Pharmacological Research 215 (2025) 107737

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

# High-Intensity Statins Promote PCSK9 Secretion and aortic valve calcification in patients with severe aortic stenosis: *In vitro* and clinical evidence

Veronika A. Myasoedova [a], Matteo Franchi [a,b], Donato De Giorgi [a], Alice Bonomi [a], Vincenza Valerio [a], Sergio Pirola [a], Niccolò Andreani [b], Valentina Rusconi [a], Francesca Bertolini [a], Ilaria Massaiu [a], Gianluca Pontone [a,c], Paolo Poggio [a,c,*,1]

[a] *Centro Cardiologico Monzino IRCCS, Milan 20138, Italy*
[b] *Unit of Biostatistics, Epidemiology and Public Health, Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan 20126, Italy*
[c] *Department of Biomedical, Surgical and Dental Sciences, University of Milan, Milan 20122, Italy*

ARTICLE INFO

*Keywords:*
Statin intensity
PCSK9
Aortic valve calcification
Aortic valve disease hospitalization

ABSTRACT

Aortic stenosis (AS) is the most common valvular disease, characterized by progressive fibro-calcific remodeling of the aortic leaflets, leading to increased morbidity and mortality. It is now well known that statins influence the production of proprotein convertase subtilisin/kexin type 9 (PCSK9), which in turn is linked to calcification. Here, we found that statins significantly increased, in a dose dependent manner, both PCSK9 secretion and valve interstitial cell (VIC) calcification, *in vitro*. These effects were blunted by PCSK9 genetic knock-down or by PCSK9 antibody neutralization. In AS patients, contrast-enhanced computed tomography evaluation showed a higher aortic valve calcium (AVC) content in patients on high-intensity statins compared to low-intensity ones, with no significant difference between low-intensity statin and non-users. At follow-up, high-intensity statin users exhibited a higher annual AVC accumulation compared to low-intensity statins and non-users. In a real-world scenario, high-intensity statin therapy was associated with a 30 % increased rate of hospitalization for non-rheumatic aortic valve disease. Our findings highlight the need for further investigation into the intricate relationship between statin therapy and aortic valve health to identify the optimal lipid-lowering strategy in the management of patients at risk of developing or afflicted by AS.

## 1. Introduction

Aortic valve stenosis (AS) represents the predominant aortic valve disease characterized by progressive fibro-calcific remodeling of the aortic valve leaflets [1]. This condition represents a significant clinical burden due to its association with increased morbidity and mortality [2], with no available pharmacological therapy. Since risk factors, similar to the atherosclerotic ones, such as dyslipidemia, obesity, hypertension, and diabetes have been identified to escalate the risk of AS, statins have been tested in this context. However, large randomized clinical trials, studying the impact of statins on AS progression, have not demonstrated a beneficial effect of statins in mitigating AS [3]. In particular, the ASTRONOMER trial found that rosuvastatin did not slow the progression of mild to moderate AS despite reducing LDL cholesterol [4]. The SEAS trial showed that simvastatin and ezetimibe lowered LDL cholesterol and coronary events but did not affect AS progression [5], as well as SALTIRE trial reported that atorvastatin reduced LDL cholesterol without halting calcific AS progression [6]. On the other end, statins have shown beneficial effect on atherosclerosis reducing the risk of adverse cardiovascular events [7]. In addition, several reports stated that long-term statins use could accelerate vascular calcification, probably enhancing plaque stability [8,9]. However, according to a recent meta-analysis, the effects of statins on coronary artery calcification (CAC) varied depending on the study type and measurement methods [10], hampering drawing conclusions. Indeed, cohort and cross-sectional studies showed a significant link between statin use and

* Correspondence to: Head of Unit for the Study of Aortic, Valvular, and Coronary Pathologies, Centro Cardiologico Monzino IRCCS, Via Carlo Parea 4, Milan 20138, Italy.
*E-mail addresses:* paolo.poggio@cardiologicomonzino.it, paolo.poggio@unimi.it (P. Poggio).
[1] Department of Biomedical, Surgical and Dental Sciences, University of Milan, Via della Commenda 10, 20122 Milan, Italy

https://doi.org/10.1016/j.phrs.2025.107737
Received 28 January 2025; Received in revised form 7 April 2025; Accepted 11 April 2025
Available online 14 April 2025

increased CAC, while randomized controlled trials did not find a significant effect [10].

Of note, statins, primarily recognized for their cholesterol-lowering effects, have been discovered to regulate the production of proprotein convertase subtilisin/kexin type 9 (PCSK9), a crucial player in cholesterol metabolism. A number of studies have proposed that statins may induce an elevation in the plasma concentration of PCSK9 [11], which in turn could potentially contribute to increased calcification rates of aortic leaflets. Indeed, recent findings have shown a causal effect of PCSK9 on aortic valve calcification (AVC), at the cellular level, in an animal model, and in genome-wide association studies [12,13].

It should also be mentioned that the FOURIER study demonstrated significant LDL cholesterol reduction and decreased cardiovascular events with evolocumab (a PCSK9 inhibitor), although its impact on AS progression was not a primary endpoint, a post-hoc analysis revealed a consistent reduction in AS events after one year of treatment [14].

Studying how statins, PCSK9, and AVC interact could lead to new treatments for managing AS. To this purpose, we conducted a series of *in vitro* experiments to investigate the potential of statins to induce PCSK9 secretion and consequently calcification of the aortic valve cells. Additionally, in our retrospective imaging study, we examined the dose-dependent effects of statins on the occurrence and progression of AVC in AS patients. Finally, to validate our hypothesis, we performed a large population-based retrospective cohort study on new users of low- or high-intensity statin therapy to evaluate the incidence of hospitalization for non-rheumatic aortic valve disease.

## 2. Materials and Methods

### 2.1. In-vitro study

Primary valve interstitial cells (VIC) have been isolated by enzymatic digestion from stenotic aortic valve leaflets, as previously described [15]. After the first *in vitro* expansion, VICs have been immortalized by two consecutive transductions using commercial lentiviral particles carrying out the encoding sequences for SV40 large T antigen (CMV-SV40LT; Genecopoeia, Rockville, MD, USA) and human telomerase reverse transcriptase (hTERT) enzyme (CMV-TERT; Genecopoeia, Rockville, MD, USA), under cytomegalovirus promoter. To verify successful transduction, we maintained in culture the cells for 30 passages.

The PCSK9 inhibition models have been generated by two different methods: i) by lentiviral transduction and ii) by antibody neutralization. The former has been obtained using commercial lentiviral particles carrying a short hairpin RNA against PCSK9 transcript (30 μl; Santa Cruz Biotechnology; Dallas, TX, USA), the latter treating cells with a neutralizing antibody against PCSK9 (0.8 ng/ml; BPS-71207, BPS Bioscience, San Diego, CA, USA).

