemic peak flow. Left ventricular mass index (LVMI) was expressed in g/m² to take into account body surface area. Forearm vascular resistances (FVR, MBP/FBF) and BMI (body weight/squared surface area) were derived from standard formulae.

Statistics. Log transformation was applied to UAE, triacylglycerol and IRI as the raw data were not distributed normally. Descriptive statistics were arithmetic means ± SD or medians and ranges for skewed data. Differences among means were tested by one-way analysis of variance (ANOVA); in the presence of a significant F-value, between-group comparisons were performed by the Scheffé’s method. Frequency distributions were analysed using χ² statistics. The intrapersonal association of variables in hypertensive subjects was tested by Pearson’s correlation coefficients. Analysis of covariance was used to take into account possible confounding influences on UAE of concomitant metric variables. Statistical significance was set at a P < 0.05. Calculations were performed using Statgraphics Plus (Manugistic, Release 1994, Rockville, MD, U.S.A.).

RESULTS

As a consequence of the selection procedure at entry, age, sex, smoking habits (see subjects and methods) and BMI (Table 1) were superimposable among the experimental groups.

UAE and cardiovascular parameters

SBP, DBP, LVMI, Rmin and FVR were higher in hypertensive patients than in control subjects (Table 1). UAE (Fig. 1), IMT max, IMT and SBP (Table 1, P < 0.01 for all) were higher in hypertensive patients with atherosclerosis than in hypertensive patients without complications. The other parameters did not differ significantly (Table 1). IMT max and UAE were correlated in the overall sample (r = 0.41, P < 0.019, n = 33) and in the hypertensive subset (r = 0.45, P < 0.034, n = 22, Fig. 2); the correlation with IMT was only borderline (r = 0.39, P < 0.11).

Plaques (IMT max ≥1.6 mm) were present in 10 out of 11 atherosclerotic patients compared with two patients with uncomplicated hypertension and four control subjects (P < 0.02). UAE in subjects with (n = 16) and without (n = 17) plaques was 17

<p>| Table 1. BMI, carotid thickness, SBP, DBP, regional and systemic haemodynamics and cardiac mass in control subjects and patients with uncomplicated essential hypertension (EH) and atherosclerotic peripheral vascular disease (EH and PVD). n = 11 per group, means ± SD. Statistical significance: *P &lt; 0.01, **P &lt; 0.02, EH patients versus control subjects. †P &lt; 0.01, uncomplicated EH patients versus EH patients with PVD. ‡P &lt; 0.01, EH patients with PVD versus uncomplicated EH patients and control subjects. |
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<table>
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<tr>
<th>Control patients</th>
<th>Patients with uncomplicated EH</th>
<th>Patients with EH and PVD</th>
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<tr>
<td>BMI (kg/m²)</td>
<td>25.0 ± 2.4</td>
<td>26.3 ± 2.9</td>
</tr>
<tr>
<td>IMT max (mm)</td>
<td>1.4 ± 0.6</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.1</td>
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<td>SBP (mmHg)</td>
<td>134 ± 10</td>
<td>150 ± 10*</td>
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<tr>
<td>DBP (mmHg)</td>
<td>80 ± 10</td>
<td>97 ± 9*</td>
</tr>
<tr>
<td>LVMI (g/m²)</td>
<td>92 ± 13</td>
<td>113 ± 20*</td>
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<tr>
<td>Rmin (units)</td>
<td>2.1 ± 0.4</td>
<td>2.6 ± 0.6*</td>
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<tr>
<td>FVR (units)</td>
<td>21 ± 5</td>
<td>34 ± 15*</td>
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(4–144) versus 13 (6–27) µg/min (P < 0.05) respectively.

**Metabolic parameters (Table 2)**

Triacylglycerol (P < 0.01) and IRI levels (P < 0.03) were higher in essential hypertensive patients with PVD than in patients without complications and control subjects. The two variables were correlated (r = 0.67, P < 0.001, n = 22) in hypertensive patients. Glucose and total, HDL- and LDL-cholesterol did not differ significantly among the study groups (Table 2).

**ACE genotype**

UAE averaged 11 (4–144), 17 (6–87) and 10 (6–122) µg/min in subjects with the DD (n = 13, 39.4%), DI (n = 13, 39.4%) and II (n = 7, 21.2%) genotype respectively (not significant). Corresponding figures for IMTm, were 1.2 ± 0.6, 1.9 ± 0.9 and 1.4 ± 0.6 mm respectively (not significant).

**Other correlates of UAE in hypertensive patients (n = 22)**

Significant univariate correlations existed between UAE and SBP (r = 0.76, P < 0.001), triacylglycerol (r = 0.75, P < 0.001) and fasting circulating insulin (r = 0.43, P < 0.03) levels. Casual DBP, cardiac mass and minimum FVR showed no correlation.

