Elevated Urinary Albumin Excretion Is Associated With Impaired Arterial Dilatory Capacity in Clinically Healthy Subjects

P. Clausen, MD, PhD; J.S. Jensen, MD, PhD, DMSc; G. Jensen, MD, DMSc; K. Borch-Johnsen, MD, DMSc; B. Feldt-Rasmussen, MD, DMSc

Background—Elevated urinary albumin excretion (UAE) predicts atherosclerotic cardiovascular disease. It is hypothesized that elevated UAE is associated with a generalized vascular dysfunction. This study tested this hypothesis for conduit arteries.

Methods and Results—Clinically healthy subjects were selected: 19 with UAE >90th percentile in the background population (6.6 µg/min < UAE < 150 µg/min) and 41 with normoalbuminuria (UAE < 6.6 µg/min). External ultrasound was used to measure the dilatory response of the brachial artery to postischemic increased blood flow (endothelium-dependent, flow-associated dilation) and to nitroglycerin (endothelium-independent, nitroglycerin-induced dilation). Plasma concentrations of the endothelial markers nitrate/nitrite, thrombomodulin, and von Willebrand factor antigen were also measured. Both flow-associated and nitroglycerin-induced dilations were significantly impaired in subjects with elevated UAE as compared with normoalbuminuric control subjects: 104.3 ± 0.6% (mean ± SEM) versus 104.3 ± 0.6% (P < 0.05) and 120.1 ± 1.5% versus 123.8 ± 1.0% (P < 0.05). No differences in the plasma concentrations of endothelial markers were found.

Conclusions—Slightly elevated UAE is associated with impaired conduit arterial dilatory capacity in clinically healthy subjects, and this impairment may be explained by a reduced dilatory response to nitric oxide of both endogenous and exogenous origin. Impaired arterial dilatory capacity may contribute to the increased cardiovascular risk in subjects with elevated UAE. (Circulation. 2001;103:1869-1874.)

Key Words: atherosclerosis ■ risk factors ■ endothelium ■ vasodilation ■ nitric oxide ■ nitroglycerin

Elevated urinary albumin excretion (UAE) is associated with increased risk of cardiovascular atherosclerotic disease in nondiabetic subjects1 as well as in type 1 and type 2 diabetic patients.2,3 The pathophysiological mechanism underlying this association is not fully understood. A higher burden of classic cardiovascular risk factors such as elevated blood pressure4 and dyslipidemia5 in subjects with elevated UAE does not appear to be of a magnitude that alone would explain the impact of elevated UAE on cardiovascular risk.

Subjects with elevated UAE are characterized by the loss of glomerular charge selectivity and size selectivity6,7 and an increased transcapillary escape rate of albumin (TER,ab).7,8 These findings have led to the hypothesis that elevated UAE is associated with a vascular dysfunction not only in the glomerulus but in the entire vascular tree.7,8 This vascular dysfunction could be predominantly of an endothelial nature, because microalbuminuria (15 µg/min < UAE < 150 µg/min) in type 1 diabetic patients is associated with elevated serum concentrations of von Willebrand factor antigen (vWFa),9 a potential marker of endothelial dysfunction.10 However, the origin of vWFa and other potential markers of endothelial dysfunction is not limited to the conduit arterial vasculature affected by atherosclerosis. Similarly, TER,ab reflects the permeability of the entire vascular tree, including the microvasculature, and not only of the conduit arteries.

To clarify whether elevated UAE is associated with conduit arterial vascular dysfunction, the dilatory capacity of the brachial artery was measured in two selected groups of clinically healthy subjects, one group with elevated UAE and the other with normoalbuminuria, by a noninvasive ultrasound method as established by Celermajer et al.11 In addition, measurements were made of the plasma concentrations of nitrate/nitrite (NOx) stable end products of nitric oxide (NO) and of other potential markers of endothelial dysfunction: thrombomodulin, the endothelial receptor for thrombin, and vWFa.

