**Carotid Atherosclerosis and Incident Atrial Fibrillation**

Karin Willeit, Raimund Pechlaner, Georg Egger, Siegfried Weger, Martin Oberhollenzer, Johann Willeit, Stefan Kiechl

**Objective**—Atrial fibrillation (AF) and atherosclerotic vascular disease are closely entangled disorders and often coexist. Whether atherosclerosis predisposes to the development of AF has not been fully elucidated.

**Approach and Results**—This study was performed within the framework of the Bruneck Study, a population-based survey with near-complete participation (932 of 1000), long-term follow-up (1990–2010), and thorough assessment of AF. The carotid arteries served as a window to systemic atherosclerosis and were scanned every 5 years. Pooled logistic regression and multistate proportional hazards models were used to identify risk predictors of incident AF and effects of AF on mortality. During follow-up, 118 new cases of AF were detected (incidence per 1000 person-years of 8.1; 95% confidence interval, 6.8–9.6). Individuals with atherosclerosis were more likely to develop AF than individuals without (odds ratio, 1.8; 95% confidence interval, 1.1–3.1; P=0.021). This finding applied to women and men and to both baseline and incident atherosclerosis during follow-up. Subjects with atherosclerosis and AF were significantly more likely to die than those with either condition alone (P=0.0034), and mortality in this group was ≈4-fold compared with individuals free of atherosclerosis and AF (hazard ratio, 4.2; 95% confidence interval, 2.6–6.8; P<0.0001).

**Conclusions**—We found that subjects with carotid atherosclerosis are at high risk of developing AF. (Arterioscler Thromb Vase Biol. 2013;33:2660-2665.)

**Key Words:** atherosclerosis • atrial fibrillation • epidemiology • population • prognosis

Atrial fibrillation (AF) is associated with a high burden of disease,1 frequently complicates heart failure,2 and accounts for a quarter of ischemic strokes.3 Prevalence of AF is on the rise,4 and healthcare expenditures are expected to increase substantially during the coming years.5 Rigorous primary prevention programs are mandatory to counteract these threatening trends, and they rely on the identification of modifiable risk factors for AF as potential targets for intervention. Several lines of evidence suggest atherosclerosis to be of relevance both to the evolution and clinical course of AF. A number of studies have reported an association of AF with coronary atherosclerosis and myocardial infarction,6,7 and the Rotterdam Study suggests an association with extracoronary (systemic) atherosclerosis as well.8 Moreover, in patients with AF, presence of atherosclerotic vascular disease was proposed to amplify the risk for thromboembolic stroke and vascular death.9–11 This excess risk may derive from more prominent atrial abnormalities or additive effects of AF and atherosclerotic vascular disease on thrombosis susceptibility and systemic inflammation.9

The aim of the present study was to further scrutinize the association between atherosclerosis and incident AF using data from a prospective population-based study with a 20-year follow-up, thorough ascertainment of AF, and repeated quantification of atherosclerosis.

**Materials and Methods**

Materials and Methods are available in the online-only Supplement.

**Results**

At study baseline, 32 of 932 subjects had a diagnosis of AF and were excluded from the following analyses. Among the remaining 900 individuals, mean age was 58.4 years, 442 (49.1%) were female, and 370 subjects (41.1%) had atherosclerotic lesions at the carotid arteries. During a median follow-up of 20 years, 118 participants developed AF—corresponding to an incidence rate of 8.1 per 1000 person-years (95% confidence interval [CI], 6.8–9.6). The age- and sex-specific incidence rates of AF obtained in our study are in agreement with those observed in other population studies7,12–15 (Figure I in the online-only Data Supplement).

Subjects with incident AF were older, more likely to have hypertension, show symptoms suggestive of chronic heart failure, and had higher levels of blood pressure, vascular cell adhesion molecule-1, and thyroxine. Baseline population characteristics according to incident AF and carotid

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**Arterioscler Thromb Vase Biol** is available at http://atvb.ahajournals.org
Atherosclerosis status are depicted in Table 1 and in Table I in the online-only Data Supplement, respectively.

Of note, 27 of the 32 men and women with baseline AF (84.4%) exhibited carotid atherosclerosis, and detection of carotid atherosclerosis antedated the diagnosis of AF in a majority of incident cases (96 of 118). In line with previous evaluations, carotid plaques in the Bruneck Study qualified as a valid surrogate of atherosclerosis in other vascular territories with an overlap as high as 82.1% to the femoral arteries and 73.2% to the abdominal aorta (fourth follow-up in 2010). Aortic diameter and plaque burden (presence of plaques in the infrarenal segment of the aorta) were higher in the case of an AF diagnosis (P=0.028 and P=0.001, fourth follow-up in 2010).

