Albuminuria in People at Least 40 Years Old: Effect of Obesity, Hypertension, and Hyperlipidemia

Patricia Metcalfe,1,2 John Baker,3 Alistair Scott,1 Chris Wild,1 Robert Scragg,4 and Evan Dryson5

Concentrations of urinary albumin and the albumin:creatinine ratio were measured in early-morning urine specimens from 5670 people older than 40 years who participated in a health screening survey of a local workforce. Sex-specific reference intervals were determined in a subgroup of 3597 people after excluding 2073 individuals with Albustix-positive proteinuria; diabetes mellitus; bacteriuria; current hypertension; body mass index ≥30 kg/m²; or serum triglyceride ≥2.5 mmol/L. The 97.5 percentile concentration for urinary albumin was 28 mg/L in men and 29 mg/L in women; for the albumin:creatinine ratio this was 2.3 g/mol in men and 2.8 g/mol in women. In the study population, the degree of albuminuria showed piecewise log-linear relationships with diastolic blood pressure (P = 0.0001) and body mass index (P = 0.0001), log-linear relationships with hypertriglyceridemia (P = 0.0001) and hypercholesterolemia (P = 0.0001), and a negative piecewise linear relationship with high-density lipoprotein (HDL) cholesterol (P = 0.0461).

Additional Keyphrases: sex-related differences · urine · reference interval · creatinine · cholesterol

Slight albuminuria (microalbuminuria) is predictive of diabetic nephropathy (1–5), increased coronary heart disease morbidity (6), early overall mortality (1, 7), and increased cardiovascular mortality (5, 7) in patients with diabetes mellitus. There is less information about the associations of slight albuminuria in nondiabetic populations. Previous studies have implicated association with hypertension (8–11), obesity (8), blood glucose concentrations (8–10), and plasma triglyceride concentrations (8, 10). The association between increased urinary albumin excretion and morbidity and mortality that has been described in non-insulin-dependent diabetic patients may also apply to the general population, in that increased cardiovascular disease morbidity (12) and early mortality (11, 12) have been reported in elderly nondiabetic subjects.

Previously, reference intervals for urinary albumin concentrations were based on results from a small number of study subjects and involved not only different methods of collection but also different modes of expressing results. The types of urine samples on which reference intervals were determined from some larger studies include early-morning urine samples from 127 English hospital and local council volunteers (13), 31 English adults (14), 105 apparently-healthy Danish adults and children (15), and 101 Japanese adult controls (16); a timed 2-h urine collection from 128 English adults (17); 24-h urine samples from 199 (18) English factory workers and 111 Finnish adults (19); and both overnight and 24-h urine samples from 374 English children (20). Because of small study numbers, the use of hospital staff, or the failure to specify the source of the study populations, the possibility of bias in sample selection cannot be excluded.

The aims of this study were to determine reference intervals and associations of albuminuria in a large population, based on assay of an early-morning urine collection, a sample type with reportedly less biological variation than random (untimed) samples or timed collections (21). We also expressed results in terms of the urinary albumin:creatinine ratio to adjust for glomerular filtration rate and free water clearance.

Subjects and Methods

Study Population

The study population comprised 5670 individuals, 4106 men and 1564 women, ages 40–78 (median 49) years, who participated in a health screening survey of a local workforce. The main criterion used was the size of the subjects' employers' companies (>50 staff of all ages), with emphasis on companies having a large number of Maori and Pacific Island employees to ensure adequate representation of these racial groups. The management of 46 companies agreed to allow their staff to participate (management response, 82%; participant response, 67%). Participants completed a self-administered questionnaire that included questions on past medical history.

After a rest of ≥15 min, blood pressure was measured twice in the sitting position with a Hawksley random zero sphygmomanometer, the disappearance of Korotkoff sounds being used to determine phase V diastolic pressure. After removal of their shoes and heavy clothing, subjects' weight was measured to the nearest 0.2 kg with a beam balance scale; height was measured to the nearest 0.5 cm.

