Endothelial Dysfunction and Mild Renal Insufficiency in Essential Hypertension

Francesco Perticone, MD; Raffaele Maio, MD; Giovanni Tripepi, Stat Tech; Carmine Zoccali, MD

Background—Mild to moderate renal insufficiency in individuals with essential hypertension is currently considered the expression of a renal microvasculopathy characterized by preglomerular arteriolar involvement and tubulo-interstitial changes. Whether endothelial dysfunction plays a role in this alteration is still undefined.

Methods and Results—We investigated the relationship between endothelial function (hemodynamic response to acetylcholine [ACh] in the forearm) and renal function in 500 patients with uncomplicated, never-treated, essential hypertension and serum creatinine within the normal range (ie, ≤1.5 mg/dL). Serum creatinine, creatinine clearance, and estimated glomerular filtration rate (GFR, by the Modification of Diet in Renal Disease formula) were related to the forearm blood flow response to ACh (all P≤0.003), and these relationships held true in multiple regression analyses that included age, gender, systolic pressure, serum cholesterol and glucose, smoking, and body mass index. Accordingly, on multiple logistic regression analysis, the risk of moderate renal dysfunction (ie, an estimated GFR <60 mL·min⁻¹·1.73 m⁻²) was 64% lower (OR 0.36, 95% CI 0.18 to 0.70) in patients in the third ACh tertile (ie, those showing the higher vasodilatory response) than in those in the first tertile (ie, showing the lower response). C-reactive protein was related directly to serum creatinine and inversely to GFR and vasodilatory response to ACh, which suggests that endothelial dysfunction is a possible mechanism linking inflammation and impaired renal function in essential hypertension.

Conclusions—An impaired vasodilatory response to ACh appears to be associated with renal function loss in patients with essential hypertension. This association suggests that systemic endothelial dysfunction is involved in mild to moderate renal insufficiency in patients with uncomplicated essential hypertension. (Circulation. 2004;110:821-825.)

Key Words: acetylcholine • endothelium • kidneys

Consistent evidence has now accrued that in individuals with essential hypertension, even minor degrees of renal insufficiency entail a high risk.1 In uncomplicated essential hypertension, a higher serum creatinine within the normal range is a strong predictor of cardiovascular morbidity, and its prognostic value persists after adjustment for several powerful confounders, including average 24-hour blood pressure and left ventricular hypertrophy.2 Endothelial dysfunction is another feature of major clinical relevance in hypertensive patients,3,4 because independently of arterial pressure levels and other risk factors, it is associated with left ventricular hypertrophy5 and predicts cardiovascular events.6

Mild to moderate renal insufficiency in individuals with essential hypertension is considered the expression of a renal microvasculopathy characterized by preglomerular arteriolar involvement (atherosclerosis) and tubulo-interstitial changes. This microvasculopathy may be triggered by sympathetic overactivity, overstimulation of the renin angiotensin system, or any factor that causes renal vasoconstriction.7 Yet afferent vasoconstriction per se is not expected to cause overt renal dysfunction as long as there is no other noxious factor. By contrast, if dysfunctional endothelium contributes to renal vasoconstriction, renal impairment may be much more likely to follow. Thus, it appears plausible that microvascular disease in the kidney may be related to an alteration involving the endothelium. In support of this possibility, hemodynamic studies exploring the renal response to the nitric oxide (NO) precursor L-arginine have consistently demonstrated an impaired renal vascular relaxation in hypertensive subjects.8–10 Such a hypothesis may also contribute to explain why both endothelial dysfunction11 and serum creatinine2 are independent predictors of adverse cardiovascular outcomes in hypertensive patients.

In the present study, we investigated the relationship between the endothelium-dependent vasodilatory response to acetylcholine (ACh) in the forearm and renal function in 500 patients with uncomplicated, never-treated, essential hypertension and serum creatinine within the normal range (ie, ≤1.5 mg/dL) who were studied in a tertiary referral center.
### Methods

The local ethics committee approved the study, and all participants gave written informed consent for all procedures.

