Urinary Albumin Excretion Is Associated with Renal Functional Abnormalities in a Nondiabetic Population

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Abstract. Microalbuminuria (MA) is an important early sign of diabetic nephropathy. Hyperfiltration and impaired filtration in relation to albuminuria has been well investigated in diabetic subjects. This study tested the hypothesis that an increased urinary albumin excretion (UAE) is associated with renal functional abnormalities also in nondiabetic subjects. The relation between UAE and creatinine clearances (Ccr) was studied in a large cross-sectional study. Subjects were divided in four groups according to UAE (mg/24 h): 0 to 15 (control), 15 to 30 (high-normal albuminuria [HNA]), 30 to 300 (MA), >300 (macroalbuminuria). An elevated filtration and a diminished filtration were defined as a Ccr exceeding or below 2× the SD of the control group corrected for age and gender. Ccr followed a parabolic trend, with a higher Ccr in the HNA as compared with control and a lower Ccr in the MA and macroalbuminuria group as compared with HNA. With each increasing UAE level, male sex, age, body mass index, minimal waist circumference, systolic and diastolic BP, plasma glucose, and a positive family history for diabetes all followed a significant linear increasing trend (P < 0.001). After adjustment for age, gender, body mass index, plasma glucose, a positive family history for diabetes, systolic and diastolic BP, antihypertensive medication, and smoking in a multivariate analysis, HNA and MA were independently associated with an elevated filtration (RR 1.8 [95% confidence interval, 1.30 to 2.51] and 1.7 [1.17 to 2.45]). Macroalbuminuria was independently associated with a diminished filtration (4.3 [range, 1.97 to 9.36]). In conclusion, an elevated UAE might be an important and early sign for progressive renal function loss in a nondiabetic population.

Microalbuminuria (MA; 30 to 300 mg/24 h) is an important early sign of incipient diabetic nephropathy in insulin-dependent diabetes mellitus (IDDM) (1,2) and, albeit less specific, in non–insulin-dependent diabetes mellitus (NIDDM) (3,4). Hyperfiltration has been observed in both IDDM (5–8) and NIDDM (9,10) and is thought to occur before the renal function loss (11). Therefore, in diabetic patients, the development of nephropathy typically follows a biphasic pattern, characterized by MA and hyperfiltration in the initial phase, followed by progression to overt proteinuria and renal function decline once overt nephropathy ensues.

Whether this biphasic pattern is typical for diabetic nephropathy or can also be observed in nondiabetic renal disease is still unknown. From animal experimental data, it has been suggested that a period of hyperfiltration might precede progressive renal function loss in nondiabetic renal disease (12,13). Furthermore, from other animal experiments, there is evidence that hyperfiltration induces proteinuria (14), suggesting that the concomitant increased intraglomerular pressure in hyperfiltration might be responsible for both albuminuria/proteinuria and the later renal function loss (15,16).

It is interesting that MA also occurs frequently in nondiabetic subjects and is associated with several risk factors, such as hypertension (17), hyperlipidemia (18,19), obesity (20), and older age (21). Little is known about the relation between albuminuria and renal function in nondiabetic subjects, especially whether nondiabetic subjects also exert early renal functional changes such as hyperfiltration, because they usually come to medical attention only after the development of renal damage. We therefore hypothesized that an increased urinary albumin excretion (UAE) is associated with early and late renal functional abnormalities, such as an elevated filtration and a diminished filtration in a nondiabetic population. To test this hypothesis, we studied renal function in relation to UAE in a large cross-sectional study of nondiabetic subjects in the city of Groningen, the Netherlands.

Materials and Methods

Study Population

This study is part of the ongoing PREVEND study (Prevention of REnal and Vascular ENd stage Disease), running in the city of Groningen, the Netherlands. All inhabitants of the city of Groningen between the ages of 28 and 75 yr (85,421 subjects) were asked to send in a morning urine sample and to fill out a short questionnaire on demographics and cardiovascular history. A total of 40,856 subjects...
(47.8%) responded. From this group, 30,890 subjects had a urinary albumin concentration of $<10$ mg/L and 9966 subjects had a urinary albumin concentration of $\geq 10$ mg/L in their morning urine sample. After exclusion of subjects with IDDM and pregnant women, all subjects with a urinary albumin concentration of $\geq 10$ mg/L ($n = 7768$) together with a randomly selected control group with a urinary albumin concentration of $<10$ mg/L ($n = 3395$) were invited for further investigations in an outpatient clinic (total $n = 11,163$). Finally, 8592 subjects completed the total screening program, rendering us the actual study cohort. The study was approved by the medical ethics committee and conducted in accordance with the guidelines of the declaration of Helsinki. All participants who attended the outpatient clinic gave written informed consent.

