The manuscript complies with the STROBE reporting guidelines for observational studies.

**Ethics statement**

At baseline written informed consent was obtained from all participants for the study and follow up of electronic health records. The Human Ethics Committee of the University of Western Australia approved the study protocol and consent form (approval number 05/06/004/H50). The Human Research Ethics Committee of the Western Australian Department of Health (DOHWA HREC) also approved the data linkage study (approval number #2009/24).

**Study population**

The participants were recruited in 1998 to a 5-year prospective, randomised, controlled trial of oral calcium supplements to prevent osteoporotic fractures, the Calcium Intake Fracture Outcome study (CAIFOS). Women were recruited from the Western Australian general population of women aged over 70 years by mail using the electoral roll, which is a requirement of citizenship. Of the 5,586 women approached, 1,500 women were recruited into the study. All participants were ambulant with an expected survival beyond 5 years and were not receiving any medication (including hormone replacement therapy) known to affect bone metabolism. Baseline disease burden and medications were comparable between these participants and the general population of similar age although these participants were more likely to be from higher socio-economic groups. In the subsequent 5 years following inclusion in the study, participants received 1.2 g of elemental calcium as calcium carbonate daily or a matching placebo. As this trial commenced and completed prior to the introduction of the clinical trials registry the trial was retrospectively registered in the Australian New Zealand Clinical Trials Registry ACTRN12615000750583. From this larger RCT an ancillary study with B-mode carotid ultrasound examination was performed at year 3 (2001). Participants were included in this study if they had simple (BMI) Framingham risk factors
assessed in 1998, lateral spine images captured as part of osteoporosis screening in 1998 or 1999 and B-mode carotid ultrasound in year 3 (892 participants).

**Biochemistry**

Fasting blood samples were collected at baseline (1998). Total cholesterol, high-density lipoprotein cholesterol and triglyceride concentrations were determined using a Hitachi 917 auto analyser (Roche diagnostics). Low-density lipoprotein cholesterol was calculated using Friedewald’s method.

**Baseline cardiovascular disease risk assessment**

The participants provided their previous medical history and current medications verified by their General Practitioner. These data were coded using the International Classification of Primary Care – Plus (ICPC-Plus) method. The coding methodology allows aggregation of different terms for similar pathologic entities as defined by the ICD-10 coding system. These data were then used to determine the presence of pre-existing diabetes (T89001-90009). Cardiovascular medications included antihypertensive medications, statins and antiplatelet medications including low dose aspirin. Previous atherosclerotic vascular disease was determined from primary discharge diagnoses from Hospital records (1980-1998). Smoking status was coded as non-smoker or ex-smoker/current smoker if they had smoked more than 1 cigarette per day for more than 3 months at any time in their life. Weight was assessed using digital scales with participants wearing light clothes and no shoes. Height was assessed using a stadiometer and the body mass index was calculated in kg/m² at baseline. Blood pressure was measured on the right arm with a mercury column manometer using an adult cuff after the participants have been seated in an upright position and had rested for 5 minutes. An average of three blood pressure readings was recorded. Mean arterial pressure was calculated using the following equation = [(2 x diastolic blood pressure) + systolic blood pressure] / 3. Measures of renal function were available in 808/892 (90.2%) of the elderly women with
estimated glomerular filtration rate calculated using the CKD-EPI equation based on serum creatinine as described previously⁴.

**Abdominal aortic calcification 24 scores (AAC24)**

Due to resource constrains bone densitometry and lateral spine images were only collected in a subgroup of the original cohort in 1998 (baseline) with the majority (82%) collected in 1999 (Year 1). All abdominal aortic calcification scores from 0 to 24 were derived from digitally enhanced lateral single-energy images of the thoraco-lumbar spine using a Hologic 4500A machine (Hologic, Bedford, MA, USA). A single experienced investigator (JTS) read all images using the established technique in 2014⁵-⁷ blinded to carotid artery measures of atherosclerosis. Severity of AAC was categorized using previously published groupings: low (AAC24 score 0 or 1), moderate (AAC24 score 2-5) and severe (AAC24 score of greater than 5)⁷. As abdominal aortic calcification was assessed more than a decade after the images were captured the study the results were not communicated to the participants.

**B-mode carotid ultrasound**

The presence of carotid focal plaques and common carotid artery intimal medial thickness (CCA-IMT) were determined at year 3 of follow up. Assessment were performed using B-mode carotid ultrasound examination by a single sonographer with a 8.0 mHz linear array transducer fitted to an Acuson Sequoia 512 ultrasound machine using a standard image acquisition protocol in 2001⁸. The far walls of the distal 2cm of the left and right common carotid arteries were examined and images were taken from 3 different angles (anterolateral, lateral and posterolateral) to account for the possibility of asymmetrical wall thickening. End-diastolic images were recorded and a semi-automated edge-detection software program was used for image analysis. The same technician performed off-line analysis of all of the images, blinded to AAC scores. After assessment of CCA-IMT and focal plaque on the right side, the process was repeated on the left side. The CCA-IMT from each of the 6 images (3 on either
side) was averaged to give an overall mean CCA-IMT. Once IMT images were recorded, the entire carotid tree (CCA, carotid bulb, internal and external carotid) was examined for the presence of focal plaque defined as a clearly identified area of focal increased thickness (≥ 1 mm) of the intima-media layer. Severity of carotid plaque was further categorized by the degree of carotid stenosis as either minimal (<25%) or moderate (≥25%).

**Statistical analysis**

Spearman’s rank correlations were used to assess the relationship between AAC24 scores and mean and maximum CCA-IMT. Differences between the CCA-IMT for the 3 categories of AAC severity were tested using ANOVA or ANCOVA adjusted for traditional cardiovascular risk factors. Differences in the prevalence of focal carotid atherosclerotic plaque and moderate carotid stenosis were tested using unadjusted and multivariable-adjusted Poisson regression with robust variance as described by Barros and Hirakata et al. The ability to identify individuals with subclinical carotid atherosclerosis was assessed using the area under the receiver operating characteristic curve C-statistic. Additionally to quantify reclassification, net reclassification and integrated discrimination improvement tests were performed. Using multivariable-adjusted models, participants were classified into three categories for carotid atherosclerotic plaque, low (< 40%), intermediate (40%-50%) or high (≥ 50%) and moderate carotid stenosis; low (< 10%), intermediate (10-15%) or high (≥ 15%). The participants were then reclassified into new risk categories with the addition of measures of AAC to the model and the net reclassification or integrated discrimination improvement calculated. Model calibration was assessed by the Hosmer–Lemeshow goodness-of-fit test, which divides the cohort into deciles of predicted risk compared to the actual risk. Calibration assessed by the Hosmer–Lemeshow goodness-of-fit test is the agreement between the models predicted event rates with the observed events and P values < 0.05 indicate poor calibration of the overall model. Multivariable-adjustments for all analyses included traditional...
cardiovascular risk factors age, body mass index (or lipids in sensitivity analysis), current smoking, prescription of antihypertensive medication, antiplatelet medication, prevalent atherosclerotic vascular disease, year of scan, prevalent diabetes, systolic blood pressure and treatment code (calcium or placebo). All analyses were undertaken using IBM SPSS Statistics Version 21 (2012, Armonk, NY: IBM Corp), STATA (version 13 StataCorp LP, College Station, TX) or SAS (Version 9.4, SAS Institute Inc., Cary, NC). P-values of less than 0.05 in two tailed testing were considered statistically significant.
References


