Transvascular and Urinary Leakage of Albumin in Atherosclerotic and Hypertensive Men

Roberto Pedrinelli, Giuseppe Penno, Giulia Dell’Omo, Simona Bandinelli, Davide Giorgi, Vitantonio Di Bello, Monica Nannipieri, Renzo Navalesi, Mario Mariani

Abstract—Increased urine albumin is associated with atherosclerotic disease and predicts cardiovascular morbidity and mortality in nondiabetic populations. This finding is frequently postulated to reflect the impact of atherosclerotic damage on glomerular and systemic capillary permeability, an interesting but as yet untested hypothesis. The transcapillary escape rate of albumin (TERalb, the 1-hour decline rate of intravenous 125I-albumin, a measure of capillary macromolecular permeability), albuminuria, lipid levels, echocardiographic wall thickness, and insulin responses to oral glucose were measured in 30 untreated dipstick-negative lean men and clinically stable atherosclerotic peripheral vascular disease; tolerance to oral glucose was a requirement for inclusion in the study. Because hypertension per se might influence TERalb, the sample included either normotensive (n=18, 118±6/72±7 mm Hg) or hypertensive (n=12, 141±7/84±6 mm Hg by 24-hour blood pressure monitoring) arteriopathic patients; 11 normal age- and gender-matched subjects (121±7/76±5 mm Hg) were used as control subjects. TERalb was higher in patients (10.7±3.2 versus 7.4±1.7%/h, P<0.013), a difference that persisted after postload glucose, insulin, and lipid levels were accounted for by covariance analysis; atherosclerosis and hypertension together did not further impair vascular permeation to albumin. In contrast with TERalb, albuminuria was elevated only in the hypertensive subgroup; the 2 variables showed no relationship, even when the data were analyzed separately in normotensive and hypertensive subgroups. Urine albumin correlated positively with 24-hour blood pressure and wall thickness. Thus, systemic capillary permeability is altered in nondiabetic atherosclerotic patients independently from blood pressure levels, but this abnormality is not reflected by proportionate changes in albuminuria. (Hypertension. 1998;32:318-323.)

Key Words: capillary permeability ■ albuminuria ■ atherosclerosis ■ hypertension, essential ■ vascular diseases

Minute increments in UAE (microalbuminuria) are associated with greater prevalence of atherosclerotic vascular disease and predict all-cause and coronary disease morbidity and mortality not only in subjects with type 1 and type 2 diabetes (see References 1 and 2 for recent data) but also unselected populations, as well as essential hypertensive patients. Thus, albuminuria may be a marker of generalized disease in the vascular wall, but the precise reasons for this relationship remain elusive. It is possible that the glomerular albumin leak reflects a widespread atherosclerotic-mediated capillary vasculopathy affecting extrarenal organs. This frequently quoted speculation seems valid for type 1 and type 2 diabetic patients; whether it applies also to nondiabetic subjects is still unclear. For this reason, we measured the TERalb (the fraction of the intravascular mass of albumin going through the vascular bed per unit of time, a parameter that estimates the integrity of systemic capillary permeability) and UAE in glucose-tolerant patients with atherosclerotic PVD. Because elevated BP may influence both UAE and TERalb, we stratified our sample according to the presence of normotension or hypertension.

Subjects
All subjects were white men. The patient group was composed of 30 patients with atherosclerotic PVD and stable intermittent claudication (pain-free walking distance >200 m on a treadmill). Angiography showed typical iliac and/or femoral atherosclerotic lesions (diffuse plaques causing stenosis and/or occlusion at arterial branch points) in all patients combined with crural alterations in some. Subjects had fasting blood glucose levels <6.7 mmol/L (120 mg/dL), HbA<sub>c</sub> <6%, and normal oral glucose tolerance (2-hour post–oral glucose load <7.8 mmol/L, 140 mg/dL). Serum creatinine was <110 μmol/L (1.2 mg/dL) and total serum cholesterol <7.8 mmol/L (300 mg/dL); there was normal urinary sediment, no urinary tract infection, BMI <30 kg/m<sup>2</sup>, and no evidence or history of congestive heart failure, advanced chronic obstructive pulmonary disease, previous amputation, pain at rest, or ischemic trophic ulcers or gangrene. Eighteen patients were normotensive on the basis of several normal casual BP determinations confirmed by 24-hour ABPM (<130/80 mm Hg<sup>24h</sup>) in the absence of antihypertensive treatment, and 12 mild-moderate hypertensive subjects were diagnosed on the basis of repeated casual BP determinations >140/90 but <160/110 mm Hg as outpatients. Angiograms had shown normal renal arteries, and renal ultrasound scanning showed normal-sized kidneys and no evidence of cortical scarring or obstructive uropathy;
routine clinical and hematological examinations excluded other secondary forms of hypertension. Hypertensive patients (n=6 never-treated) were studied after 2-week drug withdrawal (calcium channel blockers, angiotensin-converting enzyme inhibitors, or both). No patient had ever taken lipid-lowering drugs, and all had received either ticlopidine or aspirin. Eleven subjects with normal findings for physical examination, routine blood and urinary tests, BP, ECG, abdominal echography, and ankle/brachial index were the control subjects. Experimental evaluations were completed in a 2-week period. A consistent portion of our patients could not offer reliable data regarding family history of hypertension and diabetes; therefore, we did not pursue this issue any longer.