To verify the extent of VIC calcification, we cultured the cells for seven days, adding treatments every other day. In particular, we added pravastatin (Sigma-Aldrich, St. Louis, MO, USA) and atorvastatin (Sigma-Aldrich, St. Louis, MO, USA) at different concentrations without changing media. At the end of the assay, we collected supernatants to detect secreted PCSK9 by a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA) and dissolved extracellular calcium in HCl (0.6 M) to detect calcium by a commercial colorimetric assay (Abcam, Cambridge, UK). After HCl incubation, cells have been incubated in NaOH 0.1 M with sodium dodecyl sulfate (SDS; 0.1 %) in order to quantify the total protein, by a bicinchoninic acid-based colorimetric assay (ThermoFisher Scientific, Waltham, MA, USA).

### 2.2. Imaging study

To evaluate the influence of statin intensity on aortic valve calcium load, we retrospectively enrolled 295 patients with severe aortic valve stenosis who underwent both Doppler echocardiography and cardiac CT within 3 months before aortic valve surgery in Centro Cardiologico Monzino IRCCS (2011–2023). Exclusion criteria were AS due to rheumatic disease or radiotherapy, previous infective endocarditis, previous severe adverse reactions to an iodinated contrast agent, body mass index (BMI) $\geq$ 38 kg/m$^2$, and severely impaired renal function (estimated glomerular filtration rate $<$ 30 ml/min/1.73 m$^2$). Patients undergoing both surgical aortic valve replacement and transcatheter valve implantation, with both bicuspid (BAV) and tricuspid (TAV) aortic valve morphology, were included in the final analysis. This study was approved by the Institutional Review Board of Centro Cardiologico Monzino IRCCS (n. R1348/20-CCM1418) and the participants gave written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Cardiac CT (Revolution CT, GE Healthcare, WI) was performed using the following parameters: section configuration 256 $\times$ 0.625 mm, voxel size 0.625 mm, spatial resolution along the X-Y planes 0.23 mm, gantry rotation time 280 msec, and iterative reconstruction. A BMI-adapted scanning protocol (tube voltage and current) was used [16]. All patients received a 50-ml bolus of contrast (Iomeprol 400 mg/ml, Bracco, Milan, Italy). Contrast attenuation values (Hounsfield Units, HU) and contrast-to-noise ratio were measured at the level of the ascending aorta. The calcium volumes were assessed and quantified using the ImageJ (Java-based image processing program developed at the National Institutes of Health) thresholding method as previously reported by us [17,18]. Indexed contrast-enhanced CT calcium amount (iAVC) was calculated dividing the calcium volume by the aortic annular area. AVC progression was assessed by measuring the delta of iAVC per year in patients who underwent multiple CT examinations before surgery, median follow-up of 2.5 years (inter quartile range; IQR:1.22–4.64). The indexed AVC cut-off threshold of 200 mm$^2$/mm$^3$ was chosen to be as conservative as possible. This threshold was set to minimize measurement errors. By setting the cut-off at 200 mm$^2$/mm$^3$, we aimed to ensure that any observed difference in AVC load progression greater than this value would be considered real and not due to measurement variance. Hemodynamic assessment of disease progression was performed on patients who underwent multiple ultrasound evaluations with a median follow-up of 2.6 years (IQR:1.62–5.12). Instrumental examinations conducted within six months of each other were excluded from the analysis.

### 2.3. Retrospective real-world cohort study

In this retrospective cohort study, we used population-based health care utilization databases from the Lombardy region (Italy). According to the Italian law, studies entirely based on registry data do not require approval from an ethics review board. All data were completely anonymized. All procedures were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The data spanned all Regional Health Service (RHS) beneficiaries and included demographic information (sex, year of birth, start and end dates of RHS beneficiary status, vital status, and date of death) and health care data, including diagnoses and interventions [coded through the International Classification of Diseases, ninth revision clinical modification (ICD-9-CM)-classification system] from all hospitals in the region and medications prescribed by RHS physicians to outpatients, dispensed directly by territorial pharmacies [coded through the Anatomic Therapeutic Chemical (ATC) classification system and the National Marketing Authorization code system] and fully or almost fully reimbursed by the RHS. Statins are also provided free or nearly free of charge by the NHS to all citizens without time limits on treatment, in line with the therapeutic principle of treating dyslipidemia for life. A unique and pseudonymized individual identification code was used to link each database to the others. Details of the regional databases and their use for pharmacoepidemiology studies are reported elsewhere [19]. The initial cohort included all beneficiaries of the RHS who were dispensed with statin drug from January 1, 2010, to December 31, 2018.

The date of the first dispensation was labeled as "index date". Moreover, we excluded patients who were dispensed with statins in the five years before the index date (in order to only include new statins users), patients aged less than 40, those who were not beneficiaries of the RHS for at least 2 years before the index date, and those with a previous diagnosis for aortic valve disease. Patients with less than 6 months of observation after the index date were also excluded. The remaining patients comprised the final study cohort. Statins were classified into the following classes: low-intensity (Simvastatin 10 mg, Pravastatin 1 mg, and Pravastatin 20 mg) and high-intensity (Rosuvastatin $\geq$ 10 mg, Atorvastatin $\geq$ 20 mg, and Simvastatin 80 mg). To minimize possible confounding of indication, low-intensity statin users were compared with high-intensity statin users using a 1:1 high dimensional propensity score (HDPS) matching design [20]. Four data dimensions were incorporated into the algorithm. Within each data dimension, the top 50 most prevalent covariates were selected, including inpatient and outpatient diagnostic and procedural codes, or dispensed medications, alongside demographic characteristics which were forced into the model. Finally, the propensity score estimation was generated combining the four dimensions. For each patient who started low-intensity therapy, one patient was randomly selected from those who started treatment with high-intensity statins and were matched for HDPS ( $\pm$ 0.005), sex, age ( $\pm$ 1 year), and index date ( $\pm$ 1 year). Patients were followed-up from six months after the index date (in order to exclude cases of aortic valve disease who were unlikely linked to statins) until the earliest date between the clinical outcome, migration to other country, death or five years after the index date. The clinical outcome of interest was hospitalization for non-rheumatic aortic valve disease (ICD-9 424.1).

### 2.4. Statistical analyses

The data generated by the in vitro experiments were compared with GraphPad Prism (9.4.1) using the one-way ANOVA, with the Dunnett post-hoc test or test for linear trend, and for the correlation analysis, using the Pearson's correlation coefficient (rp).

The data collected in the imaging study were analyzed using IBM SPSS statistic 26 software. Continuous variables were expressed as mean $\pm$ standard error (SEM) or, when variables had a skewed distribution, as median and interquartile range, while categorical ones were reported as frequencies and percentages. For the analysis among the groups, the one-way ANOVA was performed to test a linear trend, whereas the Kruskal-Wallis test with Dunn's post-hoc test was used for comparisons between groups. A $p < 0.05$ was considered statistically significant.

Regarding the retrospective cohort study, baseline characteristics were reported as absolute frequencies and percentages and were calculated for the low- and high-intensity statins groups. The cumulative incidence of non-rheumatic aortic valve disease was estimated considering all-cause death as the competing event. Differences in survival between the two groups were assessed using Gray's test. The association between exposure to high-intensity statins (intention-to-treat approach), as compared to low-intensity statins, and the risk of non-rheumatic aortic valve disease occurrence was evaluated by the Fine and Gray regression model, also accounting for death as a competing event. Since the matched groups were still unbalanced for hypertension, the model was adjusted for hypertension. Estimates were reported as sub-distribution hazard ratios (sHR) along with their 95 % confidence intervals (CI). The SAS statistical package (SAS Institute Inc., version 9.4, Cary, NC, USA) was used for these statistical analyses. The Number needed to harm (NNH), which can be interpreted as the number of individuals who should be treated with high-intensity statins so that one of them, on average, will develop the outcome of interest, was calculated as the reciprocal of the difference in the risk of non-rheumatic aortic valve between high-intensity and low-intensity statin users [21].