**Study Design**

The screening program in the outpatient clinic consisted of two visits. At the first visit, participants completed a self-administered questionnaire regarding demographics; cardiovascular and renal history; family history on diabetes; and the use of medication for diabetes, hypertension, or hyperlipidemia. Subjects were classified as having renal disease when they were being treated for proteinuria as a consequence of renal disease, and subjects were classified as having a positive family history for diabetes when at least one first-degree relative had diabetes. After shoes and heavy clothing were removed, weight was measured to the nearest 0.5 kg with a Seca balance scale (Vogel and Halke, Hamburg, Germany). Height was measured to the nearest 0.5 cm. Minimal waist circumference was measured on bare skin at the natural indentation between the 10th rib and the iliac crest. At both visits, BP was measured in supine position, every minute, for 10 and 8 min, respectively, with an automatic Dinamap XL Model 9300 series device (Johnson and Johnson, Medical Inc., Arlington, TX). Subjects were asked to collect two consecutive 24-h urine in the last week before the second visit. The subjects were given oral and written instructions on how to collect 24-h urine, and they were instructed to postpone urine collection in case of fever, urinary tract infection, or menstruation and to refrain from heavy exercise during collection as much as possible. Furthermore, the subjects were asked to store the urine cold ($4^\circ$C) for a maximum of 4 d before the second visit. Measurements of urinary volume and albumin and creatinine concentrations were performed for each collection. At the second visit, blood was drawn, after an overnight fast, for determination of plasma glucose and serum creatinine.

**Calculations**

Systolic and diastolic BP was calculated as the mean of the last two measurements of the two visits. Body mass index (BMI) was calculated as the ratio between weight (kg) and the square of height (m) (weight/height$^2$). Body surface area (BSA) was calculated according to DuBois and DuBois (23). UAE was calculated as the mean of the two 24-h UAE. Similar to most large epidemiologic studies, we estimated GFR according to the Cockcroft and Gault formula (24), but two 24-h UAE. Similar to most large epidemiologic studies, we to DuBois and DuBois (23). UAE was calculated as the mean of the four collections.

**Laboratory Methods**

Urinary albumin concentration was determined by nephelometry with a threshold of 2.3 mg/L and intra- and interassay coefficients of variation of $\leq 4.3\%$ and $\leq 4.4\%$, respectively (Dade Behring Diagnostik, Marburg, Germany). Plasma glucose and serum and urinary creatinine were determined by Kodak Ektachem dry chemistry (Eastman Kodak, Rochester, NY). Urinary leukocyte and erythrocyte measurements were done by Nephur-test+leuco sticks (Boehringer Mannheim, Mannheim, Germany).

**Data Handling and Definitions**

In the present analysis, we started with the 8592 subjects from our actual study cohort. We excluded from this analysis 433 subjects because of erythrocyturia or leukocyturia, according to dipstick analysis (erythrocytes $>50/ul$, leucocytes $>75/ul$), or leucocytes $= 75/ul$ and erythrocytes $>50/ul$) and 239 subjects because of diabetes, which was defined as fasting plasma glucose levels of $\geq 7.8$ mmol/L or nonfasting plasma glucose levels of $\geq 11.1$ mmol/L or the use of oral antidiabetic medication. Another 46 subjects were excluded because they were treated for proteinuria as a consequence of a renal disease. Finally, 146 subjects were excluded because of missing data on albuminuria, erythrocyturia, leukocyturia, plasma glucose levels, or renal disease. Altogether, 7728 subjects were eligible for this analysis.