All participants underwent a 75-g oral glucose tolerance test, the blood samples being collected after an overnight fast and 2 h after the glucose load. Triglyceride and cholesterol were measured in the fasted serum specimen. Sera were separated from blood cells within 4

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h of phlebotomy, and all measurements were performed on the day of collection. Participants were instructed to collect a first-voided early-morning urine sample into a sterile container on the day of interview, to be used for albumin and creatinine estimation and for bacterial culture on CLED agar plates (Life Technologies, Grand Island, NY). Urine samples were refrigerated at 4 °C within 3 h of arrival at the laboratory and analyzed on the same or the following day.

Diagnostic Criteria

Diabetes mellitus and impaired glucose tolerance were diagnosed according to World Health Organization criteria (22) for epidemiological surveys: 2-h plasma glucose concentrations >11.1 and 7.8–11.1 mmol/L, respectively. Hypertension was defined as systolic blood pressure ≥160 mmHg and (or) diastolic blood pressure ≥95 mmHg (23). Bacteriuria was diagnosed in samples with a colony count >100 000 organisms/mL and (or) a leukocyte count >100/mL.

Analytical Techniques

Urinary albumin concentrations were determined by Albustix (Ames, Elkhart, IN) and by an immunoturbidimetric assay (Cambridge Life Sciences, Cambridge, UK) that has a linear standard curve between 0 and 165 mg/L and detects albumin as little as 2 mg/L. Clinical proteinuria was defined as an Albustix reading of 1+ or greater (300 mg/L). A Cobas-Fara centrifugal analyzer (Hoffmann-La Roche, Basel, Switzerland) was used to determine glucose and creatinine concentrations and serum high-density lipoprotein (HDL) cholesterol concentrations (Boehringer, Mannheim, FRG). HDL cholesterol was measured after precipitation of apolipoprotein B-containing lipoproteins with magnesium phosphotungstate (24). Total serum cholesterol and triglyceride concentrations were measured with a Chem I random-access analyzer (Technicon, Tarrytown, NY). Between-batch CVs for these assays were as follows: urinary albumin 7.4%, cholesterol 3.6%, HDL cholesterol 3.4%, triglyceride 5.4%, glucose 2.1%, and urinary creatinine 3.8%.

Statistical Methods

Blood pressure was the average of two measurements, body mass index was calculated as weight (kg) divided by the square of height (m), and the urine albumin:creatinine ratio was expressed as albumin (mg/L) divided by creatinine (mmol/L). Because of the positively skewed frequency distribution of urinary albumin concentrations, these were converted to log values for calculations (although the distribution was not completely normalized by this transformation); the results are presented as geometric means. Multiple linear-regression analysis was used to assess the joint effects of the variables associated with urinary albumin concentrations. One outlier for urinary albumin and the albumin:creatinine ratio, detected by the Dixon range statistic (25), was eliminated from reference interval calculations. Empirical likelihood ratio 95% confidence intervals were calculated for the 97.5 percentiles for urinary albumin and the albumin:creatinine ratio (26). These statistical analyses were performed with SAS (Research Triangle Park, NC) statistical software.

Piecewise linear models were fitted to urinary albumin concentrations and diastolic blood pressure, body mass index, and HDL cholesterol concentrations to obtain estimates of the value at which a significant change of slope occurred and its associated confidence interval (27). Study numbers vary for different analyses because of missing information for some studies. For all analyses, P < 0.05 (two-sided) was considered statistically significant.

We calculated bivariate curves by robust locally weighted regression (28), using 66% of the data for smoothing each x value. Confidence intervals were obtained by using the bootstrap technique (29). After randomly sampling the study population of 5425 people with replacement and calculating the weighted regression line 1000 times, we calculated an approximate 95% confidence interval as the 2.5th and 97.5th percentiles of the 1000 bootstrapped regression estimates.

