Endothelial haemostatic factors are associated with progression of urinary albumin excretion in clinically healthy subjects: a 4-year prospective study

Peter CLAUSEN, Bo FELDT-RASMUSSEN, Gorm JENSEN* and Jan Skov JENSEN*
Department of Nephrology and Endocrinology, State University Hospital, Blegdamsvej 9, DK-2100 Copenhagen O, Denmark, and
*The Copenhagen City Heart Study, Epidemiological Research Unit, Bispebjerg Municipal Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen NV, Denmark

ABSTRACT

A slightly elevated urinary albumin excretion rate (UAER), above 5–10 μg/min, is a predictor of atherosclerotic cardiovascular disease. Endothelial dysfunction is an important early feature of atherosclerosis. The plasma concentration of von Willebrand factor (vWF), a potential marker of endothelial dysfunction, predicts a subsequent increase of UAER in patients with diabetes. The aim of this study is to test the hypothesis that high concentrations of vWF as well as other haemostatic factors predict progression of UAER in clinically healthy subjects. UAER was measured together with selected markers of haemostatic function – vWF, tissue plasminogen activator (tPA), plasminogen activator inhibitor, factor VII and fibrinogen – in healthy volunteers aged 40–65 years. After a mean follow-up of 4.1 years, 64 of 74 agreed to a re-examination including re-measurement of UAER. Baseline vWF and tPA were both positively correlated to the change in UAER during follow-up ($r = 0.26$, $P = 0.04$ and $r = 0.40$, $P = 0.001$ respectively). The mean UAER increased significantly by 7.6 μg/min and 7.5 μg/min respectively in subjects with vWF and tPA above the medians at baseline ($P = 0.01$ and $P = 0.003$ respectively), whereas no changes in UAER were seen in subjects with vWF and tPA below the medians. Subjects with high tPA were also characterized by an excess of other cardiovascular risk factors at baseline. No significant differences in these risk factors were present between subjects with high or low vWF. High plasma concentrations of vWF and tPA are associated with progression of UAER in clinically healthy subjects. Both vWF and tPA are secreted by endothelial cells and the results suggest that endothelial dysfunction leads to progression of UAER.

INTRODUCTION

An elevated urinary albumin excretion rate (UAER) exceeding 15 μg/min in a timed overnight urine collection, i.e. microalbuminuria, was first introduced as a predictor of diabetic nephropathy in Type I (insulin-dependent) diabetes mellitus [1]. Microalbuminuria has later also proved to be a predictor of atherosclerotic cardiovascular disease in patients with diabetes [2,3] and in non-diabetic subjects [4]. A slightly elevated UAER, above 5–10 μg/min, is associated with an increased cardiovascular risk in studies of non-diabetic subjects.

Key words: atherosclerosis, cardiovascular disease, cardiovascular risk factor, endothelial dysfunction, microalbuminuria, tissue plasminogen activator, urinary albumin excretion, von Willebrand factor.

Abbreviations: FVIIac, factor VII activity; FVIIag, factor VII antigen; PAI, plasminogen activator inhibitor; tPA, tissue plasminogen activator; UAER, urinary albumin excretion rate; vWF, von Willebrand factor.

Correspondence: Dr P. Clausen.
The pathophysiological mechanism behind the association of slightly elevated UAER and cardiovascular risk is not fully understood. Higher levels of classical atherosclerotic risk factors such as elevated blood pressure and dyslipidemia in non-diabetic subjects with elevated UAER do not seem to be of a magnitude which alone can explain the association [8–10].