Because it has been suggested that excess cardiovascular and renal risk may already be present at levels of UAE lower than the conventional definition of MA (30 to 300 mg/24 h) (3,26), we also investigated borderline elevated UAE (15 to 30 mg/24 h). This rendered us the following groups: the control group (0 to 15 mg/24 h; $n = 5608$), the high-normal albuminuria (HNA) group (15 to 30 mg/24 h; $n = 1106$), the MA group (30 to 300 mg/24 h; $n = 932$), and the macroalbuminuria group (>300 mg/24 h; $n = 82$). Obesity was defined as having a BMI of $>30$ kg/m$^2$. Early and late renal functional abnormalities, such as elevated filtration and diminished filtration, were defined as a Ccr exceeding or below 2$\times$ the SD of the mean of the control group (9). The 2$\times$ SD borders of the mean were obtained by means of a linear regression analysis adjusted for age and gender.

**Statistical Analyses**

All calculations were performed with SPSS version 9.0 software (SPSS, Chicago, IL). Continuous data are reported as mean $\pm$ SD. In case of skewed distribution, the median with 95% confidence interval (CI) was used. All $P$ values are two-tailed. A $P$ value of $<0.05$ was considered statistically significant. Differences among the four UAE groups were assessed by $\chi^2$ analysis or ANOVA and tested for trend. To investigate the risk of an elevated filtration or a diminished filtration, logistic regression analysis was used. Stepwise logistic regression analysis was used to test the independent relation among the four albuminuria groups and an elevated or a diminished filtration. The four albuminuria groups were entered into the regression analysis, and odds ratios were estimated after adjustment for possible confounding factors. We adjusted in this analysis for age, gender, BMI, systolic and diastolic BP, antihypertensive medication, plasma glu-
Rosedale, a positive family history for diabetes, and smoking. The calculated odds ratios and 95% CI are expressed as an approximation of RR.

**Results**

The characteristics of our study group according to the four different UAE groups are shown in Table 1. Age, BMI, minimal waist circumference, systolic and diastolic BP, and glucose showed a significant linear trend with each subsequent UAE level. Similarly, the frequencies of male gender, obesity, a positive family history, and the use of antihypertensive treatment also showed a linear trend with each subsequent UAE level. These differences remained statistically significant after adjusting for age and gender. There was no significant difference in smoking among the four different albuminuria groups. Concerning the renal characteristics, serum creatinine also followed a linear trend with each subsequent UAE level (P < 0.001). In contrast, the mean values for the 24-h Ccr followed a parabolic trend with each subsequent UAE level (P < 0.001). In other words, Ccr in the HNA group was elevated as compared with the control group, whereas it showed a decreasing trend in the MA and macroalbuminuria groups as compared with the HNA group.

To investigate whether this observed parabolic pattern could still be found after adjustment for the two most important confounders, age and gender, we analyzed Ccr in a general linear model. This additional analysis supported the finding of this parabolic pattern (Figure 1).

Because the mean Ccr of a certain group is only the sum of subjects with a normal Ccr and subjects with an abnormal Ccr, this does offer optimal information about the relationship between albuminuria and an elevated or a diminished filtration as compared with normal subjects. Therefore, we also analyzed our data for subjects with an elevated or a diminished filtration. In a linear regression model, including age and gender, mean Ccr was calculated with the 2× SD borders. This is shown in Figure 2 for. Mean Ccr was 103 ± 19 ml/min per 1.73m² in males 30 yr of age and 85 ± 19 ml/min per 1.73m² in males 70 yr of age. In females 30 yr of age and 70 yr of age, the Ccr was 101 ± 18 ml/min per 1.73m² and 76 ± 18 ml/min per 1.73m², respectively. The 2× SD borders show the subjects with an elevated Ccr (n = 263) and with a diminished Ccr (n = 181), taking men and women together.

To investigate further the relation between an elevated or diminished Ccr and albuminuria, we analyzed our data in a