Results

Defining the reference population. Exclusion criteria for individuals for developing health-associated reference intervals include disease, obesity, or hypertension, or the taking of pharmacologically active agents (30). In the current study, a history of diabetes mellitus as well as the findings of Albustix-positive proteinuria, bacteriuria, current hypertension, body mass index ≥30 kg/m², and a fasted serum triglyceride ≥2.5 mmol/L was significantly correlated with increased urinary albumin concentrations. Demographic features of the study and the reference populations are compared in Table 1. There were significantly fewer Maori and Pacific Island people (P < 0.01) in the reference population than in the total study population because both ethnic groups have an increased tendency to develop obesity and hypertension in middle to later years (31).

Reference intervals. The relative contribution of vari-

<p>| Table 1. Demographic Data for 5670 Study Individuals and 3597 Reference Individuals |
|----------------------------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Sex</th>
<th>Study population</th>
<th>Reference population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>4106</td>
<td>2622</td>
</tr>
<tr>
<td>Women</td>
<td>1564</td>
<td>975</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–44</td>
<td>1727 (30.5)</td>
<td>1121 (31.2)</td>
</tr>
<tr>
<td>45–49</td>
<td>1517 (26.7)</td>
<td>960 (26.7)</td>
</tr>
<tr>
<td>50–54</td>
<td>1240 (21.9)</td>
<td>790 (21.9)</td>
</tr>
<tr>
<td>≥55</td>
<td>1186 (20.9)</td>
<td>728 (20.2)</td>
</tr>
<tr>
<td>Racial groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>4471 (78.8)</td>
<td>3187 (88.8)</td>
</tr>
<tr>
<td>Maori</td>
<td>436 (7.7)</td>
<td>143 (4.0)</td>
</tr>
<tr>
<td>Pacific Island</td>
<td>863 (11.7)</td>
<td>187 (5.5)</td>
</tr>
<tr>
<td>Asian</td>
<td>100 (1.8)</td>
<td>70 (1.9)</td>
</tr>
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CLINICAL CHEMISTRY, Vol. 38, No. 9, 1992 1803
Table 2. Effect of Exclusion Criteria on Urinary Albumin Concentrations (mg/L)*

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
<th>No. excluded</th>
<th>5870</th>
<th>5629</th>
<th>5445</th>
<th>5232</th>
<th>5029</th>
<th>4021</th>
<th>3597</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference group</td>
<td>0</td>
<td>5.2</td>
<td>5.1</td>
<td>4.9</td>
<td>4.9</td>
<td>4.7</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Albustix-positive albuminuria</td>
<td>42</td>
<td>(5.1–5.4)</td>
<td>(4.9–5.2)</td>
<td>(4.8–5.1)</td>
<td>(4.7–5.0)</td>
<td>(4.6–4.9)</td>
<td>(4.2–4.5)</td>
<td>(4.1–4.4)</td>
</tr>
<tr>
<td>Diabetes melitus</td>
<td>133</td>
<td>205.2</td>
<td>11.4</td>
<td>(9.3–14.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteriuria</td>
<td>193</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>223</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obesityb</td>
<td>1008</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipemiaa</td>
<td>423</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Values are geometric means with associated 95% confidence intervals in parentheses.

b Body mass index >30 kg/m².

a Fasting triglyceride >2.5 mmol/L.

Table 3. Reference Intervals for Urinary Albumin Concentrations and Albumin:Creatinine Ratio in 2822 Men and 975 Women

<table>
<thead>
<tr>
<th>Albuminuria, mg/L</th>
<th>Percentiles</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Men</td>
<td>0</td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Albumin:creatinine ratio, g/mol</th>
<th>Percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>0</td>
</tr>
<tr>
<td>Women</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are geometric means with associated 95% confidence intervals in parentheses.