## 3. Results

### 3.1. Statins induce PCSK9 secretion and extracellular calcium accumulation in a dose-dependent manner

It is well-established that statins can elevate plasma PCSK9 concentrations, primarily produced and secreted by hepatocytes [11,22]. However, it remains unclear whether fibroblast-like cells, such as valve interstitial cells (VIC) within the aortic valve, exhibit a similar response to statin treatment. Moreover, previous findings have linked VIC-derived PCSK9 to extracellular calcium accumulation [12].

To investigate this, we examined the effect of statins on PCSK9 secretion and calcium accumulation in VICs isolated from stenotic aortic valves. Treatment with both pravastatin and atorvastatin resulted in a dose-dependent increase in PCSK9 secretion by VICs (both p for trend $<$ 0.001) with a concomitant significantly greater calcification (both p for trend $<$ 0.001), reveling a strong and direct correlation between them ($r_p > 0.94$ and $p < 0.0001$ for both statins; Fig. 1). In particular, pravastatin started to induce PCSK9 secretion at 10 μM (fold change; FC = +1.71 $\pm$ 0.22; p = 0.0623) with a strong effect at 100 μM (FC = +2.48 $\pm$ 0.36; $p < 0.0001$), whereas atorvastatin acted at 1 μM (FC = +3.28 $\pm$ 0.62; p = 0.0016) compared to the respective untreated condition (FC = 1.00 $\pm$ 0.03). Regarding calcification, a similar effect was noted with pravastatin that increased VIC calcification both at 10 μM (FC = +1.67 $\pm$ 0.11; p = 0.0383) and 100 μM (FC = +3.22 $\pm$ 0.38; $p < 0.0001$), whereas atorvastatin at 1 μM (FC = +4.26 $\pm$ 0.82; p = 0.0003) compared to the respective untreated condition (FC = 1.00 $\pm$ 0.12). Notably, the highest tested concentration of pravastatin (100 μM) induced PCSK9 secretion and calcium accumulation to a lesser extent compared to the highest tested concentration of atorvastatin (1 μM; p = 0.0678 and p = 0.0617, respectively; Supplementary Figure S1).

In order to evaluate whether the effects of statins on VIC extracellular calcification are mediated by PCSK9, we employed two *in vitro* models: genetic PCSK9 knockdown (KD) and pharmacological neutralization of extracellular PCSK9.

Using the genetic PCSK9 KD VICs, which secrete significantly less PCSK9 (-43.45 $\pm$ 2.14 %; $p < 0.0001$; Supplementary Figure S2), pravastatin failed to induce either PCSK9 secretion or extracellular calcium deposition (Fig. 2**A** and **B**). Conversely, atorvastatin-treated PCSK9 KD VICs (1 μM) still exhibited both PCSK9 secretion (FC = +1.88 $\pm$ 0.18; p = 0.0016) and calcium deposition (FC = +1.80 $\pm$ 0.22; p = 0.0043) compared to the respective untreated condition (FC = 1.00 $\pm$ 0.10), albeit to a lesser extent than non-genetically modified VICs (Fig. 2**C** and **D**).

In our second model, when PCSK9 was blocked with a neutralizing antibody (0.8 ng/ml), we observed lower PCSK9 secretion after pravastatin treatment at 100 μM (FC = −0.99 $\pm$ 0.30; p = 0.0074) and a similar result was obtained for extracellular calcium quantification (FC = −1.02 $\pm$ 0.35; p = 0.0193; Fig. 3**A** and **B**). In contrast, atorvastatin treatment at 1 μM showed no changes in PCSK9 secretion or calcium deposition under the same conditions (Fig. 3**C** and **D**).

These results suggest that statins with different intensity or belonging to different classes may have varying capacities to induce PCKS9 secretion and calcium accumulation in VICs, highlighting the potential differential impact on AVC.

### 3.2. High-intensity statins are associated with elevated aortic valve calcium load and progression

Based on *in vitro* evidence, we sought to evaluate the role of statin intensity on AVC load and progression, measured by contrast-enhanced cardiac CT.

We evaluated 295 patients with severe AS (111 no-statin, 85 low-intensity, and 99 high-intensity statins) before aortic valve replacement (Table 1). Patients on statins were predominantly male and had a