In pooled logistic regression analysis adjusted for age and sex, carotid atherosclerosis emerged as a significant predictor of new-onset AF (odds ratio, 2.0; 95% CI, 1.2–3.3; P=0.011; Table 2). This finding was robust in multivariable analysis additionally controlling for level of soluble vascular cell adhesion molecule-1, symptoms of heart failure, and hypertension (odds ratio, 1.8; 95% CI, 1.1–3.1; P=0.021) and in a number of sensitivity analyses (sex-specific analyses, extended adjustment, exclusion of subjects with ...
previous myocardial infarction; Table 2). Remarkably, odds ratios obtained for atherosclerosis were similar to those calculated for hypertension (multivariable odds ratio, 1.8; 95% CI, 1.1–3.0).

Next, we fitted multistate proportional hazards models allowing for a separate focus on subjects with baseline atherosclerosis (1990, prevalent atherosclerosis) and those who developed carotid plaques during follow-up (1990–2010, incident atherosclerosis), and obtained comparable risk estimates in both groups (hazard ratio, 2.5; 95% CI, 1.0–6.6 and hazard ratio, 2.7; 95% CI, 1.0–7.5, respectively). Cumulative hazards for AF in women and men with prevalent or incident atherosclerosis and those free of atherosclerosis are shown in Figure 1. Cumulative incidence plots, also derived from the multistate model, carefully consider the competing risk of death and covered the life-span ≤90 years (Figure 1). Cumulative incidence of AF was the lowest in subjects free of atherosclerosis except for the elderly subjects. Lines converged and even crossed at higher ages because of the substantial survival advantage in women and men free of atherosclerosis (ie, this group more commonly survived to prime ages for AF manifestation). Lifetime risk for development of AF at an index age of 50 years (Figure 1) was 23 (17–28)% for men with baseline atherosclerosis, 34 (22–45)% for men with incident atherosclerosis, and 29 (19–39)% for men free of carotid plaques. Corresponding data for women were 26 (18–33)%; 33 (21–45)%; and 28 (18–38)%; respectively.

### Table 2. Association of Carotid Atherosclerosis With Incident Atrial Fibrillation (1990–2010) in the Bruneck Study Cohort

<table>
<thead>
<tr>
<th>Models</th>
<th>Odds Ratio of Atrial Fibrillation (95% CI)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>3.85 (2.40–6.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted for age and sex</td>
<td>1.95 (1.16–3.26)</td>
<td>0.011</td>
</tr>
<tr>
<td>Multivariable*</td>
<td>1.83 (1.09–3.06)</td>
<td>0.021</td>
</tr>
<tr>
<td>Multivariable (men)*</td>
<td>1.90 (0.88–4.12)</td>
<td>0.11</td>
</tr>
<tr>
<td>Multivariable (women)*</td>
<td>1.75 (0.87–3.52)</td>
<td>0.12</td>
</tr>
<tr>
<td>Multivariable (subjects without myocardial infarction)*</td>
<td>1.76 (1.04–2.95)</td>
<td>0.034</td>
</tr>
<tr>
<td>Multivariable extended adjustment†</td>
<td>1.99 (1.18–3.35)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Odds ratios (95% confidence intervals) were derived from pooled logistic regression analysis applying 5-year intervals (1990–1995, 1995–2000, 2000–2005, and 2005–2010) and an update of covariate levels every 5 years. The main analysis disposed of 3072 observation periods. CI indicates confidence interval.

*Adjusted for age, sex, hypertension, log-transformed soluble vascular cell adhesion molecule-1, and symptoms suggestive of heart failure (New York Heart Association classes I–IV vs 0). These variables were selected by a forward stepwise selection procedure (P\textsubscript{env} and P\textsubscript{trace} 0.05 and 0.10, respectively).

Separate models were fitted for men (observation periods, 1990–1995), women (observation periods, 1990–1995), and in subjects without previous myocardial infarction (update every 5 years; observation periods, 2900).

†These models were additionally adjusted for self-reported maximum body weight, creatinine level, log-transformed C-reactive protein, current smoking (0 vs 1), alcohol consumption (gram/d), diabetes mellitus (0 vs 1), pulse pressure, and total thyroxine level.