Effect of age and sex. Urinary albumin concentrations showed a significant decrease with age in men and women ($P = 0.0001$) (Figure 1). In contrast, the urinary albumin:creatinine ratio was slightly higher in women than men ($P = 0.1080$), because of the lower creatinine excretion among women, and declined with age in women ($P = 0.0548$) but not in men (Figure 2). To avoid the confounding effect of urinary creatinine concentra-

![Fig. 1. Effect of age and urinary albumin concentrations in 4011 men (-----) and 1416 women (-------) who were Albustix negative and abacteriuric. Also shown are the 95% confidence intervals (-----) for each group](image-url)
Fig. 2. Effect of age and albumin:creatinine ratio in 4013 men (a) and 1416 women (b) who were Albustix negative and abacteriuric. 95% confidence intervals are shown for each.

Fig. 3. Relationship between urinary albumin concentrations and body mass index in 4009 men (——) and 1416 women (-----) who were Albustix negative and abacteriuric. Also shown are the 95% confidence intervals.

Fig. 4. Relationship between urinary albumin concentrations and diastolic blood pressure in 5349 individuals who were Albustix negative and abacteriuric. 95% confidence intervals are also shown.

6 Kronmal RA. Spurious correlation and "the fallacy of the per ratio standard" revisited. Presented at the October meeting of the Medical Section of the Royal Statistical Association, 1991.

...
significant regression coefficients for age, sex, body mass index, diastolic blood pressure, and serum triglyceride concentrations (Table 4), confirming the data presented in Table 2 and Figures 1 and 3–5. We used squared terms for body mass index and diastolic pressure, to take account of their nonlinearity with urinary albumin concentrations. The net contribution of these variables accounted for 12.4% of the variation of urinary albumin concentrations. Serum total cholesterol concentrations lost significance when serum triglyceride concentrations were included in the regression model. HDL cholesterol was partially explained by serum triglyceride concentrations, but lost significance completely when body mass index was included in the model. Systolic blood pressure lost significance when body mass index was included in the model.

A multiple-regression model with the albumin:creatinine ratio as the dependent variable showed a similar contribution of variables but included an additional interaction term between age and body mass index because of the contributory effect of urinary creatinine concentration (data not shown).

**Discussion**

**Reference Intervals**

Previous published cutoff values for slight albuminuria were determined either with data from hospital clinic patients (33, 34) or from diabetic subjects who progressed to overt proteinuria (1–5). However, these results have never been corroborated in a large cross-sectional survey of the general population. Comparison with these studies is difficult because they used different collection techniques and methods of expressing results. Therefore, it was gratifying to substantiate in Table 3 the arbitrary cutoff value of 30 mg/L for microalbuminuria proposed by Mogensen (1), which was confirmed in a subsequent study by Rowe et al. (14). A higher upper limit for urinary albumin concentrations was reported by Bohn (15) and a lower upper limit was found in the study by Watts et al. (13).

Similarly, the cutoff value of 2.5 g/mol for the albumin:creatinine ratio described by Taylor et al. (33) and Woolerton et al. (34) was confirmed, but this exceeded the upper limit for the albumin:creatinine ratio found by Watts et al. (13) and Rowe et al. (14).

The sex difference in urinary albumin excretion (the concentrations from men exceeded those from women) agrees with that observed in control subjects (9) and in patients with non-insulin-dependent diabetes (7). The decrease of urinary albumin excretion with age appears to parallel the decline of the glomerular filtration rate (13). However, age and sex effects were undetectable when laboratory rounding of results and analytical variation were taken into account.

The albumin:creatinine ratio showed no significant sex difference, but there was a slight decline with age in women only. Expressing results as ratios has recently been criticized because of the additional associations of the divisor with the dividend. 6

**Associations of Albuminuria.**

**Obesity.** One cross-sectional population study showed that prevalence of albuminuria increased with increasing body mass index (8); another found slightly higher body mass indices in subjects with slight albuminuria than in those with normal albuminuria (11). A significant correlation between body mass index and urinary albumin excretion rate has also been reported previously in men with non-insulin-dependent diabetes (6). The mechanism of this association remains speculative. Weisenger et al. (35) reported nephrotic syndrome in four patients with massive obesity, but this decreased during dietary weight loss. They found minimal morphological changes on renal biopsy, as well as normal renal blood flow and normal glomerular filtration rate. On the basis of findings that increased right heart.
pressure and cardiac output improved with dietary weight loss and remission in proteinuria, they postulated that renal venous hypertension was the most likely cause of reversible proteinuria.