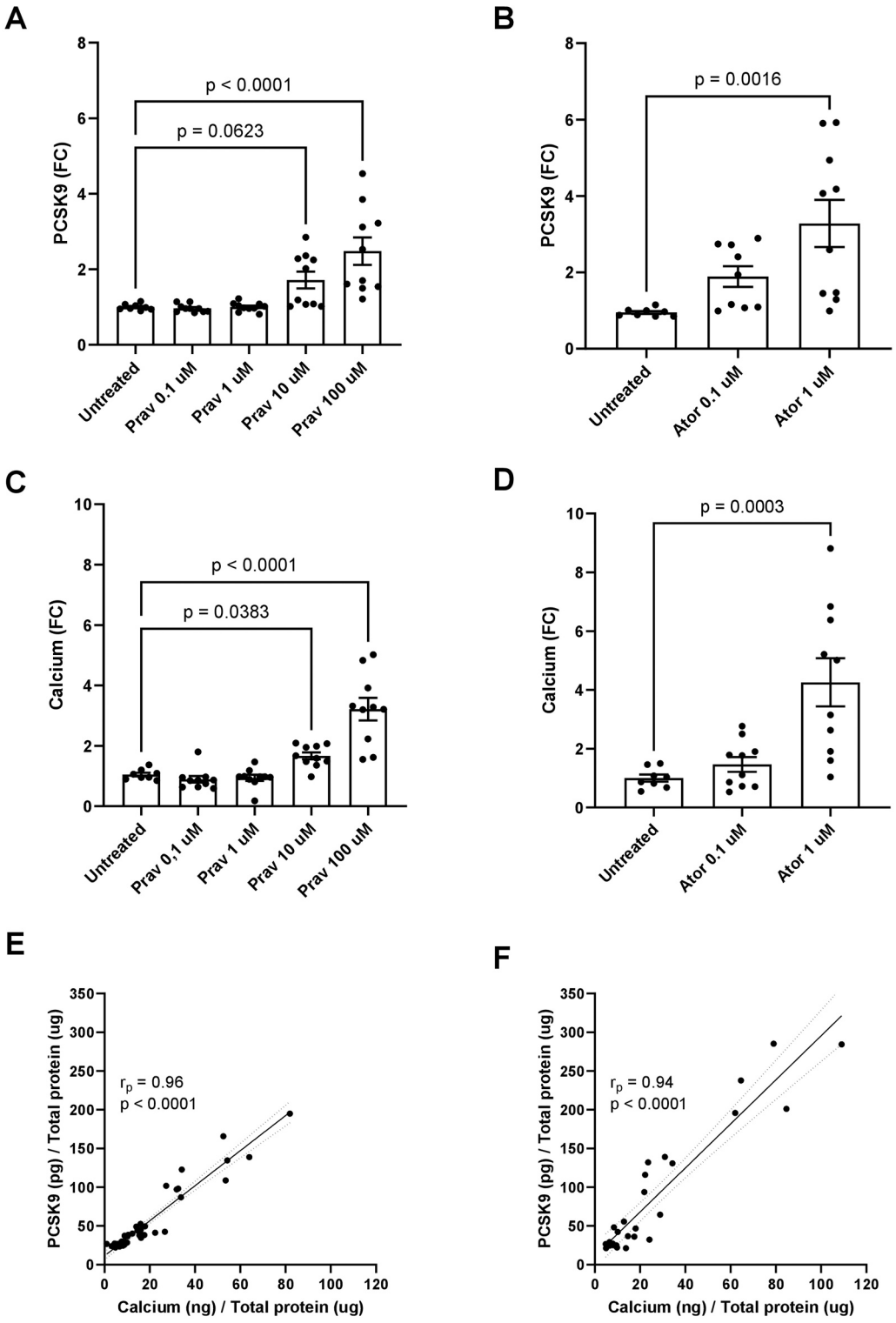


**Fig. 1. PCSK9 secretion and extracellular calcium accumulation of valve interstitial cells induced by different dose of statins.** (**A-B**) Bar graph represent the effect of statins (Prav: pravastatin; Ator: atorvastatin) on PCSK9 secretion in valve interstitial cells. (**C-D**) Bar graph represent the effect of statins on calcium accumulation in valve interstitial cells. Data were expressed in mean ± SEM, the comparisons were performed by the one-way ANOVA, with the Dunnett post-hoc test. (**E**) Correlation between PCSK9 secretion and calcium accumulation in valve interstitial cells under pravastatin treatment. (**F**) Correlation between PCSK9 secretion and calcium accumulation in valve interstitial cells under atorvastatin treatment. Correlation analyses were performed using the Pearson's correlation coefficient ($r_p$). Fold changes (FC) were calculated dividing the amount of secreted protein (pg of PCSK9 / ng of total proteins) or calcium (ng of calcium / ng of total proteins) under treatment by the secreted amount in basal condition (untreated).

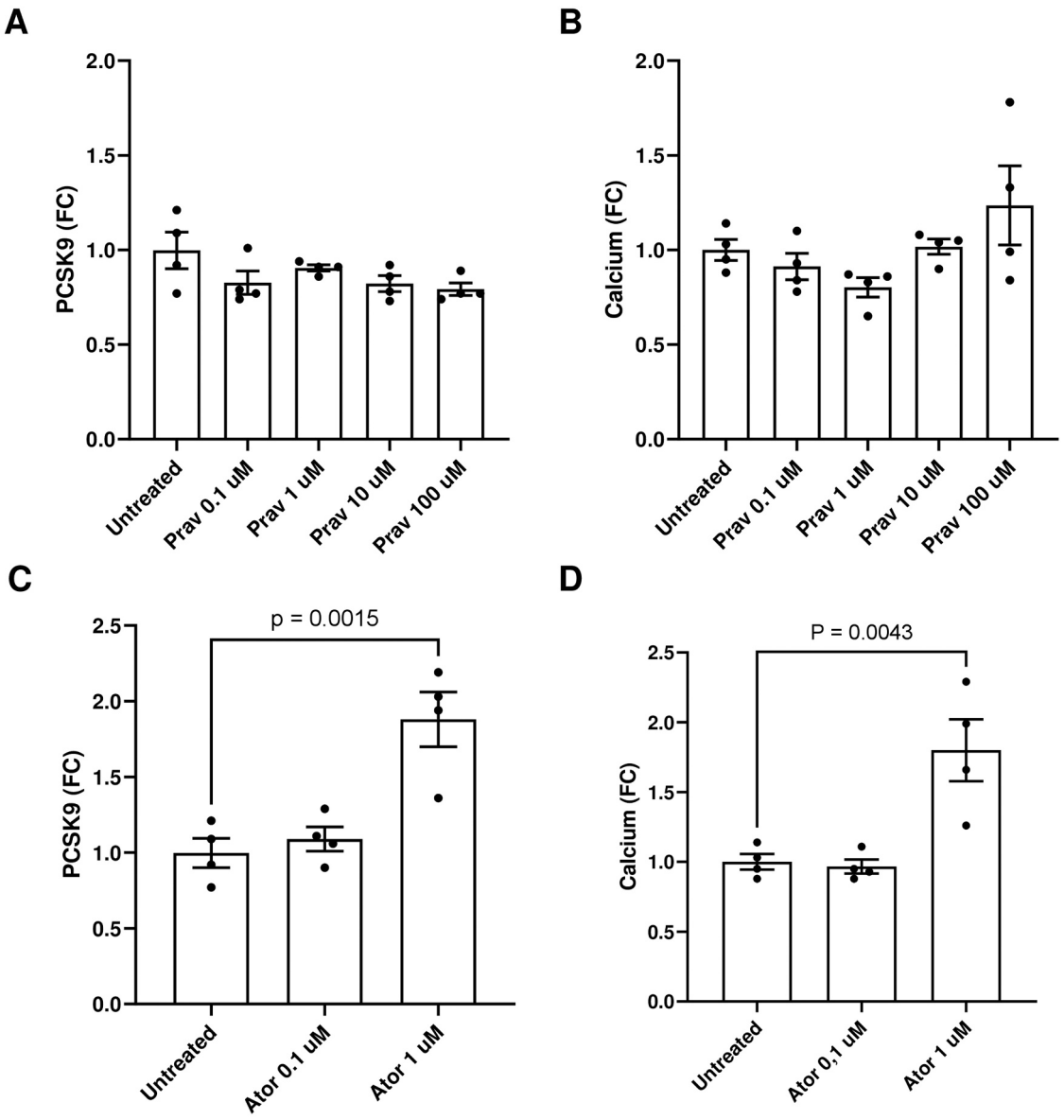


**Fig. 2. Effect of statins on genetic PCSK9 knock down (KD) valve interstitial cells.** (**A-B**) Bar graphs represent the effect of different dose of pravastatin (Prav) on PCSK9 secretion and calcium accumulation on PSCK9 KD VICs. (**C-D**). Bar graphs represent the effect of different dose of atorvastatin (Ator) on PCSK9 secretion and calcium accumulation on PSCK9 KD VICs. Data were expressed in mean ± SEM. Fold changes (FC) were calculated dividing the amount of secreted protein (pg of PCSK9 / ng of total proteins) or calcium (ng of calcium / ng of total proteins) under treatment by the secreted amount in basal condition (untreated).

higher incidence of hypertension, coronary artery disease, and peripheral artery disease. As expected, total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels were lower compared to patients without statin therapy. Notably, no differences were found between low-intensity and high-intensity statin groups.