### Patients

Five hundred patients with essential hypertension (256 men and 244 women; age range 22 to 73 years, mean 47.2 ± 11.0 years; all whites) were studied. These patients were selected from a population of ~3500 individuals referred to the hypertension clinic of the University Hospital of Catanzaro between September 1994 and January 2003. To be selected, patients had to have newly diagnosed essential hypertension with a serum creatinine ≤ 1.5 mg/dL, have an absence of proteinuria on the dipstick test, and have never received antihypertensive medications. None of the patients had a history or clinical evidence of angina, myocardial infarction, valvular heart disease, diabetes, serum cholesterol exceeding 7.25 mmol/L, peripheral vascular disease, coagulopathy, or any disease predisposing to vasculitis or Raynaud’s phenomenon. Causes of secondary hypertension were excluded by appropriate investigations, including measurement of plasma renin activity and aldosterone, Doppler studies of the renal arteries, and/or renal scintigraphy or renal angiography. The following risk factors for atherosclerosis were assessed by a dose-response curve to intra-arterial ACh infusions (0.8, 1.6, and 3.2 μg · mL⁻¹ · min⁻¹, each for 5 minutes) and SNP infusions (0.8, 1.6, and 3.2 μg · mL⁻¹ · min⁻¹, each for 5 minutes), respectively. The sequence of administration of ACh and SNP was randomized to avoid any bias related to the order of drug infusion. The drug infusion rate, adjusted for forearm volume of each subject, was 1 mL/min. For the present study, the maximal response to ACh and the area under the response curve to ACh were considered for statistical analysis.

### Indicators of Renal Function

Serum creatinine was measured in the routine laboratory by an automated technique based on the measurement of Jaffe chromogen and implemented in an auto-analyser. The glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) equation developed by Levey et al.² Standard creatinine clearance normalized to 1.73 m² was measured in a subset of 106 patients. Microalbuminuria was measured by a turbidimetric inhibition immunoassay (Boehringer Mannheim) in 164 patients. Patients who underwent creatinine clearance and microalbuminuria measurements were comparable to the whole study population in terms of demographic and anthropometric characteristics and risk factors listed in Table 1.

### CRP Measurements

CRP was measured by a high-sensitivity turbidimetric immunoassay (Behring) in a subset of 208 patients who were comparable to the whole study population with regard to the variables listed in Table 1.

### Statistical Analysis

Data are expressed as mean ± SD or as percent frequency, and comparisons between groups were made by 1-way ANOVA or the χ² test, as appropriate. Relationships between paired parameters were analyzed by Pearson product moment correlation coefficient.

To test the independent relationship between the response to ACh and renal function, we constructed multivariate models (either multiple linear or logistic regression) based on a series of traditional risk factors (age, gender, smoking, body mass index, systolic blood pressure, cholesterol, and serum glucose). Data are expressed as standardized regression coefficient (β) or as OR and 95% CI and probability value, as appropriate. All calculations were made with a standard statistical package (SPSS for Windows version 9.0.1).

### Results

All hypertensive patients showed a normal endothelium-independent vasodilation to SNP infusions (data non shown).

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### Table 1: Clinical and Biochemical Data of Patients Divided on the Basis of 3 Tertiles of Vasodilatory Response to ACh Infusion*