Controlling for the influence of SBP, triacylglycerol and fasting insulin did not abolish the difference in UAE between patients with and without atherosclerosis (Table 3).

**DISCUSSION**

The increased albuminuria independent of casual SBP, triacylglycerol and fasting insulin levels as well as the positive correlation with carotid thickness suggests that widespread atherosclerosis exerted some independent influence on UAE in our hypertensive, atherosclerotic patients. The physiopathological link between peripheral macrovascular disease and UAE is unclear; altered glomerular permeability as a reflection of a systemic capillary damage [2, 3] concomitant with atherosclerosis [4] is
isolated lower limb macrovascular disease, strongly supports the use of ultrasonographic thickness as a surrogate measure of the systemic atherosclerotic process [15]. It should also be noted that, in agreement with recent data [16], common carotid thickness showed little correlation with UAE, perhaps because coexisting hypertensive medial wall hypertrophy [17, 18] did not allow precise characterization of the relative contribution of atherosclerotic intimal thickening by ultrasonography. In contrast, the bifurcation may be more suitable for assessing atherosclerotic involvement in hypertensive patients as smooth muscle cells are much less frequent at that level [19]. Given the small size of our experimental sample, we cannot also exclude biases due to random aggregating variables. However, we were careful to stratify for several potential confounders: the groups were sex-matched and very similar in age, and specific disease-related influences were avoided by recruiting only patients with isolated atherosclerotic PVD. Smoking habits were also balanced carefully to avoid interferences from the recognized association with albuminuria [7, 20], and patients were studied after protracted drug withdrawal. The independence of UAE of the ACE gene polymorphism was important to exclude the influence of genetic factors in our patients. Our sample size, however, was too small to make any more general claims about the relationship between ACE gene polymorphism and UAE that has been shown by others in wider populations [12]. Finally, patients with uncomplicated essential hypertension provided a control for patients with advanced macrovascular disease, and the similar behaviour of baseline FVR as well as indicators of long-term structural remodelling such as cardiac mass [21] and minimum FVR [22] suggested that the two hypertensive groups did not differ markedly with regard to the degree of either end-organ target damage per se or long-term BP control by antihypertensive treatment. In contrast to the other variables, SBP was higher in hypertensive, atherosclerotic patients, and this parameter emerged as an important predictor of UAE. We do not overemphasize the relevance of these data considering that most of our patients were treated pharmacologically, and the prevailing BP regimen during treatment cannot be predicted from values measured after a 2-week drug withdrawal, especially considering that casual BP poorly reflects the 24-h pressure profile [23]. On the other hand, the data are consistent with previous studies [1], and may reflect the contribution to hypertensive albuminuria of either purely hydraulic factors [24, 25] or even less obvious mechanisms, as our data might suggest. In fact, elevated SBP without parallel elevations in DBP may be ascribed to reduced aortic compliance [26] owing to the vessel wall stiffening that is often seen in association with aortic atherosclerosis [27], which, in turn, reflects general atherosclerosis and predicts symptomatic cardiovascular disease [28, 29].

The behaviour of metabolic parameters deserves comments in at least two regards. Firstly, the finding of elevated triacylglycerol in our hypertensive patients with atherosclerosis is consistent with previous data obtained in similar patients with PVD [30]. Although we did not perform insulin stimulation or clamp studies to substantiate this possibility, hypertriglyceridaemia coupled with elevated fasting insulin levels suggests an underlying insulin resistance [31], an abnormality ascribable to either hypertension [32] or PVD as such. We favour the first hypothesis as post-load insulin levels were found not to differ between PVD patients and control subjects in previous studies [33, 34]; in fact, human ischaemic muscle shows increased insulin sensitivity [35]. The fact that the metabolic indices were in the normal range in some of our patients is not surprising, as we now know that insulin resistance is found in only some patients with essential hypertension [36]. Secondly, our data confirm the frequent finding of a slight elevation of UAE associated with fasting hyperinsulinaemia in non-diabetic patients [1], a phenomenon interpreted in the context of the insulin resistance syndromes [37]. However, this issue is not settled, as other results have indicated that microalbuminuria is more closely related to BP levels [38, 39] or body weight [40] than to hyperinsulinaemia or insulin sensitivity per se in patients with essential hypertension. In addition, microalbuminuria and hyperinsulinaemia independently predict future coronary heart disease, suggesting that independent mechanisms are operative [41]. Whether the results obtained in a general non-diabetic population apply to patients with essential hypertension remains to be elucidated.

In conclusion, our data suggest that, among several other factors, UAE levels may reflect the overall vascular status in patients with essential hypertension. Confirmation of this finding will await additional, larger studies.

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