We observed an intensity-dependent effect of statins on AVC load in patients with severe AS, with the highest AVC load present in severe AS patients under high-intensity statin treatment (none = 588.7 ± 39.19, low = 728.3 ± 58.14, and high = 817.3 ± 59.14 $mm^3/mm^2$ calcium volume; p for trend = 0.0013; Fig. 4**A**). We further investigated the impact of statin therapy on AVC load progression in 29 no-statin, 27 low-intensity, and 23 high-intensity statin-treated severe AS patients, who underwent multiple CT and echocardiographic evaluation of disease progression before surgery. No significant differences were found between the three groups regarding the clinical characteristics of the included patients (Table 2). Analyzing the variation of AVC load per year (median follow-up of 2.5 years; IQR:1.22–4.64), we found that patients receiving high-intensity statins exhibited a significant increase in calcium load (none: +69.00 ± 19.20, low: +64.85 ± 13.34, and high: +152.1 ± 36.92 $mm^3/mm^2$ calcium volume per year; p for trend = 0.021; Fig. 4**B**). Applying a threshold of an increment of 200 $mm^3/mm^2$ calcium volume per year, we observed a significantly increased incidence of AS patients exceeding this cut-off in the high-intensity statin treatment group. In particular, 6.9 %, 3.7 %, and 26 % of no-statin, low-intensity, and high-intensity statin users exceed the cut-off value, respectively (high-intensity *vs.* no-statin p = 0.057 and high-intensity *vs.* low-intensity p = 0.023). To elucidate if this AVC load accumulation was accompanied by hemodynamic changes, we assessed the standard echocardiographic parameters for AS evaluation (including aortic jet velocity, mean aortic gradient, and indexed aortic valve area), revealing the absence of a significant impact in 2.6 years of median follow-up (IQR:1.62–5.12; Fig. 4**C**).

These results corroborate the evidence that high-intensity statins may play a role in AVC presence and progression in severe AS patients.

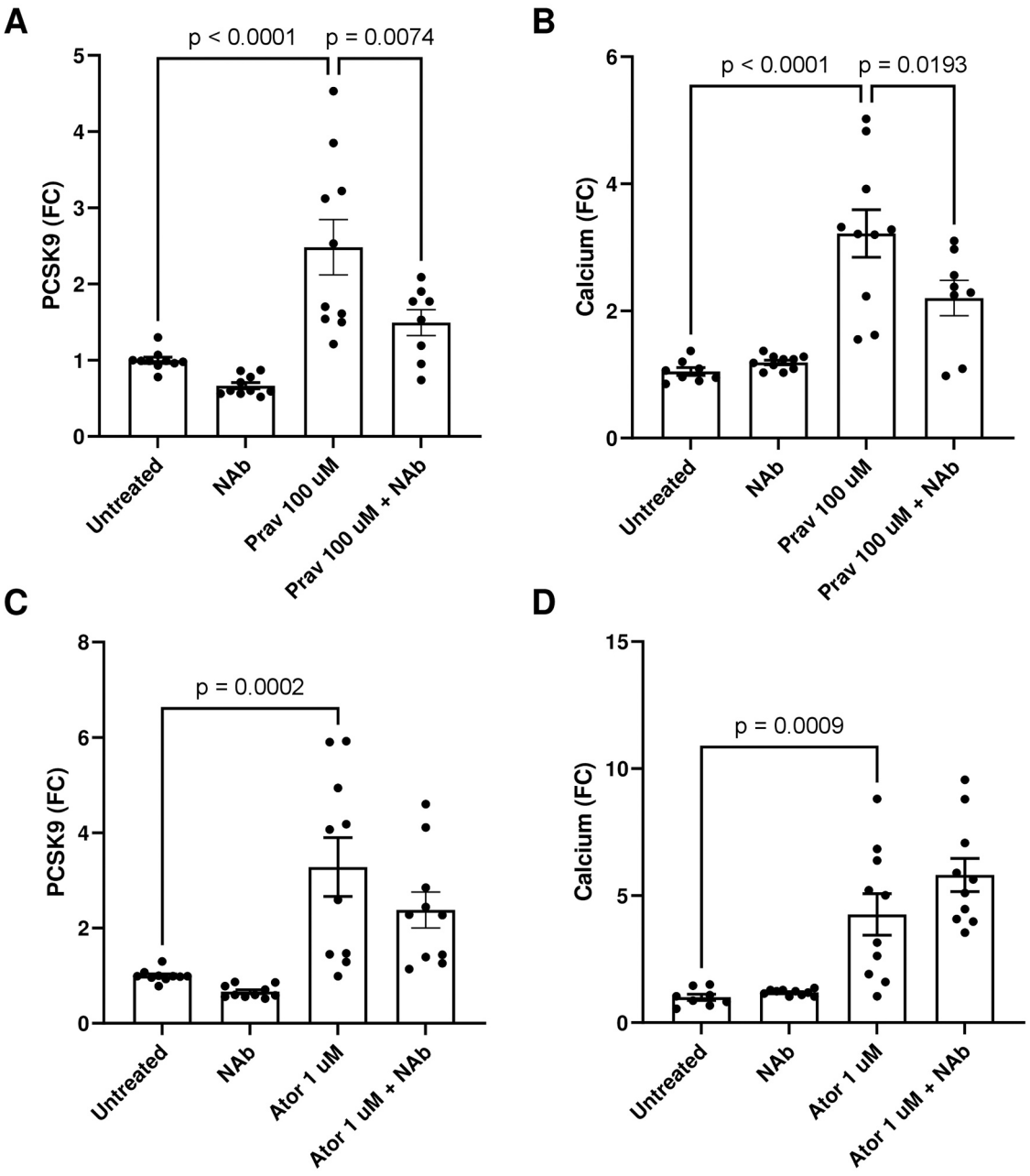


**Fig. 3. Effect of statin on PCSK9 secretion and calcium accumulation after PCSK9 blocking with a neutralizing antibody.** (**A-B**) Bar graphs represent the effect of pravastatin (Prav) with and without the PCSK9 neutralizing antibody (NAb) on PCSK9 secretion and calcium accumulation. (**C-D**) Bar graphs represent the effect of atrovastatin (Ator) with and without the PCSK9 neutralizing antibody on PCSK9 secretion and calcium accumulation. Data were expressed in mean ± SEM. Fold changes (FC) were calculated dividing the amount of secreted protein (pg of PCSK9 / ng of total proteins) or calcium (ng of calcium / ng of total proteins) under treatment by the secreted amount in basal condition (untreated).

### *3.3. High-intensity statins are associated with increased rate of hospitalization for non-rheumatic aortic valve disease in a real-world scenario*

Since increased AVC load could eventually lead to a worsening of the pathology and high-intensity statins could aggravate this situation, we evaluated the association between different intensity statins on the incidence of hospitalization for non-rheumatic aortic valve disease.

Between 2010 and 2018, a total of 850,298 new statins users were identified. After applying exclusion criteria, 196,493 new statins users (21,448 on low-intensity and 175,045 on high-intensity statins) were included in the study cohort (Supplementary Figure S3 **and** Supplementary Table S2). After matching, the two groups of 18,327 patients were well balanced for sex, age, multi-comorbidity score, and diabetes, except for the presence of hypertension, which was slightly higher in the high-intensity group (Table 3).