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

TERalb
TERalb was measured between 2 and 4 PM after a 4- to 6-hour fast and no tea, coffee, alcohol, or tobacco from the early morning. After subjects rested for 30 minutes in the supine position,125 I-labeled human serum albumin (6 to 8 Ci, 222 to 296 kBq, SARI-125 A-2; SORIN Biomedica) was injected as a bolus, and blood was withdrawn from the contralateral arm every 5 minutes during the hour following the injection. Radiiodinated albumin was freshly purified from free 125I, eluting the high-molecular-weight components into columns containing Sephadex G-25 mol/L (column PD-10; Pharma- cia); free 125I content in the injected dose was <1%. Radioactivity was measured (Cobra 5000 gamma counter; Hewlett-Packard) in duplicate in whole blood samples centrifuged for 10 minutes at 3000g. Counting time was 40 minutes with a percentage of error <20%; hematocrit level (Coulter Counter 55; Coulter Electronics) was determined in each sample. Serum albumin was measured by immunonephelometry (Behring Laser Nephelometer System; interassay variation coefficient, 5.2%).

Urinary Albumin Excretion

UAE was measured by nephelometry (Behring; limit of detection: 0.1 mg/dL; interassay variation coefficient: 3.5%). To minimize the confounding influence of daily physical activity and to facilitate the procedure, urine was collected from 8 PM to 8 AM during 3 consecutive days.

24-Hour ABPM and Cardiovascular and Renal Function Parameters

ABPM (24-hour) was performed using an oscillometric monitor (SpaceLabs 90207, SpaceLabs) on a regular work day. Recording began between 8:30 and 9 AM, with readings every 15 minutes until midnight and every 30 minutes from midnight to 8 AM.

Ankle-brachial index (the ratio between systolic BP measured at the brachial and bilateral posterior tibial artery) was measured by Doppler (Stereodop, Promeloc).

Wall thickness and chamber volumes were measured by monodimensional and bidimensional echocardiograms (Hewlett-Packard Sonos 1000) with 2.5- and 3.5-MHz transducers.

Selected Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPM</td>
<td>ambulatory blood pressure monitoring</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>blood pressure</td>
</tr>
<tr>
<td>CholHDL</td>
<td>high-density-lipoprotein cholesterol</td>
</tr>
<tr>
<td>CholLDL</td>
<td>low-density-lipoprotein cholesterol</td>
</tr>
<tr>
<td>CholTOT</td>
<td>total cholesterol</td>
</tr>
<tr>
<td>HbA1C</td>
<td>glycated hemoglobin</td>
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<tr>
<td>PVD</td>
<td>peripheral vascular disease</td>
</tr>
<tr>
<td>UAE</td>
<td>urinary albumin excretion</td>
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</table>

Serum and urine (the same samples used for UAE determination) creatinine levels were assayed by standard colorimetric methods.

Metabolic Parameters

Anthropometric measurements (height and weight) were made after each participant had removed his shoes and upper garments. Blood samples were obtained between 8 and 9 AM after an overnight fast and 15 minutes of supine rest. A glucose tolerance test was performed in the morning with a 75-g glucose load. Individuals were asked to fast for 12 to 14 hours before the test, and specimens for plasma glucose and insulin were drawn basally and at 0.5, 1, 1.5, and 2 hours after administration of the glucose load. Plasma glucose was measured by the gluco-oxidase method, and plasma insulin by radioimmunoassay (Biosource, no cross-reactivity with human pro-insulin). CholHDL, CholLDL, and triglyceride levels were assessed by enzymatic colorimetric techniques (Boehringer-Mannheim); CholHDL was calculated as [CholHDL + (CholHDL + triglyceride)/5].