Methods

Study Population
Of 7089 participants (30 to 70 years of age) in the Copenhagen City Heart Study of 1992 to 1994, 3645 collected a timed, overnight...
TABLE 1. Characteristics of Clinically Healthy Subjects With Elevated UAE and Normoalbuminuria

<table>
<thead>
<tr>
<th></th>
<th>Normoalbuminuria, n=41</th>
<th>Elevated UAE, n=19</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>UAE, μg/min</td>
<td>2.1 (0.7–6.3)</td>
<td>9.9 (7.0–89.0)</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>59±1</td>
<td>59±2</td>
<td>0.84</td>
</tr>
<tr>
<td>Sex, m/f</td>
<td>22/19</td>
<td>11/8</td>
<td>0.74</td>
</tr>
<tr>
<td>Brachial artery diameter, mm</td>
<td>3.5±0.1</td>
<td>3.5±0.1</td>
<td>0.94</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8±0.7</td>
<td>26.4±1.0</td>
<td>0.64</td>
</tr>
<tr>
<td>HbA₁₀, %</td>
<td>5.2±0.1</td>
<td>5.2±0.1</td>
<td>0.99</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/L</td>
<td>4.7 (4.1–6.3)</td>
<td>4.7 (4.4–6.9)</td>
<td>0.28</td>
</tr>
<tr>
<td>s-Creatinine, μmol/L</td>
<td>89 (67–111)</td>
<td>83 (66–109)</td>
<td>0.23</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.4±0.2</td>
<td>6.5±0.4</td>
<td>0.59</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.1±0.1</td>
<td>4.3±0.4</td>
<td>0.46</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.7±0.1</td>
<td>1.6±0.1</td>
<td>0.49</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.03 (0.39–3.74)</td>
<td>1.13 (0.37–3.54)</td>
<td>0.96</td>
</tr>
<tr>
<td>Smokers/nonsmokers</td>
<td>23/18</td>
<td>9/10</td>
<td>0.59</td>
</tr>
<tr>
<td>Present cigarette consumption of smokers, cigarettes/d</td>
<td>10 (1–35)</td>
<td>15 (1–35)</td>
<td>0.95</td>
</tr>
<tr>
<td>Lifetime cigarette consumption of all participants, pack-years</td>
<td>19 (0–61)</td>
<td>18 (0–53)</td>
<td>0.90</td>
</tr>
<tr>
<td>Alcohol intake, drinks/d</td>
<td>2 (0–15)</td>
<td>3 (0–6)</td>
<td>0.45</td>
</tr>
<tr>
<td>Lifetime alcohol intake, drink-years</td>
<td>60 (4–319)</td>
<td>84 (3–234)</td>
<td>0.43</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118±1</td>
<td>129±3</td>
<td>0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>76±1</td>
<td>79±2</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Values are mean±SEM or median (range).
pulsed-wave Doppler signal at a 70 degree angle to the vessel with the range gate (1.5 mm) in the center of the artery at rest and at the maximum within the first 15 seconds after cuff release. Volume blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle) by the heart rate and the vessel cross-sectional area (πr²).

In normal subjects, the increased flow after cuff release results in a flow-associated dilatation (FAD) of the artery, probably as a result of release of NO induced by shear stress, and the dilation can be blocked by the NO synthase inhibitor G-monomethyl-L-arginine. FAD has been shown to be closely correlated to concurrent measurements of the dilatory response of coronary arteries to invasive application of the endothelium-dependent dilator acetylcholine. Administration of the exogenous NO donor NTG results in an NTG-induced dilation (NID) through its effect on the vascular smooth muscle cells, and this dilation is independent of the endothelium.

This method of measuring conduit arterial dilatory capacity, both endothelium-dependent and endothelium-independent, can produce accurate and reproducible results. In our laboratory, the coefficients of variation for FAD and NID were 3.6% and 3.9%, respectively, for 9 scans of the same subject performed on separate days within a period of 2 weeks.

Diastolic (Korotkoff phase V) and systolic blood pressures were measured in duplicate with a standard mercury sphygmomanometer and an appropriately sized cuff after at least 15 minutes of supine rest. A mean of the two readings was taken as office blood pressure.