Another recent study showed that the association between albuminuria and obesity was lost when fasting serum insulin was included in the regression model (8). We did not confirm in the current study the possibility that the association between albuminuria and obesity might be mediated by diabetes mellitus or hypertension, because the associations between albuminuria, obesity, and diastolic blood pressure were not changed after excluding participants with diabetes mellitus.

**Blood pressure.** Our observation that diastolic blood pressure is more important than systolic blood pressure is in accordance with both the higher correlation between albuminuria and diastolic blood pressure in studies of patients with untreated essential hypertension (36, 37) and the greater statistical significance for diastolic blood pressure values in subjects with slight albuminuria (10). In contrast, population studies have demonstrated a weak correlation between urinary albumin excretion and systolic blood pressure (9), a curvilinear relationship between the albumin:creatinine ratio and systolic blood pressure (38), and higher systolic blood pressure in individuals with slight albuminuria than in subjects with normal albuminuria (8).

The mechanism of hypertension-induced slight albuminuria is unclear. Some propose that an increase in capillary hydraulic pressure in conjunction with increased filtration through stretched pores between the endothelial cells of the microvasculature is the most likely explanation for the enhanced microvascular leakage of plasma proteins in hypertensive patients (39, 40). Certainly, the rapid decrease in albumin excretion rates with treatment of hypertension (41) is more consistent with a reversible lesion rather than with structural arteriolar or glomerular changes as causes of this abnormality (36, 39).

**Hypertension.** Lipid abnormalities and atherosclerotic cardiovascular disease are common in patients with renal disease, and dyslipidemia may induce albuminuria (42). Two community studies have reported significantly higher concentrations of blood triglycerides, and a minimal alteration in blood cholesterol concentrations in individuals with slight albuminuria (8, 10). Lower HDL cholesterol concentrations were also reported in subjects with slight albuminuria than in subjects with normal albuminuria (10). Our finding that lower HDL cholesterol concentrations could be explained by hypertriglyceridermia is not surprising, given that HDL cholesterol is a product of the metabolism of triglyceride-rich lipoproteins.

**Clinical Implications**

In the current study, we observed significant relationships between albuminuria, obesity, hyperlipidemia, and hypertension in the general population. The latter three conditions have been identified as potent risk factors for cardiovascular diseases in nondiabetic populations. Their aggressive treatment should be a clinical priority in patients with slight albuminuria because of the attendant risk of accelerated macrovascular disease.

The other common interpretation of a finding of slight albuminuria is that the test must indicate underlying renal pathology in individuals with diabetes mellitus (1–5). Albuminuria is a constant finding in patients with diabetic nephropathy and almost always indicates a poor prognosis. However, our findings of a strong relationship between obesity, hypertension, hypertriglyceridermia, and slight albuminuria that is independent of diabetes mellitus suggest that important prerenal factors also contribute to albuminuria in diabetic patients. Obesity, hypertension, and hypertriglyceridermia are common associations of non-insulin-dependent diabetes mellitus, and their effects must be weighed when determining an individual’s prognosis and before subjecting patients to further investigation. Therefore, in patients with non-insulin-dependent diabetes mellitus, the coexistence of obesity, hypertension, and hypertriglyceridermia must diminish the specificity of the test in predicting irreversible diabetic renal pathology.

These reference intervals for albuminuria and the albumin:creatinine ratio in a middle-aged population confirm previous observations. The urinary albumin concentrations were associated with an adverse pattern of cardiovascular risk and support the accumulating evidence that increased urinary albumin concentrations may be a marker for cardiovascular disease.
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References