In a final step, we investigated the potential effect of AF on mortality and used the entire 1990 study population for these purposes (n=932). Again multistate proportional hazards models were fitted as illustrated in Figure 2. In brief, subjects with both atherosclerosis and AF (baseline and follow-up) were more likely to die than those with either condition alone (P=0.0034) and faced a particularly high risk of death when compared with individuals free of both conditions (hazard ratio, 4.2; 95% CI, 2.6–6.8; P<0.0001). There was, however, no formal interaction observed between atherosclerosis and AF (P for interaction=0.49) with regard to all-cause mortality. Associations did not change substantially after additional adjustment for New York Heart Association categorization and hypertension.

### Discussion

In this prospective population-based cohort with repeated high-quality assessment of atherosclerosis during a period of 20 years, both prevalent and incident atherosclerosis were significantly associated with AF risk independently of age and conventional AF risk factors (Table 2; Figure 1). A majority of individuals developed AF on an atherosclerosis background, and the strength of association equaled that observed for hypertension. Subjects with both AF and atherosclerosis were significantly more likely to die than those with either condition alone (Figure 2), although a significant interaction was not achieved.

Only 2 previous population-based studies have investigated the potential relationship between AF and carotid atherosclerosis and yielded conflicting results. In the Rotterdam Study, prominent carotid atherosclerosis at baseline translated into an enhanced risk of new-onset AF during a mean follow-up of 7.5 years (relative risk, 1.5; 95% CI, 1.1–2.1), especially in men.8 In contrast, the Cardiovascular Health Study (CHS) failed to obtain a significant association. However, the analysis accommodating carotid plaques was cross-sectional in design.16 Moreover, the CHS has been criticized for the self-report of AF diagnosis in half of the study participants. Self-report critically depends on the individual’s memory, attitude, and motivation and is thus open to detection bias.

There are several potential mechanisms underlying the relation between atherosclerosis and AF found in our study and the Rotterdam Study: (1) Carotid atherosclerosis is a valid surrogate of systemic atherosclerosis as shown previously17 and evident in the Bruneck Study (see above). Presence of atherosclerosis at large arteries including the aorta conveys heightened arterial stiffness and elevated pulse pressure—an index of the pulsatile load on the heart.18–20 Both conditions have repeatedly been demonstrated to predispose to the development of AF.21–23 The increase in systolic cardiac afterload gives rise to sustained cardiac remodeling involving ventricular hypertrophy,24 impaired ventricular relaxation,25 increased intra-atrial pressure, left atrial dysfunction, and, finally, enlargement.26 Potential consequences are atrial fibrosis, microvascular damage, and, in cases of clinical AF,27–30 (2) As an alternative mechanism, atherosclerosis in the coronary arteries may directly cause ischemia or transitory hypoperfusion in the atrium, resulting in fibrosis and occurrence of AF.27,30,31 An excess
burden of obstructive coronary artery disease is well founded in patients with AF. In a recent study, half of the patients with idiopathic AF showed coronary artery disease in computed tomography angiography. In a case series of 100 consecutive patients with AF coming to autopsy, 9 cases had a stenosis or occlusion of the nodal artery, and in half of the cases, there was no other coronary pathology. An experimental study in dogs demonstrated that atrial ischemia significantly slows atrial conduction velocity and facilitates maintenance of AF. It has to be emphasized here that causality, although appealing and plausible (see above), cannot be inferred from our epidemiological study. Also, we cannot rule out that the association between atherosclerosis and AF originates from shared pathomechanisms. This scenario, however, is unlikely given the adjustment for a large number of candidate risk factors in our study and the repeated assessment of these factors allowing the performing of a continuous update of expression levels.

The present study has notable strengths including the use of a well-characterized population-based cohort with near-complete participation (>90%), the prospective design, the long-term follow-up of 20 years, rigorous analytic methodology (multistate competing risks models), and high-quality ascertainment of atherosclerosis and risk factors. A potential limitation is the sample size, which is sufficient for the key analysis but does not allow scrutinizing the potential effects of atherosclerosis plus AF on stroke risk. Moreover, it is not feasible to capture all cases of AF in prospective population studies. Especially, asymptomatic individuals with rare episodes of paroxysmal AF may escape a proper diagnosis. Finally, we used a definition of heart failure less stringent than that usually applied resulting in rather high prevalence rates.