To assess the role of different statin potency on the incidence of hospitalization for non-rheumatic aortic valve disease, we analyzed the cumulative incidence starting from 6 months after initiation of statin therapy. After a follow-up of 4.5 years, 233 new hospitalizations for aortic valve disease occurred, of which 100 were in the low-intensity statin group and 133 were in the high-intensity statin group, corresponding to a cumulative incidence of 0.48 % and 0.65 %, respectively. An increased risk of hospitalization for non-rheumatic aortic valve disease of 34 % (95 % CI: 12–62 %) was observed in high intensity statin users, as compared to low intensity users, and this increase remained

**Table 1**
Baseline clinical characteristics of patients with severe aortic stenosis.

| Variables | All n = 295 | No-statin n = 111 | Low-intensity statin n = 85 | High-intensity statin n = 99 | p-Value |
|---|---|---|---|---|---|
| Age, years | 77.1 ± 9.1 | 76.3 ± 11.6 | 78.5 ± 7.2 | 76.8 ± 7.1 | 0.251 |
| Sex male, n (%) | 158 (54) | 41 (37) | 50 (58)* | 67 (69)# | < 0.001 |
| Body mass index, kg/m2 | 26.6 ± 4.7 | 26.2 ± 5.0 | 26.4 ± 4.3 | 27.3 ± 4.7 | 0.259 |
| Diabetes mellitus, n (%) | 76 (26) | 21 (19) | 22 (26) | 33 (33)# | 0.058 |
| Hypertension, n (%) | 241 (82) | 77 (69) | 77 (91)* | 87 (88)# | < 0.001 |
| Chronic obstructive pulmonary disease, n (%) | 46 (16) | 13 (12) | 14 (16) | 19 (19) | 0.320 |
| Smoking, n (%) | 63 (21) | 18 (16) | 21 (25) | 24 (24) | 0.246 |
| Coronary artery disease, n (%) | 130 (44) | 24 (22) | 44 (52)* | 62 (63)# | < 0.001 |
| Peripheral artery disease, n (%) | 97 (33) | 21 (19) | 30 (35)* | 46 (46)# | < 0.001 |
| Chronic kidney disease, n (%) | 38 (13) | 12 (11) | 10 (12) | 16 (16) | 0.483 |
| Left ventricular ejection fraction, (%) | 59.3 ± 9.9 | 61.0 ± 9.4 | 59.2 ± 9.7 | 57.4 ± 10.4# | 0.037 |
| Total cholesterol, mg/dL | 171 ± 40 | 188 ± 39 | 163 ± 38* | 159 ± 36# | < 0.001 |
| High-Density Lipoprotein-C, mg/dL | 55 ± 17 | 58 ± 19 | 54 ± 16 | 52 ± 15 | 0.061 |
| Triglycerides, mg/dL | 104 ± 43 | 101 ± 40 | 106 ± 46 | 106 ± 43 | 0.671 |
| Low-Density Lipoprotein-C, mg/dL | 96 ± 34 | 113 ± 32 | 88 ± 32* | 85 ± 29# | < 0.001 |

ANOVA post hoc tests, Tukey:
* Low-dose statin vs. No-statin; p-value < 0.05;
# High-dose statin vs. No-statin; p-Value < 0.05

significant even after adjusting for hypertension (30 %, 95 % CI: 7–57 %; Fig. 5). The corresponding NNH was 555 (95 % CI: 291–5764), indicating that, on average, 555 individuals treated with high intensity statin could generate a new case of hospitalization for non-rheumatic aortic valve disease.

## 4. Discussion

Our study found that high-intensity statins, besides lowering cholesterol, may also accelerate the calcification of aortic valves in AS patients. Through a series of experiments and analyses, we discovered that statins markedly increase the secretion of PCSK9 and the accumulation of calcium in VICs. This response translates into a greater burden of valve calcification, ultimately leading to more frequent hospitalization for aortic valve disease. Overall, our data highlight the need for in-depth studies evaluating statin therapy in patients with AS, considering the potential association with valve calcification and subsequent clinical outcomes.

Previous *in vitro* evidence suggests that statins can inhibit osteogenic differentiation and reduce calcium nodule deposition [23–25]. Indeed, atorvastatin has been shown to reduce alkaline phosphatase (*ALP*) expression and activity [23]. This may be related to enhanced autophagy, which then inhibits the nuclear factor kappa B (NF-κB) signaling pathway in VICs, when exposed to a pro-osteogenic stimulus [23]. Simvastatin has been shown to inhibit calcium nodule formation in a dose-dependent manner in VICs cultured on different substrates and also when stimulated with pro-fibrotic transforming growth factor beta 1 (TGFβ1) [26]. Additionally, simvastatin was able to reduce lipopolysaccharide (LPS) induced ALP and RUNX family transcription factor 2 (*RUNX2*) expression, thereby inhibiting calcium deposition in VICs [24]. Likewise, pravastatin has been shown to inhibit the formation of calcium nodules of cultured VICs in pro-fibrotic conditions [25]. However, it has also been shown that statins (e.g., simvastatin and atorvastatin), without any other concomitant treatment, were able to promote osteoblastic formation with a consequent increment in cellular mineralization [27]. Our results showed that statins directly increased calcium formation in VICs, matching the finding of the latter study. This discrepancy may be attributed to the differing culture conditions, as we did not stimulate the cells with any additional treatment, similarly to the approach of Maeda et al. [27]. This possibly reveals that when VICs are subjected to pro-fibrotic or pro-osteogenic stimuli, statin treatment could indeed ameliorate the situation. However, when VICs are derived from stenotic patients, likely with an already pre-activated osteogenic program, statins could induce cellular calcification as observed in bone cells. These findings suggest that VIC calcification under statin treatment could take different routes depending on their state at the time of isolation (e.g., normal, sclerotic, or stenotic aortic leaflets) as well as culture conditions, highlighting the need for further investigation.

Nevertheless, statin therapy has been proven a valuable tool to reduce atherosclerosis progression and prevent cardiovascular events [28]. However, in patients taking statin therapy, a significant and dose-dependent increase of circulating PCSK9 levels was noted, independent of statin type, treatment duration, and statin lipophilicity [11, 29,30]. The observed increase in PCSK9 under statin treatment can be attributed to the activation of the sterol regulatory element binding transcription factor 2 (*SREBF2*) under cholesterol depletion, which in turn binds to the promoter region of PCSK9 and induces its expression [11,31]. Thus, based on our *in vitro* evidence and the aforementioned clinical studies, we may infer that AS patients treated with low- and high-intensity statins could exhibit increased PCSK9 levels compared to non-statin users, with a more pronounced effect in the latter group. However, it should be noted that both genetic and pharmacological inhibitions of PCSK9 revealed a different effect of pravastatin and atorvastatin on PCSK9 secretion and calcium accumulation in VICs. While pravastatin (hydrophilic) completely abolished the increase of PCSK9 and calcium, atorvastatin (lipophilic) did not. These discrepancies could be due to statin class differences and related cellular pathway interference, as previously observed by Godoy et al. [32].