<table>
<thead>
<tr>
<th>Tertile</th>
<th>Maximal Vasodilatory Response to ACh Infusion</th>
<th>P</th>
<th>r (r²)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Age, y (³214%)</td>
<td>0.02</td>
<td>-0.17 (&lt;0.001)</td>
</tr>
<tr>
<td>II</td>
<td>Male gender, n (%)</td>
<td>0.53</td>
<td>-0.19 (&lt;0.001)</td>
</tr>
<tr>
<td>III</td>
<td>Body mass index, kg/m²</td>
<td>0.004</td>
<td>-0.15 (0.001)</td>
</tr>
<tr>
<td></td>
<td>Systolic blood pressure, mm Hg</td>
<td>0.35</td>
<td>-0.06 (0.21)</td>
</tr>
<tr>
<td></td>
<td>Diastolic blood pressure, mm Hg</td>
<td>0.47</td>
<td>-0.03 (0.49)</td>
</tr>
<tr>
<td></td>
<td>Pulse pressure, mm Hg</td>
<td>0.59</td>
<td>-0.05 (0.29)</td>
</tr>
<tr>
<td></td>
<td>Heart rate, bpm</td>
<td>0.96</td>
<td>0.04 (0.36)</td>
</tr>
<tr>
<td></td>
<td>Smokers, n (%)</td>
<td>0.53</td>
<td>-0.03 (0.57)</td>
</tr>
<tr>
<td></td>
<td>Cholesterol, mmol/L</td>
<td>0.31</td>
<td>0.02 (0.64)</td>
</tr>
<tr>
<td></td>
<td>Glucose, mmol/L</td>
<td>0.31</td>
<td>-0.10 (0.02)</td>
</tr>
</tbody>
</table>

*On the basis of maximal response to ACh, patients were ranked and grouped into 3 tertiles (first tertile: lower response; second tertile: intermediate response; third tertile: higher response).

†Correlation coefficient (r) and P value of relationship between maximal response to ACh (as a continuous variable) and each variable listed in the Table.

Data are expressed as mean ± SD or as percent frequency, and comparisons among groups were made by 1-way ANOVA or χ² test, as appropriate.
On the basis of the maximal response to ACh, patients were grouped into 3 tertiles (first tertile: lower response; second tertile: intermediate response; third tertile: higher response). As shown in Table 1, patients in the third tertile (ie, those displaying the higher vasodilatory response to ACh) were younger, with a lower preponderance of males and a lower body mass index than for patients in the other 2 tertiles. The 3 tertiles did not differ with regard to arterial pressure and heart rate, serum glucose and cholesterol, or the proportion of smokers.

Serum creatinine was significantly lower in patients in the third tertile than in those in the other 2 tertiles (Figure 1). Conversely, estimated GFR was progressively higher from the first to the third tertiles (Figure 1). Similar results were obtained when the 2 indicators of renal function were analyzed in relationship to the vasodilatory response to ACh as a continuous variable (creatinine-ACh \( r = -0.32, P < 0.001 \); estimated GFR-ACh \( r = 0.19, P < 0.001 \)) or in terms of area under the ACh curve (\( P < 0.001 \) by 1-way ANOVA). In contrast, serum creatinine and estimated GFR were identical when patients were grouped according to the maximal response to SNP (both \( P = \text{NS} \)). When the relationship between endothelial function and variables listed in Table 1 was tested with the maximal response to ACh as a continuous variable (Table 1, last column), the results did not differ materially from those based on ACh as a categorical variable except with regard to the ACh-glucose link. Creatinine clearance (\( r = 0.28, P = 0.003 \)), microalbuminuria (\( r = -0.17, P = 0.03 \)) and serum CRP (Figure 2) were all related to ACh. Serum CRP was significantly related to serum creatinine (\( r = 0.22, P = 0.001 \)) and to estimated GFR (Figure 2). Microalbuminuria was related to serum creatinine (\( r = 0.21, P = 0.008 \)) but not to estimated GFR (\( r = -0.07, P = 0.35 \)).

**Multivariate Analyses**

To test the independence of the associations between markers of renal function and ACh from other risk factors, we performed multiple regression analyses that included demographic and cardiovascular risk factors. As shown in Table 2, response to ACh ranked as the first correlate of both serum creatinine and estimated GFR.

