**SUPPLEMENTARY MATERIALS**

**Bisphosphonates Use is Associated with Increased Coronary Artery Calcification in the General Population: The Rotterdam Study**

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**Figure S-1:** Flow diagram of study participant at baseline and follow-up

Total population of 3,229 were invited for a non-contrast MDCT scan



Not participated

N= 775

Scans that had artifacts and lacked prescription information were excluded; N= 111 and N= 14

Completed scans

N= 2,524 (78% response)



 

**Baseline population of the study**

**N= 2,399**

Unavailable population

N=814

Participants were invited for a follow-up scan

N= 1,599



Not participated

N=648

Completed scans

N= 951 (59.5% response)

Scans that had artifacts and lacked prescription information were excluded; N=89 and N=11

N=648



**Follow-up population of the study**

**N= 851**

**Methods S-1**: Assessment of arterial calcification

Noncontrast CT images were obtained using 16-slice (n=739) or 64-slice (n=1660) multidetector scanners (Somatom Sensation 16 or 64; Siemens, Forchheim, Germany). A cardiac scan (within a single breath hold) was acquired from the apex of the heart to the tracheal bifurcation, and another scan covered the area from the aortic arch to the intracranial vasculature (1 cm above the sella turcica). The field of view for both scans was optimized for visualization of blood vessels. Detailed scan parameters have been reported elsewhere (1, 2).

Coronary artery calcification (CAC), aortic arch calcification (AAC), and extracranial carotid artery calcification (ECAC) were quantified using commercially available software (Syngo CalciumScoring; Siemens). We evaluated the left main, left anterior descending, left circumflex, and right coronary arteries. The aortic arch was assessed from its origin (the slice where the ascending and descending aorta merge into the inner curvature of the arch) to the first centimeter of the common carotid, vertebral, and subclavian arteries beyond the origin of the vertebral arteries. Calcification in the extracranial carotid artery was measured bilaterally within three centimeters proximal and distal to the bifurcation.

Intracranial carotid artery calcification (ICAC) was assessed from the horizontal segment of the petrous internal carotid artery up to the circle of Willis. Because automated software is not available for this region (due to the close proximity of calcification to the skull base), we used a semiautomated scoring method described in detail elsewhere (1). This approach allowed manual delineation of calcified areas and calculation of calcification volume by multiplying the number of pixels above the calcium threshold (130 Hounsfield units) by the pixel size and slice increment. Trained readers, blinded to participant characteristics, performed all scoring. The interrater reliability of this method was excellent (intraclass correlation coefficient = 0.99), as reported previously (3).

No significant difference was found between the left and right internal carotid artery calcification volumes. Therefore, the sum of both sides was used for ECAC and ICAC in all analyses (3)

**Method S-2:** Measurement of covariates

Information on cardiovascular risk factors was obtained through standardized home interviews, physical examination, and blood sampling. Systolic and diastolic blood pressure were measured twice at the right arm using a random-zero sphygmomanometer, and the average of measurements was used. Hypertension was defined as having a systolic blood pressure higher than 160 mmHg or a diastolic blood pressure higher than 100 mmHg or taking blood pressure medication. Diabetes was defined as the use of anti-diabetic medications or a fasting glucose level greater than 7.1 mmol/l. Smoking behavior was categorized as current smoking and non-smoking. Waist circumference was measured midway between the lower rib margin and the iliac crest with participants in a standing position without heavy outer garments and with emptied pockets, breathing out gently. Hip circumference was recorded as the maximum circumference over the buttocks. Waist-to-hip ratio (WHR) was consequently calculated as the ratio of waist circumference over hip circumference. History of prevalent CVD was defined as a history of myocardial infarction, percutaneous transluminal coronary angioplasty (PCI), coronary artery bypass graft (CABG), or stroke. The information was collected either at the study's inception (1990–1993) or at the time each individual joined the study as described previously (4). Femoral neck bone mineral density (BMD) was measured using DXA, dual energy x-ray absorptiometry as one of the indices to identify osteoporosis.

**Method S-3:** Assessment of Bisphosphonate use, the cumulative duration

Prescription episode calculated by dividing the total number of dispensed tablets by the prescribed daily number. Additionally, for dosage comparisons, we calculated the average daily dose for each participant, expressed in the standardized ‘defined daily dose’ (DDD) in accordance with World Health Organization guidelines (5).