Figure 1. Cumulative hazard of atrial fibrillation (AF) and cumulative incidence of atrial fibrillation by atherosclerosis status and sex, estimated under consideration of the competing risk of death. Top, Prevalent and incident atherosclerosis entailed a similar and significantly elevated hazard of AF. Bottom, The cumulative incidence of AF is a function of both AF hazard and mortality. Therefore, AF incidence curves do not exactly correspond to the AF hazard curves. In particular, the risk of death was substantially increased in subjects with prevalent atherosclerosis, which markedly reduced their lifetime AF incidence simply because they did not survive until manifestation of AF.
Overall, our study demonstrates a strong and independent association between atherosclerosis and AF but cannot prove causality. Nevertheless, it is appealing to hypothesize that rigorous prevention of atherosclerosis through lifestyle modification and medical therapy could confer benefit in the context of AF both in terms of disease manifestation and prognosis.

Sources of Funding

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Disclosures

None.

References


**Significance**

Our study yields evidence for a role of atherosclerosis in the manifestation of atrial fibrillation (AF). We obtained a significant association between carotid plaques and incident AF, which emerged as independent of classic AF and cardiovascular risk factors. Subjects with atherosclerosis and AF were significantly more likely to die than those with either condition alone. The prospective population-based Bruneck Study with its high-quality assessment of atherosclerosis during a period of 20 years served as a database for the analysis. Causality cannot be inferred from our study. Nevertheless, the data intend stimulating further research in this field and raise hope that rigorous prevention of atherosclerosis may help in fighting the expected AF epidemic.
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Supplemental Figure Ia: Incidence of Atrial Fibrillation (Per 1000 Person-Years) by Age Group in Men. The red line indicates incidence rates in the Bruneck Study while other colors visualize incidence rates from previous population-based studies (Renfrew/Paisely\textsuperscript{1}, ARIC\textsuperscript{2}, Rotterdam\textsuperscript{3}, Framingham\textsuperscript{4}, CHS\textsuperscript{5}).
Supplemental Figure Ib: Incidence of Atrial Fibrillation (Per 1000 Person-Years) by Age Group in Women. The red line indicates incidence rates in the Bruneck Study while other colors visualize incidence rates from previous population-based studies (Renfrew/Paisely\textsuperscript{1}, ARIC\textsuperscript{2}, Rotterdam\textsuperscript{3}, Framingham\textsuperscript{4}, CHS\textsuperscript{5}).
References


### Supplemental Table I: Baseline Characteristics of Study Subjects Free of Atrial Fibrillation at Baseline (n=900). Data Are Presented Separately for those with and without Baseline Carotid Atherosclerosis.

<table>
<thead>
<tr>
<th>Carotid Atherosclerosis (1990)</th>
<th>No (n=530)</th>
<th>Yes (n=370)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.0±9.4</td>
<td>66.1±8.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female sex, n (%)</td>
<td>288 (54.3)</td>
<td>154 (41.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Anthropometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>24.9±3.6</td>
<td>25.0±4.0</td>
<td>0.72</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.9±12.3</td>
<td>67.9±12.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.66±0.09</td>
<td>1.65±0.09</td>
<td>0.021</td>
</tr>
<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein, nmol/L</td>
<td>13.7 (8.0-27.4)*</td>
<td>14.9 (9.9-29.7)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Osteoprotegerin, pmol/L</td>
<td>3.4±0.9</td>
<td>4.1±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soluble VCAM-1, ng/ml</td>
<td>604.5 (486.6, 775.1)*</td>
<td>650.8 (565.5, 930.4)*</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Life-style and vascular risk variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>124 (23.4)</td>
<td>96 (25.9)</td>
<td>0.38</td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td>25.7±35.6</td>
<td>40.3±47.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>15 (2.8)</td>
<td>44 (11.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.4±0.5</td>
<td>5.7±1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.4±0.7</td>
<td>5.8±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>262 (49.4)</td>
<td>292 (78.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>138.9±18.7</td>
<td>154.7±22.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>87.6±10.0</td>
<td>90.7±9.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>51.4±11.6</td>
<td>64.0±16.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-Cholesterol, mmol/L</td>
<td>1.5±0.4</td>
<td>1.5±0.4</td>
<td>0.46</td>
</tr>
<tr>
<td>LDL-Cholesterol, mmol/L</td>
<td>3.5±1.0</td>
<td>3.8±1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tT&lt;sub&gt;4&lt;/sub&gt;, nmol/L</td>
<td>118.0±75.3</td>
<td>117.0±26.1</td>
<td>0.57</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>78.5±12.1</td>
<td>84.0±62.4</td>
<td>0.093</td>
</tr>
<tr>
<td><strong>Cardiovascular disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of myocardial infarction, n (%)</td>
<td>7 (1.3)</td>
<td>10 (2.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>NYHA ≥ 1, n (%)</td>
<td>54 (10.2)</td>
<td>141 (38.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean maximum CCA IMT (1995), mm</td>
<td>0.83±0.18</td>
<td>1.12±0.26</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented are mean±SD or numbers (%). VCAM-1, vascular cell adhesion molecule-1; HDL, high density lipoprotein; LDL, low density lipoprotein; tT<sub>4</sub>, total thyroxin level; NYHA, New York Heart Association classes 1-4 vs. 0; CCA, common carotid artery and IMT, intima-media thickness – this variable was not available in 1990.