Previous studies have shown the increased urinary loss of albumin to be a marker of systemic transvascular albumin leakiness, which may reflect a generalized vascular dysfunction [11–14]. The vascular endothelium serves a central role in the regulation of vascular tone, haemostasis, fibrinolysis, permeability of macromolecules, and the synthesis of growth factors and subendothelial matrix components [15]. Endothelial dysfunction is considered to be an important early part of the atherosclerotic process [16]. The plasma concentration of von Willebrand factor (vWF), a potential marker of endothelial dysfunction [17], is positively correlated to UAER in diabetes mellitus as well as arterial hypertension [18–22], and in Type I diabetes elevated plasma vWF precedes the development of microalbuminuria [20]. In order to investigate the possible influence of haemostatic factors on progression of UAER in clinically healthy subjects, plasma concentrations of vWF as well as a number of other haemostatic factors were measured in a population of volunteers. The concentrations of these factors were correlated to UAER at baseline and to changes in UAER during a 4-year follow-up period to test the hypothesis that high concentrations of vWF as well as other haemostatic factors predict progression of UAER in clinically healthy subjects.

SUBJECTS AND METHODS

Study population
All subjects were recruited from The Copenhagen City Heart Study 1992–1994, an epidemiological study of cardiovascular disease and its known and potential risk factors [23,24]. In total, 3645 subjects collected a timed overnight urine sample. Eighty-seven clinically healthy 40–65-year-old participants with either normoalbuminuria (UAER < 6.6 μg/min (the 90th percentile in the total population), n = 28) or an elevated UAER [6.6 μg/min < UAER < 150 μg/min (upper limit to avoid patients with subclinical renal or urinary tract disease), n = 59] were included in the present study as well as cross-sectional studies of UAER and cardiovascular risk factors [9,13,14]. Only subjects with a negative urinalysis (leucocyte esterase, nitrite, haemoglobin and glucose) were included to ensure accuracy of UAER measurements. In short, the group with elevated UAER comprised all clinically healthy subjects aged 40–65 years who accepted the invitation and in whom elevated UAER was confirmed by two extra urine samples. Clinically healthy subjects with normoalbuminuria were invited at random to constitute an age- and sex-matched group, and normoalbuminuria was also confirmed by extra urine samples. Exclusion criteria were arterial hypertension, diabetes, atherosclerotic cardiovascular disease and regular consumption of medicine [9,13,14].

After a mean follow-up of 4.1 years, 86 could be traced and 74 (86%) of these were still clinically healthy according to inclusion criteria. Sixty-four (86%) of these agreed to a re-examination. The total follow-up time was 261.1 person-years. The subjects included in follow-up were also taking part in cross-sectional studies of UAER and cardiovascular pathophysiology [10]. All subjects included gave their informed consent to participation. The study was performed in accordance with the Second Helsinki Declaration and was approved by the regional ethics committee.

METHODS

All blood samples were obtained in the morning after a 10-h fast and tobacco abstinence. After a 1-h rest while recumbent venous blood samples were drawn without stasis.

Plasma vWF, factor VII antigen (FVIIag), tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI)-1 concentrations were measured by ELISA methods [25–28]. Factor VII activity (FVIIac) was measured using a modified one-stage clotting assay with rabbit brain thromboplastin and a normal human standard. Factor VII-deficient plasma for substrate was prepared from normal human plasma by immunoadsorption using a monoclonal antibody raised against plasma-derived factor VII [29]. Plasma fibrinogen concentration was measured by a photometric method [30]. Serum creatinine and blood glucose concentrations were measured by colorimetric methods, and serum albumin by an ELISA method [31]. Serum total cholesterol, high-density lipoprotein-cholesterol and triacylglycerol concentrations were measured using enzymic colorimetric methods (Boehringer Mannheim, Germany). Low-density lipoprotein-cholesterol concentration was calculated by the formula of Friedewald.

Concentrations of urinary albumin and urinary β2-microglobulin were measured in timed overnight urine collections using ELISA methods [31,32]. Intra- and inter-assay coefficients of variation were less than 5 and 10% respectively. Excretion rates of albumin and β2-microglobulin were calculated on the basis of urinary concentrations, urinary volume and reported collection period. Glomerular filtration rate was measured by 51Cr-EDTA as described by Brøchner-Mortensen [33,34]. Height and weight were recorded and body mass index was calculated (weight/height2). Systolic and diastolic
blood pressure were measured four times at baseline and twice at follow-up after at least 15 min in the recumbent position, and the average was recorded. Smoking habits were recorded.