As part of the grouping procedure, urinary albumin concentration (UAC) was measured by an ELISA technique as previously described. The intra-assay and interassay coefficients of variation were both 5.5%. UAE was calculated from urinary volume, UAC, and self-reported length of the overnight urine collection time period.

Venous blood samples were drawn without stasis. Plasma NOx was measured with a 2-step commercial assay kit (R&D). The first step was the conversion of nitrate to nitrite by nitrate reductase. The second step was the addition of the Griess reagents to convert a colored azo compound measured by photometric absorbance. All samples were analyzed within the same assay. The intra-assay coefficient of variation was 5.2% (n=8). Plasma thrombomodulin was measured with a commercial ELISA assay kit (Diagnostics Stago). All samples were analyzed within the same assay. The intra-assay coefficient of variation was 4.8% (n=8). Plasma vWFa concentration was measured by an in-house ELISA method with rabbit polyclonal antibody against human vWFa. The samples were analyzed consecutively in different assays. The interassay coefficient of variation was 6.4% (n=14). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured by enzymatic colorimetric methods (Boehringer Mannheim). LDL cholesterol concentration was calculated by Friedewald’s formula. HbA1c was measured with a Hitachi autoanalyzer by a combined turbidimetric and photometric method. Nondiabetic reference interval for HbA1c is 4.2% to 6.3%. The plasma concentrations of albumin, creatinine, potassium, and sodium, the serum concentration of thyroid-stimulating hormone, the blood concentrations of hemoglobin and glucose, and the leukocyte and thrombocyte counts were all measured by standard laboratory methods.

Height and weight were recorded and body mass index (BMI) was calculated (weight/height²).

Information was recorded regarding present and former smoking and drinking habits. Study subjects were classified as smokers and nonsmokers, and for each subject, an estimate of present cigarette consumption and of lifetime cigarette consumption was made. The number of “pack-years” (20 cigarettes/d in 1 year) was used for lifetime cigarette consumption. Only a few subjects had no alcohol intake, and estimates of present alcohol intake and lifetime intake (1 drink/d in 1 year = 1 “drink-year”) were made.

Statistical Analysis

Normally distributed continuous variables are given as mean±SEM and nonnormally distributed continuous variables as medians (range). Comparisons of continuous variables between groups were done by unpaired Student’s t test or Mann-Whitney U test for normally and nonnormally distributed variables, respectively. Comparisons of arterial diameters in different stages of the scan protocol were done by paired Student’s t test. Fisher’s exact tests were used to compare distribution of categorical variables. ANCOVA tests were performed to incorporate differences in covariates between the groups in the analyses. Correlations were sought by simple linear regression analyses. Nonnormally distributed variables were logarithmically transformed before inclusion in regression analyses. A probability value of <0.05 was considered significant.

Results

Both FAD and NID were significantly impaired in subjects with elevated UAE (Figure 1 and Figure 2).

The baseline resting arterial diameter, the diameter during ischemia, and the diameter before the administration of NTG were identical in both groups (Table 2).

FAD and NID were closely associated in subjects with normalalbuminuria (r=0.49, P=0.001, FAD=71.8±0.26×NID), and although the association was not significant in the smaller number of subjects with elevated UAE, the regression line was identical (r=0.40, P=0.10, FAD=73.2 ± 0.24×NID), as was the regression
line in the combined material (r=0.49, P=0.00008, FAD=69.9+0.27×NID). No significant difference in the maximum flow increase after deflation of the cuff was found between the two groups: 445% (183% to 942%) versus 332% (150% to 1052%) (P=0.10).

No subject had evidence of atherosclerotic plaques in the brachial artery on ultrasound scanning.

No differences were found between control subjects and subjects with elevated UAE in the plasma concentrations of NOx (25 [14 to 47] µmol/L versus 25 [16 to 177] µmol/L, P=0.20), vWFa (1.07±0.06 IU/mL versus 1.00±0.07 IU/mL, P=0.50), or thrombomodulin (43 [28 to 93] µg/mL versus 40 [28 to 74] µg/mL, P=0.58).

