Albuminuria as risk factor for initiation and progression of carotid atherosclerosis in non-diabetic persons: the Tromsø Study

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Aims High levels of microalbuminuria have been associated with severe atherosclerosis. In this prospective, population-based study, we examined whether urinary albumin-to-creatinine-ratios (ACR) in the lower range were associated with the initiation and progression of atherosclerosis.

Methods and results Carotid ultrasonography and measurements of ACR, fibrinogen, monocytes, white cell count, and well-established cardiovascular risk factors were performed in 4037 non-diabetic subjects, 2203 without, and 1834 with pre-existing plaques at baseline. After 7 years new ultrasound measurements were performed. In subjects without pre-existing plaques, 884 had developed at least one plaque during follow-up. Baseline ACR was significantly related to the area of the novel plaques ($P$ for linear trend = 0.009 over the baseline ACR quartiles, after multiple adjustments). The relationship with ACR was clearly modified by fibrinogen ($P$ = 0.001, for the interaction ACR x fibrinogen). Subjects with high levels of both ACR and fibrinogen developed plaques with the largest area. In subjects with pre-existing plaques, ACR was related to plaque-progression ($P$ for linear trend = 0.026, after multiple adjustments). In these individuals, the interaction between fibrinogen and ACR on plaque-growth appeared only in those with minimal atherosclerosis at baseline.

Conclusion ACR is positively related to plaque-initiation and plaque-growth. This relationship is substantially modified by fibrinogen in previously plaque-free subjects.

KEYWORDS Atherosclerosis; Carotid arteries; Fibrinogen; Microalbuminuria; Plaque; Ultrasonography

Introduction

Microalbuminuria is a urinary finding thought to reflect generalized endothelium dysfunction along the vascular tree, including the glomeruli. Numerous studies have found that microalbuminuria predicts cardiovascular events, especially in subjects with hypertension and diabetes. Recent studies have shown that the risk of cardiovascular diseases (CVD), all-cause mortality, and mortality caused by CVD are increased also at levels well below the usually defined cut-off levels for pathological albuminuria, independently of diabetes.

Microalbuminuria is not only related to symptomatic vascular disease, but also to early signs of atherosclerosis. Even in non-diabetic subjects, microalbuminuria has been found to associate with carotid atherosclerosis, and in a recent population-based cross-sectional study, it was shown that atherosclerosis was associated with urinary albumin-to-creatinine-ratios (ACR) at levels far below what is termed microalbuminuria. However, because no similar longitudinal studies of individuals in the general community have been performed, it is not known whether even low levels of ACR predict the development of atherosclerosis.

In the present population-based, prospective study of non-diabetic individuals, we examined whether low levels of ACR were associated with the development of new carotid artery plaques in previously plaque-free subjects and plaque-growth in subjects with pre-existing plaques. However, because inflammation is widely accepted as essential in all stages of the atherosclerotic process and recent cross-sectional studies have found a significant interaction between the presence of microalbuminuria and measures of vascular inflammation in relation to cardiovascular risk factors, we also assessed the possible interactions with some measures of inflammation (fibrinogen, monocytes, and white blood cell count).

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Methods

The Tromsø Study is a population-based, longitudinal study of inhabitants in the municipality of Tromsø, Norway. The Regional Ethical Committee has approved the study, and the participating subjects have given informed consent. At the fourth survey in 1994–95, all inhabitants aged 55–74 years, and 5–10% samples of the other 5-year birth cohorts older than 24 years of age were invited to an ultrasonographic examination of the right carotid artery. In the age groups 25–54, 55–74, and 75–84 years, 1751, 7158, and 148 subjects were invited and 1205, 5617, and 80, respectively, participated. In total 6902 (76% of the eligible population) attended, and 6727 persons were examined by ultrasound. Among the 2203 persons without pre-existing plaques, 1319 (60%) had no plaques and 2248 had plaques at baseline. In the present analyses we excluded 821 subjects; 122 persons reporting diabetes and/or use of medication for diabetes, 88 persons with missing measurements of either ACR, fibrinogen, or plaque-area, 542 with bacteruria or haematuria on any day when urine samples were collected or macroalbuminuria (ACR >25 mg/mmol), and 69 persons who withdrew their data from the analysis. Thus, 4037 subjects (2203 with and 1834 with plaques at baseline) were included.