At follow-up a repeated UAER was measured in a timed overnight urine collection as well as follow-up measurements of systolic and diastolic blood pressure, body mass index, serum creatinine, fasting blood glucose, serum total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol and triacyl-glycerols.

**Statistical analysis**

Normally distributed continuous variables are presented as means (S.D.) and non-normally distributed variables as means (20–80th percentiles). The distribution of UAER and changes in UAER were both positively skewed. Therefore, correlations were sought by Spearman’s non-parametric rank correlation analysis, and changes from baseline to follow-up values of UAER were tested by Wilcoxon’s matched pairs rank sum test. Differences between groups in baseline variables were tested by Student’s unpaired t-test or the Mann–Whitney test for normally or non-normally distributed continuous variables respectively, and by Fisher’s exact test for dichotomous variables. \( P < 0.05 \) (two-tailed) was taken as statistically significant. The analyses were performed using the statistical software package STATISTICA version 5.0 (StatSoft Inc.*, U.S.A.).

**RESULTS**

vWF and tPA concentrations at baseline were both positively correlated to the change in UAER during 4.1 years of follow-up (\( r = 0.26, P = 0.04 \) and \( r = 0.40, P = 0.001 \)), although they were not related to the UAER at baseline (\( r = 0.11, P = 0.38 \) and \( r = -0.07, P = 0.59 \)). The other haemostatic factors (FVIIag, FVIIac, fibrinogen and PAI-1) did not correlate significantly to either UAER at baseline or change during follow-up (Table 1). After a mean of 4.1 years, UAER had increased significantly by 7.6 (\( -0.6–9.0 \) \( \mu \)g/min [mean (20–80th percentiles)], \( P = 0.01 \) and 7.5 (\( -0.5–7.8 \) \( \mu \)g/min, \( P = 0.003 \) in subjects with vWF or tPA above the medians at baseline (1.12 units/l and 5.20 ng/ml respectively), whereas no change in UAER \( 0.0 \) (\( -2.1–2.4 \) \( \mu \)g/min, \( P = 0.84 \) and 0.5 (\( -2.6–1.6 \) \( \mu \)g/min, \( P = 0.64 \) was seen in subjects with vWF or tPA below the medians (Figures 1 and 2). No significant differences in baseline variables

---

**Table 1** Correlations between haemostatic factors at baseline and UAER at baseline and changes in UAER during follow-up in clinically healthy subjects (\( n = 64 \))

<table>
<thead>
<tr>
<th><strong>UAER</strong></th>
<th><strong>Correlation coefficient</strong></th>
<th><strong>P value</strong></th>
<th><strong>Change in UAER</strong></th>
<th><strong>Correlation coefficient</strong></th>
<th><strong>P value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF</td>
<td>0.11</td>
<td>0.38</td>
<td>0.26</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>tPA</td>
<td>-0.07</td>
<td>0.59</td>
<td>-0.40</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>-0.17</td>
<td>0.17</td>
<td>0.19</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>FVIIag</td>
<td>-0.09</td>
<td>0.48</td>
<td>0.11</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>FVIIac</td>
<td>-0.01</td>
<td>0.91</td>
<td>0.09</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.21</td>
<td>0.09</td>
<td>0.14</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

© 1999 The Biochemical Society and the Medical Research Society
Table 2  Follow-up time and baseline variables in clinically healthy subjects with vWF above or below the median (1.12 units/ml)
Continuous variables are expressed as means (S.D.) or means (20–80th percentiles). LDL, low-density lipoprotein; HDL, high-density lipoprotein; GFR, glomerular filtration rate.