**Table S-1**: Characteristics of the follow-up population by bisphosphonate use

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Total** | | | **Total**  **N=851** | **Ever- users**  **N=147** | **Never-users**  **N=430** |
| Ever users | | N (%) | 147 (17.3%) | 147 (100%) | 0 (0%) |
| Current bisphosphonates users | | N (%) | 20.0 (2.4%) | 20.0 (13.6%) | N/A |
| Initiators | | N (%) | 95 (11.2%) | 95 (64.6%) | 0 (0%) |
| Cumulative duration of bisphosphonates use (ever users) from inception (years) | | Mean (SD)  Median [Min, Max] | 0.84 (2.66)  0 [0, 25.0] | 4.86 (4.65)  4.05 [0.00, 25.0] | N/A |
| Cumulative duration of bisphosphonates use among initiators (years) | | Mean (SD)  Median [Min, Max] | 2.91 (2.73)  2.08 [0.03, 12.03] | 2.91 (2.73)  2.08 [0.03, 12.03] | N/A |
| Average daily defined dose of bisphosphonates from inception | | Mean (SD)  Median [Min, Max] | 0.42 (3.28)  0 [0, 90.0] | 2.45 (7.58)  0.98 [0, 90.0] | N/A |
| Alendronate users (ever users) | | N (%) | 91 (10.7%) | 91 (61.9%) | N/A |
| Risedronate users (ever users) | | N (%) | 59 (6.9%) | 59 (40.1%) | N/A |
| Men | | N(%) | 391 (45.9%) | 38.0 (25.9%) | 353 (50.1%) |
| Age (years) | | Mean (SD)  Median [Min, Max] | 78.9 (4.21)  78.0 [73.0, 100] | 79.5 (4.37)  79.0 [73.0, 94.0] | 78.7 (4.17)  78.0 [73.0, 100] |
| Interval between the baseline and follow up CT scans | | Mean (SD)  Median [Min, Max] | 13.6 (0.512)  14.0 [13.0, 15.0] | 13.6 (0.498)  14.0 [13.0, 15.0] | 13.6 (0.514)  14.0 [13.0, 15.0] |
| Interval between inception and follow up CT scan (years) | | Mean (SD)  Median [Min, Max] | 18.8 (2.87)  18.0 [15.0, 27.0] | 18.8 (2.80)  18.0 [15.0, 26.0] | 18.9 (2.88)  18.0 [16.0, 27.0] |
| Presence of calcification | CAC | N (%) | 780 (91.7%) | 133 (90.5%) | 647 (91.9%) |
| AAC | N (%) | 826 (97.1%) | 142 (96.6%) | 684 (97.2%) |
| ECAC | N (%) | 756 (88.8%) | 131 (89.1%) | 625 (88.8%) |
| ICAC | N (%) | 780 (91.7%) | 133 (90.5%) | 647 (91.9%) |
| Calcification volume | CAC (mm3) | Mean (SD),  Median [Min, Max] | 504 (723)  209 [0, 4390] | 394 (628)  121 [0, 3260] | 527 (740)  222 [0, 4390] |
| AAC (mm3) | Mean (SD),  Median [Min, Max] | 1650 (2440)  870 [0, 27900] | 1710 (2120)  931 [10.1, 14100] | 1640 (2500)  848 [0, 27900] |
| ECAC (mm3) | Mean (SD),  Median [Min, Max] | 251 (482)  112 [0, 8610] | 219 (311)  108 [0, 1980] | 258 (511)  113 [0, 8610] |
| ICAC (mm3) | Mean (SD),  Median [Min, Max] | 504 (723)  209 [0, 4390] | 394 (628)  121 [0, 3260] | 527 (740)  222 [0, 4390] |

Follow up characteristics of the population were measured at the time of the follow up computed tomography (CT) scan. The follow up CT has been conducted after an average of 13.6 years from the baseline CT. Continuous variables are presented as mean, standard deviation (SD), median, minimum, and maximum [Min, Max], and categorical variables are presented as absolute numbers (percentage).

Ever use of bisphosphonates is defined as having at least one bisphosphonate prescription from inception until the time of the follow up CT scan. Current use is defined by a prescription that covers the time of the CT scan.