In 1994–95, information about smoking habits, prevalent diabetes mellitus, angina pectoris, previous MI, stroke, treatment for hypertension, and physical activity was collected from self-administered questionnaires, and measurements of height, body weight, blood pressure, non-fasting serum lipids, and counts of white blood cells and monocytes were done as described previously. Fibrinogen was measured using the PT-Fibrinogen reagent (Instrumentation Laboratory, Italy). Urine samples from the first morning urine from three consecutive days were used to assess microalbuminuria. Albumin and creatinine were measured by turbidimetry on a Mountain View, CA, USA) equipped with a linear array transducer. The between- and within-sonographer agreement on plaque area was substantial, with values of 0.72 (0.60–0.84) and 0.76 (0.63–0.89), respectively, and similar results at follow-up. With regard to the plaque-area, the intraobserver mean arithmetic difference (SD) for sonographer 1 was 0.2 (3.1) mm² and the limits of agreement were −5.9 to 6.3 mm². The corresponding values for sonographer 2 were 0.01 (3.8) mm² and −7.5 to 7.5 mm². The between- and within-sonographer mean arithmetic difference (SD) of the plaque-area was −1.0 (4.4) mm² and the limits of agreement were −9.6 to 7.6 mm².

Statistical analysis

Differences between groups were tested using ANCOVA. Trends over several groups were tested using multiple regression analyses. When means of ACR were compared, the values were log-transformed before statistical testing was performed.

The participants (n = 4037) were divided into groups according to quartiles of ACR. Multiple linear regression analyses were performed in order to evaluate the impact of ACR (measured as a log-transformed variable or divided into quartiles) on the total area of new plaques and the change in the area of pre-existing plaques. Adjustments were done for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, other use of medication for hypertension and smoking which are all well-established risk factors for CVD. Additional adjustments were done for fibrinogen, monocyte, and white blood cell count and, in separate analyses, also for baseline plaque-area in subjects with pre-existing plaques. Because the analyses of the subjects without plaque at baseline included a large number of individuals with no plaques at follow-up in 2001, we performed similar analyses among subjects who had developed new plaques. The assumptions of this model were assessed by residual analysis. The interaction terms ‘ACR × fibrinogen’, ‘ACR × monocytes’, and ‘ACR × white blood cell count’ were included in the models in separate analyses. The interaction analyses with inflammation parameters were done including ACR as log-transformed continuous variable. For subjects without pre-existing plaques at baseline the joint effect of ACR and fibrinogen was demonstrated using ANCOVA.

In order to describe more accurately the dependence of mean plaque-area on log-transformed ACR, taking into account possible non-linearities in the relationship, a non-parametric local regression analysis was carried out using the Loess procedure. The smoothing parameter was set equal to 0.75. A two-sided P-value < 0.05 was considered statistically significant.

The data were analysed using the Windows 14.0 version of SPSS. Local regression was performed by applying Proc Loess in SAS, version 9.1.

Results

Subjects without plaques at baseline

Among the 2203 persons without pre-existing plaques, 1319 subjects still had no plaques at follow-up 7 years later, while 884 had developed at least one plaque. The baseline characteristics according to plaque status (no plaques and quartiles of plaque-area) are presented in Table 1. Significant trends over levels of plaque-area were found with respect to age, sex, systolic and diastolic blood pressure, serum total plaques were stored on super-VHS videotape. B-mode images were subsequently digitized and transferred to a personal computer with the use of a commercially available video grabber card (Matrox Meteor II). Measurements of plaque-area were made with the use of the Adobe Photoshop software (version 7.0), by tracing the perimeter of each plaque with a cursor. Total carotid plaque-area was defined as the sum of the plaque-areas in the six possible sites in each person.