Data Analysis

Plasma 125I-albumin concentration (cpm/mL) was plotted on a semilogarithmic scale, and the transcapillary escape rate (%/h) was calculated from the monoeponential disappearance rate constant of the 125I curve from 10 to 60 minutes.13 UAE (µg/min) was the average of 3 consecutive collections (mean variation coefficient of triplicate measurements, 24%). According to standard criteria, microalbuminuria was defined as a value between 20 and 200 µg/min. ABPM values were the mean of the overall 24-hour readings after artifact editing. Plasma volume (mL/1.73 m2) was determined by extrapolation to zero time of the disappearance curve corrected for the injected dose of tracer obtained by weighing the syringes before and after the injection. Two-hour area under the curve of postload plasma insulin and glucose was calculated by the trapezoidal rule. BMI (body weight/surface area, squared) and creatinine clearance (mean variation coefficient of triplicate measurements, 17%) were derived according to standard formulas.

Statistics

Log transformation was applied to TERalb, UAE, plasma insulin, and triglycerides because the raw data were not distributed normally. Descriptive statistics were mean±SD or medians and ranges for skewed data. Differences among means were tested by 1-way ANOVA, correcting for potential confounders by ANCOVA. A multiple range test was used to evaluate differences between means. Intraindividual association of variables in hypertensive subjects was tested by Pearson’s correlation coefficients. Statistical significance was set at P<0.05. Calculations were performed using Statgraphics Plus (Manugistic Inc, release 1997).

Results

Age and prevalence of current smokers did not differ among the 3 groups; 24-hour ABPM in normotensive PVD patients was closely comparable to that of normal subjects and higher in hypertensive arteriopathic patients, as expected. Ankle-brachial index, a measure of hemodynamic severity,17 was similarly reduced in patients. Myocardial walls were thicker in hypertensive subjects; cardiac and renal function was normal (Table 1).

Atherogenic lipid levels were elevated in patients; levels of CholHDL and HbA1C did not differ (Table 2).

Patients showed hyperinsulinemia and hyperglycemia after oral glucose, a trend more evident in hypertensive subjects, who also showed greater BMI (P<0.08) (Table 2).

TERalb
TERalb was higher (10.7±3.2 versus 7.4±1.7%/h, P<0.013) in atherosclerotic patients. The difference persisted after accounting for BP, postload glucose and insulin, lipid level,
Urinary Albumin Excretion

and UAE level by ANCOVA (Table 3). Serum albumin concentration, hematocrit level, and plasma volume were comparable in the 3 groups (Table 2).

TERaLb values did not differ between normotensive and hypertensive patients (Figure 1, left).

Urinary Albumin Excretion

UAE was similar in normotensive arteriopathic patients and control subjects and was elevated (P<0.004) in the hypertensives (Figure 1, right), in whom microalbuminuria was present in 5 subjects (median UAE, 125 μg/min; range, 26 to 198 μg/min).

Table 2. Levels of Lipid, Glucose, Insulin, Plasma Volume, Hematocrit, and Serum Albumin in Controls and Normotensive and Hypertensive Atherosclerotic Patients

<table>
<thead>
<tr>
<th>Evaluation Variables</th>
<th>Controls</th>
<th>NT+ATH</th>
<th>EH+ATH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CholLDL, mmol/L</td>
<td>5.3±1.2</td>
<td>6.1±0.8*</td>
<td>6.2±1.1*</td>
</tr>
<tr>
<td>CholHDL, mmol/L</td>
<td>3.6±1</td>
<td>4.2±0.8*</td>
<td>4.3±0.9*</td>
</tr>
<tr>
<td>Chol tot, mmol/L</td>
<td>1.07±0.1</td>
<td>1.02±0.3</td>
<td>0.95±0.2</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.27 (0.6–3.5)</td>
<td>1.53 (0.9–4.1)*</td>
<td>1.66 (1.1–5.4)*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.3±0.5</td>
<td>5.4±0.3</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.6±0.6</td>
<td>5.0±0.3</td>
<td>5.2±0.8</td>
</tr>
<tr>
<td>AUC glucose, (mmol/L×2h⁻¹)×10³</td>
<td>7.7±1.7</td>
<td>8.7±1.5</td>
<td>9.5±1.2*</td>
</tr>
<tr>
<td>Fasting insulin, pmol/L</td>
<td>56 (34–102)</td>
<td>58 (31–178)</td>
<td>81 (36–161)</td>
</tr>
<tr>
<td>AUC insulin, mmol/L×2h⁻¹</td>
<td>4.2 (2.5–10.9)</td>
<td>7.4 (3.2–18.2)*</td>
<td>10 (3.9–20.3)*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4±2</td>
<td>25.2±2.7</td>
<td>27.1±1.4</td>
</tr>
<tr>
<td>Plasma volume, mL/1.73 m²</td>
<td>3310±781</td>
<td>3284±633</td>
<td>3189±697</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>35.6±7.8</td>
<td>38±4.4</td>
<td>37.6±3.7</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42±1.8</td>
<td>44±3</td>
<td>43±3.5</td>
</tr>
</tbody>
</table>

Values are mean±SD or median (range). NT+ATH indicates normotensive with atherosclerosis; EH+ATH, essential hypertensive with atherosclerosis; and AUC, area under the curve.