PCSK9 was found to be an independent predictor of calcific aortic valve disease [33,34] and recent investigations have highlighted the role of PCSK9 in aortic valve calcification [12,35]. A direct effect of PCSK9 on the development and progression of AS has been suggested by the study of aged *Pcsk9*$^{-/-}$ mice that exhibit lower AVC than wild type ones; moreover, *in vitro* experiments showed that *Pcsk9*$^{-/-}$ VICs were partially protected from calcification [13]. Of note, in humans, PCSK9 is highly expressed in calcified aortic valves, probably due to the expression of VIC-related PCSK9 [12]. Indeed, in human VICs subjected to a pro-osteogenic medium, PCSK9 levels increased and a neutralizing antibody anti-PCSK9 significantly reduced calcium accumulation [12]. Our results are in line with these findings since the inhibition of PCSK9, either genetic or with a neutralizing antibody, showed a reduction in VIC calcification.

It is well-known that AVC is associated with worsening cardiovascular events and all-cause mortality [36–39]. However, considering vascular calcification, the calcium formation within a coronary plaque can enhance features that reduce the risk of rupture, thereby increasing plaque stability [40,41]. To this regard, statins have been shown to reduce the risk of cardiovascular events, not only through their primary role represented by their lipid-lowering effect, but probably due to alternative mechanisms that act on plaque stabilization [28, 42–45]. One possible explanation, recently questioned [10], is that long-term use of statins may increase calcium deposition in atherosclerotic plaque [8, 44,46,47]. However, investigations on the progression of aortic valve

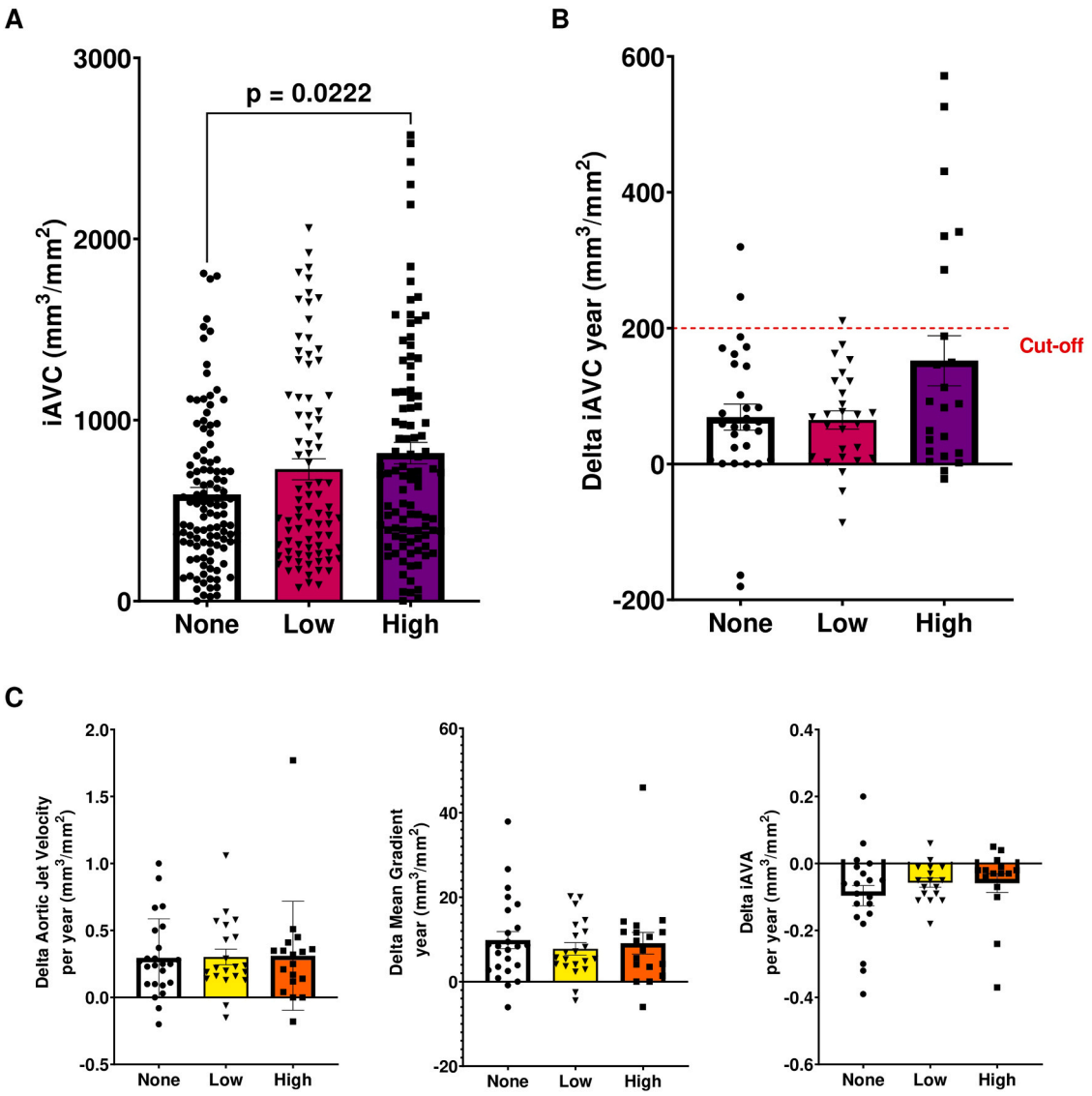


**Fig. 4. Statins and aortic valve calcium load and progression measured by computed tomography (CT).** (**A**) Bar graph represents the indexed aortic valve calcium (AVC) load measured by CT in patients with severe aortic stenosis (AS) who did not receive statin therapy (None), who received low-intensity statin therapy (Low: Atorvastatin ≤ 10 mg 20 %, Simvastatin ≤ 20 mg 64 %, Rosuvastatin ≤ 5 mg 7 %, and Pravastatin ≤ 40 mg 9 %), and patients who received high-intensity statin therapy (High: Atorvastatin ≥ 20 mg 65 %, Simvastatin ≥ 40 mg 7 %, and Rosuvastatin ≥ 10 mg 28 %). (**B**) Bar graph represents the progression of indexed AVC delta per year measured by CT in AS patients without statins and patients with low- and high-intensity statins (Low: Atorvastatin ≤ 10 mg 11 %, Simvastatin ≤ 20 mg 56 %, Rosuvastatin ≤ 5 mg 15 %, and Pravastatin ≤ 40 mg 18 %; High: Atorvastatin ≥ 20 mg 78 %, Simvastatin ≥ 40 mg 9 %, and Rosuvastatin ≥ 10 mg 13 %). The red dotted line represents the increment of indexed AVC cut-off set at 200 $mm^2/mm^3$. (**C**) Bar graphs represent the effect of statin use on progression of standard echocardiographic parameters for AS evaluation (delta of aortic jet velocity, mean aortic gradient, and indexed aortic valve area per year).

disease did not provide evidence supporting a protective effect of statin therapy. Indeed, results of observational studies and randomized clinical trials did not show any positive effect of statin use on aortic valve structure, function, or calcification as well as for clinical outcomes [3, 48]. In contrast, our results indicate that patients with severe AS who take high-intensity statins had an increased AVC burden, compared to those on low-intensity or no-statins therapy. However, the classical echocardiography parameters of AS severity were stable and in line with previous findings. These observations may indicate that AVC load builds up before critically influencing hemodynamics changes, thus requiring a long observational period to unveil if changes in echocardiographic parameters occur. To overcome this possible limitation, our real-world data analysis, on more than 35,000 patients followed up for 5 years, revealed a higher incidence rate of hospitalization for non-rheumatic aortic valve disease in new statins users receiving high-intensity therapy compared to those on low-intensity therapy.