The between- and within-sonographer agreement on plaque occurrence was substantial, with values of 0.72 (0.60–0.84) and 0.76 (0.63–0.89), respectively, and similar results at follow-up. With regard to the plaque-area, the intraobserver mean arithmetic difference (SD) for sonographer 1 was 0.2 (3.1) mm² and the limits of agreement were −5.9 to 6.3 mm². The corresponding values for sonographer 2 were 0.01 (3.8) mm² and −7.5 to 7.5 mm². The between- and within-sonographer mean arithmetic difference (SD) of the plaque-area was −1.0 (4.4) mm² and the limits of agreement were −9.6 to 7.6 mm².

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plaque-free individuals, with a total of 2203 individuals included in the analyses. The results from the analyses of initiation and progression of plaques at baseline are displayed in Table 1. The characteristics of individuals without plaques at baseline are presented in Table 1. The results show that the plaque-area increased by 1.5 mm² when adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking. For each standard deviation (SD) increase in the log-transformed ACR-level, the plaque-area increased by 1.3 mm² when adjusted for age and sex (P < 0.001), and by 0.9 mm² when adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking (P < 0.001). After further adjustments for fibrinogen, monocyte, and white blood cell count, the area increased by 1.0 mm² (P < 0.001). The trends tended to be stronger in men than in women, but they did not differ significantly.

In a similar analysis within the subgroup of individuals who had developed new plaques at follow-up in 2001 (n = 884), we found that for each SD increase in the log-transformed ACR-level, the plaque-area increased by 1.5 mm² when adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking (P = 0.001).
**Subjects with pre-existing plaques at baseline**

Table 4 shows the baseline characteristics of the 1834 persons with pre-existing plaques. When stratified according to changes in plaque-area, significant trends over the groups were found with respect to age, sex, systolic and diastolic blood pressure, prevalence of current smoking, prevalence of CVD, ever use of medication for hypertension, ACR, and monocytes.

Table 5 displays the relationship between the ACR-groups and the total plaque-area in 1994 as well as the changes between 1994 and 2001. Plaque-growth was independently related to ACR when adjusted for age and sex. The picture was similar after multiple adjustments.

For each SD increase in the log-transformed ACR-level, the change in plaque-area (between 1994 and 2001) increased by 1.1 mm² when adjusted for age and sex \( (P = 0.005) \), and by 0.8 mm² when adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking \( (P = 0.06) \). After further adjustments for fibrinogen, monocytes, and white blood cell count, the area increased by 0.8 mm² \( (P = 0.06) \), 0.6 mm² \( (P = 0.16) \), and 0.8 mm² \( (P = 0.04) \), respectively, for each SD increase in the log-transformed ACR-level. The relationship between ACR and plaque-area growth was somewhat stronger when adjustment for the area of the plaques at baseline was included in the model \( (1.5 \text{ mm}^2 \text{ increase per SD increase in log-transformed ACR-level and } P < 0.001 \text{ when adjusted for age and sex, and } 1.0 \text{ mm}^2 \text{ and } P = 0.01 \text{ when adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking}) \). There was no significant difference between men and women regarding the rate of plaque growth.

Figure 2 shows the relation between change in mean plaque-area and log-transformed ACR in subjects with plaque at baseline, as estimated by local regression. The curve reflects an underlying increasing relationship, but the results are less convincing than in subjects without plaque at baseline. However, the particular strength of the relationship among the few subjects with relatively high ACR levels is similar.