<p>| Table 1. Population characteristics according to urinary albumin excretiona |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Control (0 to 15 mg/24 h)</th>
<th>High Normal (15 to 30 mg/24 h)</th>
<th>Microalbuminuria (30 to 300 mg/24 h)</th>
<th>Macroalbuminuria (&gt;300 mg/24 h)</th>
<th>Uncorrected P Value</th>
<th>Corrected P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>5608 (72.6%)</td>
<td>1106 (14.3%)</td>
<td>932 (12.1%)</td>
<td>82 (1.1%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>47.2</td>
<td>59.7</td>
<td>65.9</td>
<td>67.1</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>47 ± 12</td>
<td>51 ± 13</td>
<td>55 ± 13</td>
<td>59 ± 12</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.5 ± 3.9</td>
<td>26.6 ± 4.2</td>
<td>27.7 ± 4.7</td>
<td>29.8 ± 5.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Minimal waist circumference</td>
<td>86.2 ± 12.1</td>
<td>91.1 ± 12.8</td>
<td>95.4 ± 13.7</td>
<td>99.7 ± 12.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>11.7</td>
<td>18.5</td>
<td>25.7</td>
<td>39.0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 17</td>
<td>135 ± 20</td>
<td>142 ± 24</td>
<td>152 ± 25</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72 ± 9</td>
<td>77 ± 10</td>
<td>80 ± 11</td>
<td>83 ± 10</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive treatment (%)</td>
<td>7.3</td>
<td>13.9</td>
<td>19.8</td>
<td>36.3</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.7 ± 0.6</td>
<td>4.9 ± 0.7</td>
<td>5.0 ± 0.8</td>
<td>5.1 ± 0.8</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive family history for diabetes (%)</td>
<td>17.5</td>
<td>18.3</td>
<td>21.0</td>
<td>20.7</td>
<td>&lt;0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>44.0</td>
<td>45.6</td>
<td>46.7</td>
<td>45.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/24 h)</td>
<td>7.2 (5.6–9.8)</td>
<td>19.6 (17.1–23.5)</td>
<td>53.3 (38.3–86.9)</td>
<td>496.9 (354.5–766.9)</td>
<td></td>
<td></td>
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<tr>
<td>Serum creatinine (µmol/L)</td>
<td>83 ± 13</td>
<td>84 ± 15</td>
<td>90 ± 26</td>
<td>115 ± 110</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Creatinine clearance (ml/min per 1.73 m²)</td>
<td>92.7 ± 19.9</td>
<td>97.7 ± 22.1</td>
<td>90.8 ± 23.0</td>
<td>79.2 ± 27.7</td>
<td>&lt;0.001</td>
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</tr>
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a The mean ± standard deviation is given, except for urinary albumin excretion, which is expressed as median with the 25th and 75th percentiles.

b Age- and gender-corrected P value.

c All P values are for linear trend except for creatinine clearance, which is for parabolic trend.
logistic regression analysis (Table 2). We first analyzed our data adjusting only for age and gender. After also adjusting for all other possible confounders, such as BMI, plasma glucose, a positive family history for diabetes, BP, antihypertensive medication, and smoking, the results remained the same. An elevated filtration was associated with albuminuria, BMI, and plasma glucose, whereas it was inversely associated with the use of antihypertensive medication. Furthermore, a diminished filtration was associated with albuminuria, BMI, male gender, and antihypertensive medication, whereas it was inversely associated with plasma glucose. HNA and MA were associated with an increased RR for an elevated filtration (RR 1.8 [95% CI, 1.30 to 2.51], \( P < 0.001 \); and RR 1.7 [95% CI, 1.17 to 2.45], \( P < 0.01 \), respectively). Macroalbuminuria was also associated with an increased RR for an elevated filtration (RR 1.8 [95% CI, 0.64 to 5.23]), but this was not significant probably because of the small sample size (\( n = 82 \)). Conversely, macroalbuminuria was associated with an increased RR for a diminished filtration (RR 4.3 [95% CI, 1.97 to 9.36], \( P < 0.001 \)), whereas HNA was associated with a decreased RR for a diminished filtration (RR 0.3 [95% CI, 0.13 to 0.59], \( P < 0.001 \)). In other words, HNA and MA are characterized by an elevated filtration, whereas macroalbuminuria is characterized by a diminished filtration. Moreover, a diminished filtration is not observed in association with HNA.

**Discussion**

This is the first, albeit cross-sectional, study that might shed some light on renal functional abnormalities in nondiabetic subjects in an early phase. Because diabetic subjects are usually followed for their diabetic disease, the course of their renal function is usually well known. Nondiabetic subjects come to medical attention only after the development of renal damage, which explains why little is known about early renal functional abnormalities in these subjects.