No differences were found between the groups with regard to age, sex, baseline brachial arterial diameter, BMI, HbA1c, blood glucose, serum creatinine, serum lipids, or smoking and drinking habits (Table 1). Nor were there any differences in hemoglobin, leukocyte or thrombocyte counts, urea, potassium, sodium, or thyroid-stimulating hormone (data not shown). Systolic blood pressure was slightly but significantly higher in subjects with elevated UAE. Diastolic blood pressure was also higher in subjects with elevated UAE, but the difference was not significant (Table 1). If systolic blood pressure was included in covariant analyses, the differences between the two groups with regard to both FAD and NID were of only borderline significance (P=0.06). The differences in FAD and NID remained significant despite introducing diastolic blood pressure as a covariant (P=0.03 and P=0.04).

Both FAD and NID were identical in normoalbuminuric subjects with systolic or diastolic blood pressures above or below the median values in this group (120 and 75 mm Hg). FAD in subjects with systolic blood pressure above the median was 104.3±0.9% versus 104.1±0.7% in subjects with systolic blood pressure below (P=0.43). NID in subjects with systolic blood pressure above the median was 124.3±1.2% versus 123.6±1.7% in subjects with systolic blood pressure below (P=0.66). Similarly, FAD was 104.5±0.7% versus 104.0±1.2% (P=0.73), and NID was 123.8±1.1% versus 123.8±2.4% (P=1.00) in subjects with diastolic blood pressure above or below the median.

No association was found between systolic blood pressure and either FAD or NID in the combined material (r=−0.13, P=0.32, and r=−0.09, P=0.51). Similarly, no associations were found if data were analyzed separately for the two groups (data not shown). In addition, no associations were found between diastolic blood pressure and the dilatory capacity (data not shown).

Discussion

In this study, a UAE above the 90th percentile in the population (6.6 µg/min < UAE < 150 µg/min) was associated with impaired conduit arterial dilatory capacity in clinically healthy subjects. The association was established in a highly selected population of clinically healthy subjects, thereby reducing potential confounders, and the impaired arterial dilatory capacity associated with elevated UAE in the present study cannot be explained by differences in baseline vessel diameter or differences in classic cardiovascular risk factors such as sex distribution, age, BMI, serum lipids, fasting blood glucose, and so forth, between subjects with elevated UAE and normoalbuminuric control subjects.

However, in accordance with previous observations, elevated UAE was associated with elevated blood pressure within the normal range (hypertension was an exclusion criterion). However, the difference in blood pressure between
the two groups alone is not likely to explain the demonstrated difference in arterial dilatory capacity. First, no association was found between blood pressure and either FAD or NID. Second, normoalbuminuric control subjects in the present study had the same FAD and NID independent of whether they had a systolic or diastolic blood pressure above or below the median blood pressure in this group. Third, the differences in FAD and NID were of borderline significance even when systolic blood pressure was introduced as a covariant, and the differences in both FAD and NID were significant despite the introduction of diastolic blood pressure as a covariant. Nevertheless, the differences in blood pressure between subjects with elevated UAE and normoalbuminuric control subjects may influence the data on arterial dilatory capacity.

In addition to higher blood pressure, subjects with elevated UAE may also be characterized by a higher burden of subclinical atherosclerosis because tests designed to elucidate this such as the ECG stress test and ultrasound measurements of the intima-media thickness of the carotid arteries were not a part of the present study.

Theoretically, insulin resistance in subjects with elevated UAE could also be part of the established association between UAE and conduit arterial dilatory capacity. However, we have previously demonstrated in a similar study population that clinically healthy subjects with elevated UAE have a normal glucose tolerance.17 Whatever the precise mechanism, the results of the present study indicate that elevated UAE is associated with an arterial dysfunction not limited to the glomerulus and other microvessels but also present in the conduit arteries. Measurement of UAE may therefore contribute to estimation of vascular status and cardiovascular risk.