To estimate the risk of moderate renal dysfunction (ie, estimated GFR < 60 mL \( \cdot \) \( \text{min}^{-1} \cdot \text{1.73 m}^2 \)) associated with an impaired response to ACh, we performed a multiple logistic regression analysis (Table 3). In this analysis, the risk of moderate renal dysfunction was 64% lower (OR 0.36, 95% CI 0.18 to 0.70) in patients in the third tertile (ie, those showing the higher vasodilatory response) than in those in the first tertile.

**Discussion**

In a large series of patients with uncomplicated, untreated essential hypertension and serum creatinine within the normal range, the vasodilatory response to ACh in the forearm was independently related to indicators of renal function (serum creatinine, creatinine clearance, and estimated GFR), and patients with more compromised vasodilatory responses were characterized by a substantial increase in the risk of moderate renal insufficiency. These associations suggest that mild to moderate renal insufficiency in patients with uncomplicated essential hypertension is affected in part by endothelial dysfunction, independent of arterial pressure.
TABLE 2. Multivariate Analysis of Indicators of Renal Function

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Serum Creatinine</th>
<th>Estimated GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>ACh</td>
<td>−0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>Male gender</td>
<td>0.04</td>
<td>0.38</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.03</td>
<td>0.54</td>
</tr>
<tr>
<td>Age</td>
<td>0.02</td>
<td>0.63</td>
</tr>
<tr>
<td>Glucose</td>
<td>−0.002</td>
<td>0.96</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>−0.005</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Data are expressed as standardized regression coefficients (β) and P values. Because age and gender are included in the formula for the calculation of estimated GFR, these 2 variables were not introduced in the estimated GFR model.

The kidney is a well-known target of hypertension, and there is now substantial evidence that renal dysfunction in patients with otherwise uncomplicated essential hypertension is associated with increased cardiovascular risk.1,2,5,11–14 Recent studies have shown that in patients with essential hypertension, the prognostic power of serum creatinine for adverse cardiovascular outcomes extends into the normal range.2 and that at such levels, a more marked age-dependent decline in the GFR is strongly associated with left ventricular concentric remodeling and hypertrophy.17 A creatinine level between 107 μmol/L (1.2 mg/dL) and 133 μmol/L (1.5 mg/dL) is included as a criterion for risk stratification in the European Society of Hypertension–European Society of Cardiology guidelines.18 and an estimated GFR ≤60 mL·min⁻¹·1.73 m⁻² is listed as a major risk factor in the guidelines of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure.19 Furthermore, even though creatinine is a less-than-ideal marker of the GFR, increased serum creatinine predicts end-stage renal disease, and recent guidelines by the American Kidney Foundation20 now recommend GFR estimates based on serum creatinine as a fundamental tool for risk stratification of kidney diseases.

The cause of mild renal dysfunction in essential hypertension is still incompletely understood. An intriguing hypothesis by Johnson et al? holds that patients with essential hypertension develop a subtle, acquired renal injury, mediated by transient renal vasoconstriction or by transient increases in blood pressure that eventually induce microvascular and tubulo-interstitial injury. Because a similar arteriolopathy can be induced in the rat by the NO inhibitor N²-nitro-¹-arginine methyl ester,21 it appears likely that the endothelium is a crucial target of this vasculopathy.