*P<0.05, †P<0.01, ‡P<0.001 vs controls.

Table 3. ANCOVA For TERaLb

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F Ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covariates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABPM, systolic</td>
<td>0.022</td>
<td>1</td>
<td>0.022</td>
<td>1.26</td>
<td>&lt;0.27</td>
</tr>
<tr>
<td>ABPM, diastolic</td>
<td>0.035</td>
<td>1</td>
<td>0.035</td>
<td>2.00</td>
<td>&lt;0.17</td>
</tr>
<tr>
<td>AUC glucose</td>
<td>0.022</td>
<td>1</td>
<td>0.022</td>
<td>1.29</td>
<td>&lt;0.26</td>
</tr>
<tr>
<td>AUC insulin</td>
<td>0.0046</td>
<td>1</td>
<td>0.0046</td>
<td>0.26</td>
<td>&lt;0.61</td>
</tr>
<tr>
<td>CholLDL</td>
<td>0.026</td>
<td>1</td>
<td>0.026</td>
<td>1.52</td>
<td>&lt;0.23</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.0027</td>
<td>1</td>
<td>0.0027</td>
<td>0.16</td>
<td>&lt;0.69</td>
</tr>
<tr>
<td>UAE</td>
<td>0.0018</td>
<td>1</td>
<td>0.0018</td>
<td>0.10</td>
<td>&lt;0.75</td>
</tr>
<tr>
<td>Vascular status*</td>
<td>0.16</td>
<td>2</td>
<td>0.08</td>
<td>4.62</td>
<td>&lt;0.018</td>
</tr>
<tr>
<td>Residual</td>
<td>0.55</td>
<td>31</td>
<td>0.017</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AUC indicates area under the curve.

TERaLb in atherosclerotic vs control subjects.

Correlations

TERaLb and UAE values were unrelated (Figure 2). The same negative result was obtained in the 2 patient subgroups (normotensive arteriopathic patients: r = −0.14, P < 0.57; hypertensive arteriopathic patients: r = −0.37, P < 0.24) when analyzed separately.

TERaLb did not correlate (n = 41) with any of the numeric parameters listed in Tables 1 and 2, including systolic (r = 0.14, P < 0.4) and diastolic (r = −0.08, P < 0.5) ABPM. In the same set of data, UAE correlated directly with values for ABPM (systolic: r = 0.50, P < 0.0001; diastolic: r = 0.53, P < 0.0001) and wall thickness (interventricular septum thickness: r = 0.50, P < 0.0008; posterior wall thickness: r = 0.44, P < 0.004).

Discussion

The two main and original results of this cross-sectional case-control study carried out in glucose-tolerant men with
normal renal function and comparable prevalence of active smokers were (1) increased TERalb in atherosclerotic patients compared with normal age-matched control subjects and (2) dissociated behavior of UAE and TERalb.