The possible interactions between ACR and our markers of inflammation (fibrinogen, monocytes, and white blood cell count) were tested. The interaction-term ‘fibrinogen × ACR’ was highly significant \( (P = 0.004) \), adjusted for age and sex, and \( P = 0.001 \), after adjustments for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking). The interaction terms for monocytes and white blood cell count were not statistically significant \( (P = 0.33 \text{ and } P = 0.37, \text{ respectively}) \). Table 3 shows that subjects with high levels of both ACR and fibrinogen developed novel plaques with the largest total area.

Among the 884 subjects without plaques at baseline but with plaques at follow-up, 567 subjects had developed one plaque, 235 had developed two plaques, and 82 had developed more than two plaques. The number of new plaques was strongly related to the total area of the plaques \( (r = 0.67 \text{ in all subjects}) \). The overall results are similar regardless of which indicator of atherosclerosis (number of plaques or total area of the plaques) is used as the dependent variable.

**Discussion**

The present study showed a relationship between ACR and the development of carotid atherosclerosis in non-diabetic persons. The effect was similar for initiation of novel atherosclerosis (no plaque at baseline) and for progression of already established atherosclerosis. In subjects with no plaque at baseline, the effect of ACR on plaque growth was clearly modified by fibrinogen. In persons with established atherosclerosis, however, the interaction between fibrinogen and ACR on plaque growth was present only in those with minimal atherosclerosis at baseline.

Our study is the first population-based prospective study that examines the relationship between ACR and the
development of artery plaques in persons without diabetes. Most, albeit not all, previous cross-sectional studies of non-diabetic subjects, either in the general population or in selected (mainly hypertensive) groups of subjects, also showed that microalbuminuria and atherosclerosis were associated. Atherosclerosis was generally assessed as an increased intima-media thickness (an indicator of general atherosclerosis), and only one study examined the relationship to the size of artery plaques (a measure of more advanced atherosclerosis).

The reason why higher levels of ACR seem to be associated with the risk of developing extensive atherosclerosis is still to be elucidated but, as suggested by Furtner et al., it is not unlikely that the progression of atherosclerosis is stimulated in subjects with endothelial leakiness because the entrance of lipoproteins and other pro-atherogenic mediators is facilitated.

We found that fibrinogen modified the relationship between ACR and the development of plaques in previously plaque-free subjects. Other prospective studies have not examined this possible interaction, but similar effects were seen in a cross-sectional study where C-reactive protein modified the relationship between microalbuminuria and blood pressure. In another study of non-diabetic hypertensive men, microalbuminuria accompanied by evidence of subclinical inflammation was strongly associated to metabolic abnormalities, whereas isolated microalbuminuria seemed to represent a more benign profile. The authors hypothesized that 'inflammatory microalbuminuria' may precede and perhaps predispose to the development of
cardiovascular abnormalities. Our results seem to support this hypothesis, as the joint effect of microalbuminuria and fibrinogen on plaque growth was most pronounced in the earliest phase of atherosclerosis development.

Interestingly, inflammatory markers seem to differ with regard to modification of ACR and plaque formation, as no such effect was observed for monocytes or white blood cell count. However, fibrinogen has several other pathophysiological effects related to atherosclerosis, such as stimulation of platelet aggregation and increase in blood viscosity, chemotaxis, proliferation of smooth muscle cells, and formation of platelet-rich thrombi.19,20 Only a weak correlation ($r = 0.09$) was found between ACR and fibrinogen, which indicates that they probably do not reflect the same underlying process.

There is always a possibility that a significant result has been achieved by chance, especially when multiple comparisons are performed. To reduce this risk one may therefore adjust for multiple comparisons. This policy is debated, however.21 As particular tests were performed because of the hypothesis stated before the study was initiated, we did not adjust for multiple comparisons. It has been shown that adjustments for baseline values in some common situations may induce an overestimation of the relationship between the predictor and changes over time,22 and we therefore presented the changes of the plaque-area in subjects with pre-existing plaques both with and without adjustments for the area at baseline. The relationships were basically similar, however. The ANCOVA including persons without plaques was non-standard in the sense that common distributional assumptions could not be fully satisfied. Tests carried out excluding these persons, still based on a fairly large data set, indicated that our results were robust against such deviations. However, because of the problems inherent in the specification of the relationship between ACR and plaque-area, we also explored this association by non-parametric local regression.