* Median (inter-quartile range) is given for highly skewed variables.
Materials and Methods

Study Subjects
The Bruneck Study is a prospective, population-based cohort study on cardiovascular disease, which started in 1990. The study population consisted of 1000 subjects randomly selected and stratified by age and sex from inhabitants aged 40 to 79 living in Bruneck (n=4739), Northern Italy. A total of 932 subjects accepted to participate in the long-term follow-up survey and had a baseline ECG. Follow-up examinations were performed every 5 years (1995, 2000, 2005, 2010) and participation rates exceeded 90% each. The study protocol was approved by the Ethics Committees of Verona and Bolzano. Written informed consent was obtained from all participants before entering the study.

Ascertainment of AF
The diagnosis of AF was made according to the Minnesota coding system (Minnesota code 8.3) by two cardiologists from electrocardiograms (ECG) recorded a) at baseline and during the 4 follow-up study visits (standard 12-lead ECG), b) in the outpatient clinic and during hospitalization and, c) at the general practitioners office. We had access to virtually all ECGs recorded on study participants over the period of 20 years. In addition, medical records and discharge letters were obtained from the Bruneck hospital and reviewed for a diagnosis of AF. The situation in Bruneck is unique in that medical care is primarily provided in the Bruneck hospital to which residents of Bruneck usually are referred by general practitioners for diagnostic tests. Additionally, population mobility in this area has been low over the past 20 years. In total, 334 ECGs were performed per 1000 person-years of follow-up and 39 participants had one or more 24-hour Holter recordings. AF was diagnosed if paroxysmal or persistent AF or atrial flutter was present on ECG. The date of first occurrence of AF was recorded. We did not consider cases of AF if they occurred postoperatively or transiently in the context of acute coronary syndrome.

Assessment of Atherosclerosis
At baseline and during follow-up examinations (1995, 2000, 2005, 2010), carotid artery ultrasound scans were recorded for all subjects in a supine position using a duplex ultrasound system with a 10-MHz transducer and a 5-MHz Doppler. Four segments of the right and left carotid artery were examined in multiple planes: proximal common carotid artery (15 to 30 mm proximal to the carotid bulb), distal common carotid artery (<15 mm proximal to the carotid bulb), proximal internal carotid artery (carotid bulb and the initial 10 mm of the vessel), distal internal carotid artery (>10 mm above the flow divider). A plaque was defined as an echo structure protruding into the lumen with focal broadening of the vessel wall of at least 0.5 mm relative to adjacent segments. The extent of carotid atherosclerosis was quantified by a scoring system adding the maximum axial thickness of atherosclerotic plaques (in millimeters) on the near and far wall at each of the 8 vessel segments. This study uses a contrast of 0 (no plaque) versus 1 (one or more plaque in the 8 segments described). This categorization was found to be highly reproducible (inter-observer kappa-coefficient for the plaque definition, 0.84 indicating very good agreement). Intima-media thickness (IMT) was assessed at the far wall in plaque-free sites of the common carotid arteries (in 1995, 2000, 2005 and 2010) as the distance between the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface. The mean maximum IMT was calculated as the average of maximal IMT from the left and right sides.