**Increased TERalb in Atherosclerotic Patients**

Our data identify a defect that is likely localized at the capillary level where most of the albumin permeation takes place, although the contribution to this increase from various organs and tissues with different permeability cannot be identified precisely. This result confirms the existence of a systemic microvascular involvement in atherosclerotic PVD, an issue approached by other investigators by evaluating vasorelaxant potential of resistance and conduit lower limb arteries, both scarcely representative of the most distal segments of the systemic microcirculation. Increased transmural pressure difference, increased area of the microcirculatory bed, and increased microvascular permeability. The first factor was unlikely to play any major role because the abnormal microvascular albumin leakage characterized patients with normal BP, in whom preserved cardiac function also allowed the exclusion of an influence of increased postcapillary resistance. Quite surprisingly in the light of previous results, hypertension combined with atherosclerosis was not associated with further additional increments in capillary permeability. However, ex post facto calculation of statistical power showed that our sample size (n=12 hypertensive versus n=18 normotensive) had the statistical power to safely exclude (β<0.20) only differences ≥3%/h. Thus, the negative result could be due to insufficient statistical power, but other explanations are conceivable. For example, TERalb may have a ceiling or be sensitive only to frankly elevated pressor regimens as opposed to the mild-moderate values present in our hypertensive patients. Type of previous antihypertensive medication and/or length of treatment might also affect the transvascular leakage of albumin, but we cannot deny or support any of the above possibilities. Increased microcirculatory area due to opening of nonperfused capillaries could theoretically increase TERalb, a hypothesis not to be refuted a priori, even though the data on capillary rarefaction in hypertension suggest the opposite inference, if anything. Overall, exaggerated capillary permeability seems a more likely explanation for the increased transvascular albumin leakage shown in this group of atherosclerotic patients. Changed permeability of the vascular wall due to reduced concentration of anionic glycosaminoglycans is possible, and altered permeability due to systemic atherosclerotic endothelial dysfunction is also conceivable. Yet, the available evidence on this topic has been gathered mainly through evaluation of vasorelaxation to nitric oxide–releasing drugs; how TERalb relates to it is unknown. As reported previously, no significant correlation was found with total and LDL cholesterol levels, suggesting that lipids may not influence capillary permeation of albumin. We also evaluated the insulinemic response to oral glucose, since insulin may play a role in the transfer of macromolecules from the blood to the extracellular space after food intake. Endogenous insulin levels did not appear to influence TERalb, in indirect agreement with the ineffectiveness of exogenous infusion of the hormone on vascular permeability. It was also of interest that the emerging pattern of hyperinsulinemia combined with slight, albeit still normal, hyperglycemia in our arteriopathic patients confirmed previous data suggestive of some defect in glucose disposal in this clinical condition.

**Dissociated Behavior of UAE and TERalb**

Coexistence of normal UAE with elevated TERalb and lack of any intraindividual relationship between the 2 parameters does not support the concept based on inferential evidence of increased urine albumin as a direct reflection of altered
systemic microvascular permeability, a conclusion limited to permeability quantified through TER alb determination and to nondiabetic patients with atherosclerotic PVD. On the basis of these data, one is led to postulate an indirect link between microalbuminuria and atherosclerosis through adverse changes in cardiovascular risk factors. As a matter of fact, microalbuminuria was found only in the hypertensive components of this sample, and in this as well as our previous studies, UT UAE correlated positively with both 24-hour ABPM and wall thickness, a long-term sensor of afterload. Inappropriate matching for BP levels might explain why Jensen found parallel elevation in UAE and TER alb in his pooled series of normotensive and hypertensive patients with severe atherosclerotic vascular disease. Furthermore, exaggerated insulin response and lower tolerance to glucose load (in itself suggestive of a greater insulin resistance) did characterize our hypertensive arteriopathic patients in whom microalbuminuria was also highly prevalent. This behavior may suggest that, as in non–insulin-dependent diabetic subjects, renal excretion of albumin increases for still-unclear reasons in the presence of greater degrees of insulin resistance. However, coexisting hypertension and insulin resistance could not account for the association of microalbuminuria with carotid atherosclerosis in recent studies. Furthermore, the systemic implications of albuminuria are underlined by accruing evidence showing independent links between UAE and vascular disease in nondiabetic subjects. Several possibilities can be imagined to reconcile the initial working hypothesis with our negative results. Due to the complex regulation of intraglomerular hemodynamics, an abnormal permeability might still be compensated for by other control mechanisms, such as modulation of afferent and efferent arteriolar tone and/or modifications of the mesangial cell contractile tone. Thus, microalbuminuria could identify only those more advanced systemic vasculopathies bound to trigger new clinical events, while TER alb could be sensitive even to milder, more stable forms of disease. This assumption is reasonable because the mutual relationships and the exact interpretation of the different indices of vascular damage are still undetermined and most likely not uniform. For example, the transvascular leakage of albumin, a variable of still-unknown prognostic power, was increased in non–insulin-dependent diabetics both with and without nephropathy. On the other hand, von Willebrand factor level, a recognized predictor of cardiovascular events and a circulating marker of endothelial dysfunction, was elevated only in microalbuminuric subjects with either essential hypertension or diabetes. Second, abnormalities in TER alb and UAE might react with different time rates in response to the development of microvascular atherosclerotic damage; our patients were recruited cross-sectionally at unknown but most likely variable points of the individual clinical course. Third, albuminuria and TER alb might identify different coexisting kinds of systemic vascular impairment such as endothelial dysfunction versus abnormal macromolecular permeability of the extracellular matrix for anionic proteins, respectively, a problem never addressed so far. In conclusion, systemic capillary permeability is altered in nondiabetic atherosclerotic patients independently from BP levels, but this abnormality is not reflected by proportionate changes in albuminuria. However, more studies are needed to understand in full the mechanisms that connect microalbuminuria and vascular disease.

Acknowledgment
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References


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