The present study has several strengths. It is a longitudinal, population-based study comprising a large group of subjects with a high attendance rate. The eligible persons had their carotid arteries examined by ultrasound, a measure that correlates well with atherosclerosis in other arterial territories and is associated with clinical CVD.23,24 A strict definition of albuminuria was used, in that urine samples from all patients were cultured, and patients with bacteruria were excluded from the analysis. A limitation of the study is, however, that we were not able to follow all subjects throughout the study period. Of the eligible subjects, 73% were re-examined. The persons who did not attend the 2001–02 examination, but fulfilled our inclusion criteria had higher ACR levels at baseline than the participants who attended. They also had a higher prevalence of CVD, higher blood pressure, higher fibrinogen, monocyte, and white blood cell count; more of them had used medication for hypertension and a larger proportion was current smokers. It is therefore not unlikely that they had developed more and larger plaques, and non-participation may, therefore, have weakened the true relationship between these variables. Moreover, random misclassifications may have occurred due to measurement errors of ACR and plaque assessments, which again may lead to weaker relationships between them.

We conclude that in subjects without diabetes or macroalbuminuria there is a linear relationship both between ACR and plaque initiation and ACR and plaque growth.

### Table 5

<table>
<thead>
<tr>
<th>ACR groups</th>
<th>n</th>
<th>Plaque-area in 1994, mean (95% CI)</th>
<th>Change in plaque-area 1994–2001, mean (95% CI)</th>
<th>Change in plaque-area 1994–2001, mean (95% CI)</th>
<th>Change in plaque-area 1994–2001, mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00–0.36 (mg/mmol)</td>
<td>398</td>
<td>18.5 (16.8–20.0)</td>
<td>4.1 (2.3–5.8)</td>
<td>4.6 (2.8–6.4)</td>
<td>4.5 (2.8–6.2)</td>
</tr>
<tr>
<td>0.37–0.53 (mg/mmol)</td>
<td>450</td>
<td>17.5 (15.9–19.0)</td>
<td>5.9 (4.2–7.5)</td>
<td>6.0 (4.3–7.7)</td>
<td>5.5 (3.9–7.0)</td>
</tr>
<tr>
<td>0.54–0.85 (mg/mmol)</td>
<td>458</td>
<td>20.0 (18.7–21.8)</td>
<td>5.3 (3.7–7.0)</td>
<td>5.4 (3.7–7.0)</td>
<td>5.7 (4.1–7.2)</td>
</tr>
<tr>
<td>0.86–24.82 (mg/mmol)</td>
<td>528</td>
<td>20.8 (19.4–22.2)</td>
<td>8.1 (6.5–9.6)</td>
<td>7.6 (6.0–9.2)</td>
<td>7.9 (6.4–9.4)</td>
</tr>
<tr>
<td>$P_{\text{trend}}$</td>
<td>0.004</td>
<td>0.002</td>
<td>0.026</td>
<td>0.004</td>
<td></td>
</tr>
</tbody>
</table>

The plaque-areas and the changes are presented according to ACR levels (defined by ACR quartiles for the total group of participants).

- $^{a}$Adjusted for age and sex.
- $^{b}$Adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension and smoking.
- $^{c}$Adjusted for age, sex, systolic blood pressure, serum total cholesterol, serum HDL-cholesterol, ever use of medication for hypertension, smoking, and plaque-area at baseline.

![Figure 2](image-url)  

**Figure 2** The relationship between log-transformed values of ACR and change in mean plaque-area for subjects with plaque at baseline, as estimated by local regression analysis.
This relationship is substantially modified by fibrinogen in previously plaque-free subjects.

Acknowledgements

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Conflict of interest: none declared.

References