The femoral arteries were scanned 40 mm proximal and 10 mm distal to the bifurcation in the superficial and deep branches (in 1995, 2000, 2005 and 2010). The above-mentioned criteria for plaque definition were used. Sonographic evaluation of the infrarenal
aorta was performed in 2010 with a 3.5-MHz curved array probe in longitudinal and transverse images to assess the maximum antero-posterior diameter. Presence of plaque was categorized as a dichotomous variable (no plaque, plaque) in order to analyze the overlap between atherosclerosis in the carotid and femoral arteries and the abdominal aorta.

All examinations and evaluations were performed by the same trained sonographer who was blinded to the participants clinical and laboratory information and had no information on the respective ECGs.

**Assessment of other Variables**

Participants were evaluated with regards to their medical history, life-style behaviors, socio-economic status and vascular risk factors using standardized questionnaires and interviews. Subjects were classified as current smokers or non-smokers. The number of pack-years was documented for each smoker and former smoker. Daily alcohol intake was quantified in grams per day. Physical examination data included weight and height measurements. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Life time maximum body weight was assessed by the subjects self-report. Blood pressure was taken in a sitting position after 10 minutes at rest and means of three independent measurements were used in this study. Hypertension was defined as systolic blood pressure over 140 or diastolic blood pressure over 90 or the use of antihypertensive drugs. Pulse pressure was defined as the difference between systolic blood pressure and diastolic blood pressure. Diabetes mellitus was diagnosed when fasting plasma glucose exceeded 7.8 mmol/L or when participants were under anti-diabetic medication. Myocardial infarction was deemed confirmed when World Health Organization criteria for definite disease status were met. The New York Heart Association classification was used to classify symptoms suggestive of heart failure but no systematic confirmation by X-ray or echocardiography was performed. Venous blood samples were taken in the morning after participants had fasted and abstained from smoking for at least eight hours. Serum concentrations of total and HDL cholesterol and triglycerides were measured with enzymatic techniques. Soluble vascular cell adhesion molecule-1 (VCAM-1) concentration was assessed in duplicate by commercially available enzyme-linked immunosorbent assay kits (Bender MedSystems, Milan, Italy). Intra- and inter-assay coefficients of variation were 4.8% and 11.2%, respectively. All other laboratory parameters including total (free plus protein-bound) thyroxine (tT4) were measured by standard methods, as described previously.

**Statistical analysis**

Calculations were performed with R (version 3.0.0, R Foundation for Statistical Computing, Vienna) and SPSS statistical software (version 18.0, SPSS Inc, Chicago, Ill). Descriptive statistics are reported as count (percentage) for dichotomous variables and mean ± SD for continuous variables. Person-years of follow-up for each participant were accrued from the 1990 baseline until diagnosis of AF, death or October 1st, 2010, whichever came first. Multivariable pooled logistic regression analysis, as proposed by D’Agostino et al, was used to test the association between carotid atherosclerosis and AF. This approach has been shown to be asymptotically equivalent to Cox regression analysis with time-dependent covariates in the case of short intervals between re-evaluations and low rates of events. We focused on 5-year intervals (1990-1995, 1995-2000, 2000-2005, and 2005-2010) and updated covariate levels for each 5-year period. On top of this conventional approach we employed multi-state proportional hazards models to further elaborate the relationship between atherosclerosis and incident atrial fibrillation and to assess the impact of AF and atherosclerosis on mortality. This type of analysis can be considered as a sequence of competing risks models, naturally accommodates the dynamics of sequential events, and allows for unbiased estimation of
cumulative incidence functions in the presence of competing risks through an Aalen-Johansen-type estimator. The multi-state model allows for initial (baseline), transient (drop in and possibly out), and absorbing (definite) states. Our AF model featured two initial states (prevalent atherosclerosis, no prevalent atherosclerosis), one transient state (incident atherosclerosis), and two absorbing states (incident AF, all-cause death), while our mortality model disposed of four initial states (prevalent atherosclerosis, prevalent AF, both and neither), three transient states (incident atherosclerosis, incident AF, atherosclerosis plus AF), and one absorbing state (all-cause death). In both models, sex was included as transition-specific covariate for all transitions and age was used as the time scale. The proportional hazards assumption was tested by computing the significance level of the correlation coefficient between Kaplan-Meier-transformed survival time and scaled Schoenfeld residuals for all variables. The Markov assumption, relevant to transient states, was tested by including the time point of entering the transient state as transition-specific covariate for transitions to the absorbing states. Both the proportional hazards and the Markov assumption were found tenable in our models. Statistical power was too low for fitting additional models targeting thromboembolic stroke.
References