**Abbreviations:**

N/A: not applicable, CAC: coronary artery calcification, AAC: aortic arch calcification, ECAC: extracranial internal carotid artery calcification, ICAC: intracranial internal carotid artery calcification, N/A: not applicable

**Table S-2**: Characteristics of baseline population by follow-up participation status

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Total** | | | **With follow-up CT**  **N=851** | **Without follow-up CT**  **N=1548** |
| Ever BPs use\* | | N (%) | 61.0 (7.2%) | 163 (10.5%) |
| Age (years) | | Mean (SD)  Median [Min, Max] | 65.3 (4.13)  65.0 [59.0, 85.0] | 71.0 (6.99)  70.0 [59.0, 98.0] |
| Female | | N(%) | 460 (54.1%) | 796 (51.4%) |
| Diabetes mellitus | | N(%) | 86.0 (10.1%) | 233 (15.1%) |
| Waist to hip ratio | | Mean (SD)  Median [Min, Max] | 0.896 (0.0897)  0.898 [0.663, 1.15] | 0.918 (0.0918)  0.915 [0.648, 1.21] |
| Hypertension | | N(%) | 336 (39.5%) | 863 (55.7%) |
| Current smoking | | N(%) | 110 (12.9%) | 279 (18.0%) |
| Prevalent CVD | | N(%) | 29.0 (3.4%) | 129 (8.3%) |
| Interval between inception and the baseline CT (years)\* | | Mean (SD)  Median [Min, Max] | 5.27 (2.67)  4.00 [1.00, 14.0] | 6.62 (3.38)  4.00 [1.00, 13.0] |
| Cumulative duration of BPs (years)\* | | Mean (SD)  Median [Min, Max] | 0.121 (0.740)  0 [0, 9.05] | 0.188 (1.02)  0 [0, 12.0] |
| Average defined daily dose of BPs use\* | | Mean (SD)  Median [Min, Max] | 0.217 (3.13)  0 [0, 90.0] | 0.235 (2.48)  0 [0, 84.0] |
| Statin use\* | | N (%) | 165 (19.4%) | 423 (27.3%) |
| Vitamin K antagonists use\* | | N (%) | 54.0 (6.3%) | 238 (15.4%) |
| HDL cholesterol (mmol/L) | | Mean (SD)  Median [Min, Max] | 1.47 (0.393)  1.43 [0.670, 3.15] | 1.43 (0.397)  1.37 [0.680, 3.59] |
| Non-HDL cholesterol (mmol/L) | | Mean (SD)  Median [Min, Max] | 4.33 (0.917)  4.29 [1.73, 7.20] | 4.19 (0.976)  4.16 [1.21, 7.77] |
| Femoral neck bone mineral density (g/cm²)\* | | Mean (SD)  Median [Min, Max] | 29.0 (3.4%)  0.925 [0.480, 1.41] | 129 (8.3%)  0.925 [0.398, 1.45] |
| Alendronate use \* | | N (%) | 39.0 (4.6%) | 117 (7.6%) |
| Risedronate use\* | | N (%) | 21.0 (2.5%) | 39.0 (2.5%) |
| Presence of calcification | CAC | N (%) | 636 (74.7%) | 1332 (86.0%) |
| AAC | N (%) | 748 (87.9%) | 1474 (95.2%) |
| ECAC | N (%) | 551 (64.7%) | 1201 (77.6%) |
| ICAC | N (%) | 639 (75.1%) | 1328 (85.8%) |
| Calcification volume | CAC (mm3) | Mean (SD),  Median [Min, Max] | 149 (370)  15.4 [0, 5100] | 328 (595)  83.2 [0, 6920] |
| AAC (mm3) | Mean (SD),  Median [Min, Max] | 340 (683)  105 [0, 7240] | 942 (1410)  395 [0, 11900] |
| ECAC (mm3) | Mean (SD),  Median [Min, Max] | 51.9 (126)  6.20 [0, 1800] | 134 (237)  40.2 [0, 2830] |
| ICAC (mm3) | Mean (SD),  Median [Min, Max] | 64.3 (123)  19.5 [0, 1320] | 145 (210)  59.9 [0, 1720] |

The baseline population (N = 2,399) included participants who underwent baseline CT imaging. The table shows characteristics of the population at cohort inception, including BPs use, statin use, vitamin K antagonist use, femoral neck bone mineral density, and arterial calcification measured at baseline CT (the baseline). These variables which measured at baseline are marked with an asterisk (\*). Continuous variables are presented as mean, standard deviation (SD), median, minimum, and maximum [Min, Max], and categorical variables are presented as absolute numbers (percentage).

Prevalent CVD was described as a history of myocardial infarction, percutaneous transluminal coronary angioplasty (PCI), coronary artery bypass graft (CABG), or stroke. Non- HDL cholesterol level was calculated as total cholesterol minus HDL cholesterol.

**Abbreviations:**

N/A: not applicable, CAC: coronary artery calcification, AAC: aortic arch calcification, ECAC: extracranial internal carotid artery calcification, ICAC: intracranial internal carotid artery calcification, N/A: not applicable

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