Other groups that used the same ultrasound method as in the present study have demonstrated impaired arterial dilatory capacity in subjects with a number of cardiovascular risk factors including smoking,11 hypercholesterolemia,11 uncomplicated diabetes and hypertension,16,19 and hyperhomo-cyst(e)inemia.12 In contrast to the findings in the present study, the dilatory impairment in these studies was restricted to or most pronounced for FAD, suggesting that the vascular dysfunction associated with these risk factors is predominantly of an endothelial nature. In this respect, the isolated elevated UAE in the present study was associated with an equal impairment of the endothelium-independent (NID) and the endothelium-dependent (FAD) dilatory capacities.

However, in a recent large study of 800 subjects not including measurements of UAE, FAD and NID were independently associated with each other,29 and this is confirmed in our much smaller but more selected group of normoalbuminuric clinically healthy subjects and the association seems identical in clinically healthy subjects with elevated UAE.

The equal impairment of FAD and NID in the present study may be explained by an impaired dilatory response to NO of both endogenous and exogenous origin.

Theoretically, an impaired dilatory response to NO may be caused by decreased bioavailability to the smooth muscle cells, by mechanical forces in or around the arterial wall limiting the dilatory ability, or by dysfunction of the smooth muscle cells. Decreased bioavailability is unlikely to explain our findings because the impairment of the dilatory response to NTG (NID) was similar to the impairment of FAD, and NTG is first converted to NO inside the smooth muscle cells.31 No plaques were observed in the arteries studied; nevertheless, microscopic structural changes in the vascular walls of clinically healthy subjects with elevated UAE could alter the dilatory capacity, as could functional changes of the smooth muscle cells.

On the basis of measurements of FAD and NID, the vascular dysfunction present in clinically healthy subjects with slightly elevated UAE does not appear to be predominantly of an endothelial nature. This is further supported by the identical plasma levels of NOx, the stable end products of NO, and of the identical plasma levels of the potential endothelial markers vWFa and thrombomodulin in the two groups.

By measurement of various plasma or serum markers, elevated UAE has been found to be associated with vascular/endothelial dysfunction in diabetes.9,22 Only a few studies have more directly investigated a possible association between functional endothelial/vascular dysfunction and UAE. In a small study, Zenere et al33 found, with an ultrasound method similar to that used in the present study, an impaired vasodilatory effect of both increased flow and NTG in 8 microalbuminuric type 1 diabetic patients as compared with 10 otherwise comparable normoalbuminuric patients. Similarly, Lekakis et al18 found in another ultrasound study of arterial vasodilatory capacity that whereas only FAD was impaired in normoalbuminuric patients, both FAD and NID were impaired in 5 patients with microalbuminuria. In contrast, Taddei et al,24 by using a plethysmographic method, found no difference in the dilatory response to endothelium-dependent and endothelium-independent vasodilators between hypertensive patients with either microalbuminuria or normoalbuminuria.

Whereas the ultrasound method used in the present study measures only the conduit arterial dilatory capacity, the relative contributions of conduit arteries, resistance arteries, the venous circulation, and the interstitial fluid compartments to results by plethysmography are unclear.25 This could explain the difference between that study and ours.

Another explanation might be that associations between UAE and vascular dysfunction differ in patients with essential hypertension as compared with diabetic patients and healthy subjects. However, when other estimates of endothelial dysfunction are used, elevated UAE and endothelial dysfunction also have been found to be associated in hypertensive patients.26

**Conclusions**

A slightly elevated UAE (>6.6 μg/min; median, 9.9 μg/min) is associated with impaired conduit arterial dilatory capacity in clinically healthy subjects. Flow-associated (endothelium-dependent) and NTG-induced (endothelium-independent) dilations are equally impaired, indicating an impaired response to NO of both endogenous and exogenous origin, which could be caused by structural changes of the arterial wall. The
impaired arterial dilatory capacity may contribute to the increased cardiovascular risk in subjects with elevated UAE.

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References
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