This study shows that the GFR in nondiabetic subjects might have a parabolic pattern that is associated with the different levels of UAE. In other words, the MA group has more subjects with an elevated filtration, whereas in the macroalbuminuria group more subjects with a diminished filtration can be found. It is interesting that the association between an elevated filtration and albuminuria was even more pronounced in subjects with a UAE thus far considered to be normal: 15 to 30 mg/24 h.

One could argue that those subjects with an elevated filtration or a diminished filtration have such a filtration only by coincidence, because of miscollection or because of the variability of the measurement. To test the validity of our data, we also analyzed our data after excluding subjects by the Bland-Altman method. The Bland-Altman method investigates the agreement between two measurements, which should measure the same. When there was more than a \( 2 \times \) SD difference of the

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*Figure 1.* Creatinine clearance (Ccr) according to the four different albuminuria groups. The error bars represent the 95% confidence intervals. The numbers in the bars represent the age-and gender-adjusted mean of the Ccr (ml/min per 1.73m²).

*Figure 2.* Ccr (ml/min per 1.73m²) as a function of age, with the two lines representing the upper and lower \( 2 \times \) SD borders of the mean according to the regression analysis. (A) Males. (B) Females. The subjects above the upper line represent subjects with an elevated filtration, and the subjects below the lower line represent subjects with a diminished filtration.
The finding that HNA is associated with a lower risk for a diminished filtration emphasizes the finding that this group is mainly characterized by an elevated filtration.

The parabolic pattern in Ccr as shown in Figure 1 seems similar to the widely known parabolic or biphasic pattern in renal function as observed in diabetes. Although the renal hemodynamic abnormalities in diabetes are likely to be specific for the diabetic condition (32), it has been suggested that determinants of progressive renal function loss are similar for patients with established nondiabetic and diabetic renal disease (33).

One of the underlying mechanisms responsible for the observed early renal functional abnormalities in our nondiabetic subjects may be the consequence of insulin resistance. As mentioned before, subjects with an elevated UAE had higher BP, higher BMI, higher minimal waist circumference, and higher plasma glucose levels. These characteristics have been related to the insulin resistance syndrome (34). The insulin resistance syndrome may not only underlie NIDDM but can also be found in nondiabetic subjects (34). Within this context, MA has been argued to be part of the insulin resistance syndrome in diabetic (35–38) and nondiabetic subjects (19,39). The underlying mechanism for hyperfiltration or an impaired filtration might be related to hyperinsulinemia, associated with insulin resistance. In both animal (40) and human experimental research (41,42), hyperinsulinemia has been reported to influence GFR and has even been related to hyperfiltration. It has also been argued that insulin may also be responsible for an altered permeability of the glomerular membrane for albumin (43).

Because this study population after the selection procedure was enriched for albuminuria, one could argue that the population was also enriched for prediabetes. After correction in a multivariate model for characteristics of prediabetes, i.e., plasma glucose, BMI, systolic and diastolic BP, and a diabetic family history, the results remained the same. Therefore, there seems to be a robust effect of albuminuria on renal function, independent of prediabetes or insulin resistance. It is, however, difficult to unravel the responsibility of each unique part on renal function in a cross-sectional study. Nevertheless, these
were nondiabetic subjects with early renal functional abnormalities in relation to albuminuria, which has not been shown before.

Apart from the observed elevated filtration in the HNA and microalbuminuric subjects, we observed an independent association with a diminished filtration in macroalbuminuric subjects. Taking together this parabolic pattern in Ccr, it is tempting to speculate that the group with “hyperfiltration” will eventually progress to renal functional impairment. However, we conducted only a cross-sectional study and cannot draw that conclusion without a longitudinal follow-up. When a longitudinal follow-up can confirm that subjects with an elevated filtration will indeed progress to renal functional impairment with a concomitant increase in UAE, an elevated UAE might be an important and early sign for progressive renal function loss in a nondiabetic population.

In conclusion, we found an association between UAE and renal function, i.e., an elevated Ccr in the stages of slightly elevated albuminuria and a lower Ccr in groups with higher UAE. Moreover, in these nondiabetic subjects, increased UAE is associated with a metabolic profile resembling the insulin resistance syndrome. Screening for albuminuria may identify nondiabetic subjects who are at risk for developing renal functional loss.

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References