<table>
<thead>
<tr>
<th></th>
<th>vWF ≤ median (n = 32)</th>
<th>vWF ≥ median (n = 32)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follow-up time (years)</td>
<td>4.2 (0.5)</td>
<td>4.0 (0.5)</td>
<td>0.17</td>
</tr>
<tr>
<td>UAERbaseline (µg/min)</td>
<td>4.8 (1.4–7.7)</td>
<td>7.9 (1.9–7.7)</td>
<td>0.25</td>
</tr>
<tr>
<td>Males (%)</td>
<td>56</td>
<td>59</td>
<td>1.00</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 (49–60)</td>
<td>59 (49–63)</td>
<td>0.37</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>120 (5)</td>
<td>123 (14)</td>
<td>0.66</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>70 (9)</td>
<td>71 (9)</td>
<td>0.72</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 (22–27)</td>
<td>26 (22–28)</td>
<td>0.42</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>53</td>
<td>72</td>
<td>0.20</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.9 (4.4–5.5)</td>
<td>4.7 (4.3–5.2)</td>
<td>0.29</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>41.6 (2.9)</td>
<td>42.6 (4.4)</td>
<td>0.35</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>6.0 (1.0)</td>
<td>6.0 (1.0)</td>
<td>0.90</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mmol/l)</td>
<td>4.0 (0.8)</td>
<td>4.1 (1.0)</td>
<td>0.75</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>1.4 (0.4)</td>
<td>1.4 (0.4)</td>
<td>0.72</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>1.3 (0.7–1.7)</td>
<td>1.1 (0.7–1.5)</td>
<td>0.63</td>
</tr>
<tr>
<td>GFR (ml·min⁻¹·1.73 m⁻²)</td>
<td>102 (15)</td>
<td>99 (16)</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>80 (12)</td>
<td>82 (13)</td>
<td>0.45</td>
</tr>
<tr>
<td>Urinary β₂-microglobulin excretion rate (µg/min)</td>
<td>9.3 (2.4–8.8) × 10⁻²</td>
<td>8.1 (3.4–8.1) × 10⁻²</td>
<td>0.15</td>
</tr>
</tbody>
</table>

with a potential impact on atherosclerotic risk were found between subjects with vWF above the median and subjects with vWF below the median (Table 2). In contrast, subjects with high tPA were older and were characterized by male predominance, and higher blood pressure, body mass index, serum lipoproteins and fasting blood glucose at baseline compared with subjects with low tPA (Table 3). The change in UAER during follow-up was not significantly associated with concomitant changes in systolic and diastolic blood pressure, body...
mass index, fasting blood glucose, serum creatinine or serum lipoproteins (Table 4).

**DISCUSSION**

This study shows that high plasma levels of vWF antigen and tPA antigen are associated with progression in UAER in clinically healthy subjects during a follow-up period of 4 years. In contrast, other haemostatic factors (FVIIag, FVIIac, fibrinogen and PAI-1) are not significantly associated with changes in UAER.

A limitation of the study is that baseline and follow-up UAER were both measured only once. Although the use of overnight sampling tends to decrease the intra-individual variation, it is still considerable [38]. This limitation is likely to induce type II errors, but nevertheless predictive effects of vWF and tPA on changes in UAER were observed. Since no cross-sectional correlations between the plasma concentrations of the haemostatic factors and UAER were found at baseline, we decided not to re-measure the concentrations at follow-up. Hence, from our results it is not possible to illustrate whether increases in UAER were accompanied by concomitant changes in the haemostatic factors as seen in patients with diabetes [20,21]. The aim of this study was to investigate potential associations between baseline plasma concentrations of haemostatic factors and later changes in UAER in clinically healthy subjects without medical conditions that might influence UAER. Therefore, 12 subjects who developed such medical conditions during the follow-up period were excluded. The total group of non-attendants did not differ from the attendants with regard to any of the baseline variables (results not shown). The initial total cohort will be followed in order to illustrate the impact of UAER, plasma haemostatic factors and classical atherosclerotic risk factors on mortality and cardiovascular disease.