A reduced response to ACh has been documented in the forearm vasculature of patients with essential hypertension compared with healthy normotensive controls.11,12,21,22 Endothelial dysfunction is largely unrelated to arterial pressure levels in essential hypertension, and other risk factors, such as overweight, smoking, and hypercholesterolemia, may contribute to impair endothelial function when present in hypertensive patients.25 Interestingly, it is well demonstrated that endothelium-dependent vasodilatation in coronary and peripheral arteries is a closely related phenomenon,26 which indicates that endothelial dysfunction represents a systemic disorder involving both conduit arteries and small resistance vessels in the extremities. Studies of endothelial function in the kidney vasculature are laborious to perform, mainly because the stimulation and blockade of NO synthase by these substances have prolonged effects on the renal vasculature,27 and to date, there is no direct proof that endothelial dysfunction in the forearm or the coronary circulation is paralleled by a similar hemodynamic dysfunction in the kidney. However, the systemic nature of endothelial dysfunction,2 the parallelism between endothelial dysfunction in various vascular beds,26 and the fact that endothelial dysfunction in the forearm28 and in the coronary circulation29 is predictive of adverse cardiovascular outcomes plausibly suggest that endothelial dysfunction in the forearm is also paralleled by a similar alteration in renal vasculature. If so, the arterial pressure–independent link between the GFR and the vasodilatory response to ACh we found in the present study suggests that systemic endothelial dysfunction may be a mechanism contributing to mild renal dysfunction in essential hypertension. Endothelium-derived factors modulate not only vascular tone but also processes such as inflammation, cell proliferation, and fibrosis. A microinflammatory process signaled by serum CRP recently has been found to be associated with a subtle reduction in creatinine clearance in a large population study in Groeningen.30 Interestingly, in the present study, serum CRP was related inversely with the vasodilatory response to ACh and GFR. These associations suggest that endothelial dysfunction may be an intermediate mechanism mediating the effect of inflammation on renal function. Microalbuminuria is an important early marker of renal dysfunction, which is also considered an indicator of a generalized endothelial dysfunction.31 In the present study, microalbuminuria was related to the vasodilatory response to

TABLE 3. Multiple Logistic Regression Analysis of Renal Dysfunction (GFR <60 mL·min⁻¹·1.73 m⁻²)

<table>
<thead>
<tr>
<th>Units of Increase</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response to ACh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile I</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tertile II</td>
<td>0.68 (0.38–1.22)</td>
<td>0.20</td>
</tr>
<tr>
<td>Tertile III</td>
<td>0.36 (0.18–0.70)</td>
<td>0.003</td>
</tr>
<tr>
<td>BMI</td>
<td>1 kg/m²</td>
<td></td>
</tr>
<tr>
<td>1.09 (1.02–1.17)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1 mmol/L</td>
<td></td>
</tr>
<tr>
<td>1.16 (0.85–1.58)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Systolic pressure</td>
<td>10 mm Hg</td>
<td></td>
</tr>
<tr>
<td>0.96 (0.82–1.11)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>1 mmol/L</td>
<td></td>
</tr>
<tr>
<td>0.89 (0.58–1.37)</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0=no; 1=yes</td>
<td>1.18 (0.60–2.32)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Seventy-three (15%) of 500 patients had an estimated GFR <60 mL·min⁻¹·1.73 m⁻².

*Reference group.

Because age and gender are included in the formula for the calculation of estimated GFR, these 2 variables were not introduced in the multiple logistic regression analysis.
ACh, but similar to a recent study in patients with essential hypertension, it was unrelated to the GFR.

The present study has limitations. First, we adopted a cross-sectional design that does not enable us to establish the direction of causality, and therefore our observations remain to be confirmed in prospective observational and interventional studies. Second, ours is a tertiary referral center, and therefore patients enrolled in this survey represent a selected population not representative of primary care. Third, we estimated the GFR by the MDRD formula. Although of recognized validity in epidemiological studies and in the screening of renal diseases, this formula is based on age and gender, ie, 2 factors that may confound the relationship between GFR and the vasodilatory response to ACh. This possibility is suggested by the fact that the link between estimated GFR and such a response was weaker than that between serum creatinine or creatinine clearance and the same outcome measure. Finally, for logistical reasons and because of the methodological uncertainties that still surround the interpretation of the renal vasodilatory response to NO agonists and antagonists, we did not study renal endothelial function directly.

In conclusion, an impaired vasodilatory response to ACh appears to be a risk marker for renal failure loss in patients with essential hypertension. Furthermore, high serum CRP is associated with an impaired vasodilatory response to ACh and renal function impairment in these patients. Further studies are warranted to clarify the nature of these links that may lead to renal dysfunction, ie, to a crucial cardiovascular risk factor in essential hypertension.

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References

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