The predictive effect of high tPA on progression of UAER in this study may be due to higher age, male predominance, dyslipidaemia or elevated blood pressure, body mass index and fasting blood glucose in subjects with high tPA. As a potential marker of endothelial dysfunction the association between this factor and classical cardiovascular risk factors is not surprising and does not, in our view, invalidate the conclusions of the study. Furthermore, high vWF was associated with increases in UAER although subjects with high vWF did not have a significantly worse atherosclerotic risk profile or deteriorated glomerular or tubular renal function at baseline compared with subjects with low vWF.

vWF enhances platelet adhesion and factor VIII availability and increased plasma levels may induce a procoagulant state [17]. tPA is the major activator of plasminogen in plasma [36], but increased plasma concentrations of tPA indicate predominantly increased tPA/PAI-1 complexes thus reflecting decreased fibrinolytic activity [37]. Both vWF and tPA are, in contrast to the other haemostatic factors measured, secreted by endothelial cells [17,38,39] and may reflect the endothelial function [17,40]. Hence, both vWF and tPA can influence progression of UAER both due to their association with the haemostatic balance and as a result of their reflection of the endothelial function. In Type I diabetes elevated plasma vWF precedes the development of microalbuminuria [20], and in Type II (non-insulin-dependent) diabetes mellitus the development of microalbuminuria is associated with the baseline level of and change in vWF [21]. These observations are in accordance with the results of the present study of clinically healthy subjects. vWF and tPA are both associated with increased cardiovascular risk [41,42] and the predictive effect of elevated UAER on cardiovascular risk [2–7] may in part be due to the association with elevated vWF and tPA.

In Type I and Type II diabetes mellitus and hypertension, vWF has been reported to be positively correlated to UAER in cross-sectional analyses [18–22]. No baseline cross-sectional correlations between vWF or tPA and UAER were found in the present study. The low UAER in clinically healthy subjects compared with diabetic or hypertensive patients is one possible explanation. Another explanation could be a combined effect of these diseases and endothelial dysfunction on UAER.

Using blood flow responses to intra-arterial infusions of endothelium-dependent and endothelium-independent vasodilators as an indicator of endothelial status, endothelial dysfunction is associated with cholesterol levels even in the normal range [43]. Hence, the association in this study between endothelial haemostatic factors at baseline and changes in UAER during follow-up could have been confounded by concomitant changes

<table>
<thead>
<tr>
<th>Change in:</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>−0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>0.13</td>
<td>0.29</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.04</td>
<td>0.71</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>−0.06</td>
<td>0.62</td>
</tr>
<tr>
<td>Serum creatinine (µg/min)</td>
<td>0.12</td>
<td>0.33</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>−0.13</td>
<td>0.30</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mmol/l)</td>
<td>−0.10</td>
<td>0.42</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>−0.09</td>
<td>0.46</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>0.04</td>
<td>0.76</td>
</tr>
</tbody>
</table>

* LDL, low-density lipoprotein; HDL, high-density lipoprotein.*

**Table 4 Correlations between changes in UAER during follow-up and concomitant changes in other cardiovascular risk factors (n = 64)**
in serum lipids. However, no significant associations between the changes in serum lipids and the changes in UAER were found.

In conclusion, high plasma concentrations of vWF antigen and tPA antigen are associated with progression of UAER in clinically healthy subjects, whereas this could not be demonstrated for other haemostatic factors in this study. Both vWF and tPA are secreted by endothelial cells and the results suggest that endothelial dysfunction leads to progression of UAER in clinically healthy subjects.

ACKNOWLEDGMENTS

The study was supported by grants from the Danish Heart Foundation, the Foersom Foundation, the Danish Medical Research Foundation, the Foundation of A. P. Møller for the advancement of Medical Science, the Foundation of P. A. Messerschmidt and wife, and the Danish Medical Research Council. We thank Ms. Marja Deckert, Steno Diabetes Center, for technical assistance.

REFERENCES

22 Pedrinelli, R., Giampietro, O., Carmassi, F. et al. (1994) Microalbuminuria and endothelial dysfunction in essential hypertension. Lancet 344, 14–18

Received 4 January 1999/18 February 1999; accepted 23 March 1999