These findings may be of clinical interest since novel and effective lipid-lowering drugs, such as PCSK9 inhibitors, have emerged, providing different pharmacological options to reduce low-density lipoprotein cholesterol in patients at risk of developing severe AS. In addition, the efficacy of these drugs in reducing AS-related events has already been shown [14]. This does not negate the proven benefits of statin therapy in post-aortic valve replacement patients, as recently suggested by the latest European Association for Cardiothoracic Surgery (EACTS; 2024) guidelines [49].

Our study has several limitations. First, we analyzed only two types of statins (pravastatin and atorvastatin) in our *in-vitro* study. However, this choice was done considering their varying classes and potencies. Second, our imaging study included retrospective data on calcium accumulation in patients with AS. It is warranted to study the evaluation of calcium accumulation in patients with non-severe AS, ranging from mild to moderate, in a prospective manner. Third, we included only

**Table 2**
Clinical characteristics of patients with severe aortic stenosis in follow-up cohort.

| Variables | All n = 79 | No-statin n = 29 | Low-intensity statin n = 27 | High-intensity statin n = 23 | p-Value |
|---|---|---|---|---|---|
| Age, years | 71.4 ± 10.6 | 68.9 ± 12.6 | 74.2 ± 8.5 | 71.4 ± 10.6 | 0.181 |
| Sex male, n (%) | 57 (72) | 17 (59) | 21 (78) | 19 (83) | 0.118 |
| Body mass index, kg/m2 | 27.3 ± 4.9 | 28.3 ± 5.1 | 27.2 ± 5.5 | 26.3 ± 3.7 | 0.353 |
| Diabetes mellitus, n (%) | 22 (28) | 9 (31) | 7 (26) | 6 (26) | 0.058 |
| Hypertension, n (%) | 71 (90) | 25 (86) | 25 (93) | 21 (91) | 0.713 |
| Chronic obstructive pulmonary disease, n (%) | 14 (18) | 5 (17) | 6 (22) | 3 (13) | 0.704 |
| Smoking, n (%) | 18 (23) | 7 (24) | 6 (22) | 5 (22) | 0.977 |
| Coronary artery disease, n (%) | 40 (51) | 13 (45) | 15 (56) | 12 (52) | 0.722 |
| Peripheral artery disease, n (%) | 21 (27) | 7 (24) | 7 (26) | 7 (30) | 0.878 |
| Chronic kidney disease, n (%) | 6 (8) | 5 (17) | 1 (0.4) | 0 (0) | 0.483 |
| Left ventricular ejection fraction, (%) | 57.9 ± 10.1 | 56.6 ± 9.8 | 59.2 ± 9.7 | 59.4 ± 9.8 | 0.610 |
| Total cholesterol, mg/dL | 154 ± 40 | 144 ± 42 | 155 ± 26 | 167 ± 49 | 0.170 |
| High-Density Lipoprotein-C, mg/dL | 51 ± 16 | 45 ± 13 | 55 ± 15 | 55 ± 20 | 0.051 |
| Triglycerides, mg/dL | 98 ± 42 | 93 ± 37 | 97 ± 40 | 106 ± 49 | 0.531 |
| Low-Density Lipoprotein-C, mg/dL | 87 ± 36 | 82 ± 36 | 79 ± 22 | 104 ± 45 | 0.058 |

ANOVA post hoc tests, Tukey: * Low-dose statin vs. No-statin; p-value < 0.05; # High-dose statin vs. No-statin; p-Value < 0.05;

**Table 3**
Characteristics of real-world cohort patients included in 1:1 match analysis.

| | Low-Intensity statins n = 18.736 | | High-Intensity statins n = 18.736 | | p-Value |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Female | 11,366 | 60.5 | 11,336 | 60.5 | 1.000 |
| Male | 7400 | 39.5 | 7400 | 39.5 | |
| Age | | | | | |
| < 70 years | 3368 | 18.0 | 3380 | 18.0 | 0.9982 |
| 70–84 years | 5030 | 26.9 | 5027 | 26.8 | |
| > 84 years | 5914 | 31.6 | 5902 | 31.5 | |
| MCS (multi comorbidity score) | 4424 | 23.6 | 4427 | 23.6 | |
| Low | 14,707 | 78.5 | 14,753 | 78.7 | 0.1248 |
| Medium | 3475 | 18.5 | 3374 | 18.0 | |
| High | 554 | 3.0 | 609 | 3.3 | |
| Diabetes | 3354 | 17.9 | 3351 | 17.9 | 0.9677 |
| Hypertension | 10,144 | 54.1 | 10,353 | 55.3 | 0.0301 |

patients who underwent multiple CT and echocardiographic evaluations for clinical reasons, excluding instrumental examinations conducted within six months of each other. Although we were able to evaluate only 27 % of patients at follow-up, our results constitute a real-world evaluation. Fourth, in our real-world data, we assessed hospitalization for non-rheumatic aortic valve disease as the endpoint without specifying the type of aortic valve disease, whether insufficiency or stenosis. However, given that AS is the most common aortic valve disorder, and considering the number of subjects included, we assumed that majority of hospitalizations for new statins users were primarily due to AS. Finally, given the paucity of individual clinical characteristics available in the regional health care utilization databases, we cannot exclude that unmeasured residual confounding may have affected our results.

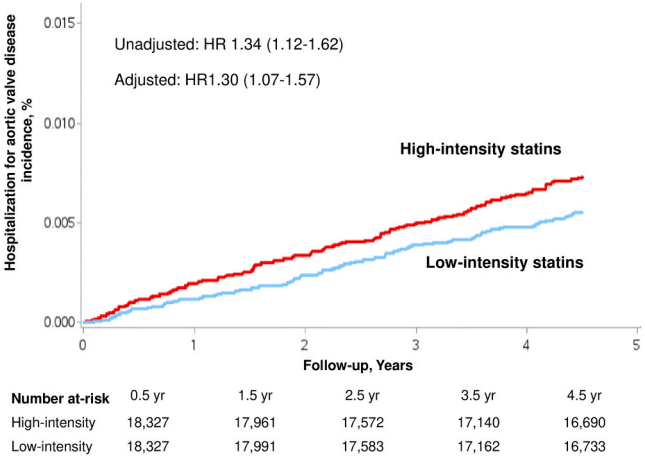


**Fig. 5. Effect of statin potency on the incidence of hospitalization for non-rheumatic aortic valve disease.** Cumulative incidence of hospitalization for non-rheumatic aortic valve disease in new low- and high intensity statin users after 4.5 years of follow-up. HR: hazard ratio.

## 5. Conclusions

In summary, high-intensity statin therapy is associated with elevated PCSK9 secretion and increased aortic valve calcification, as evidenced by *in-vitro* experiments and CT imaging evaluation, in patients with severe AS. Additionally, real-world data, from a substantial patient cohort, support our findings by revealing a higher incidence rate of hospitalization for non-rheumatic aortic valve disease among patients receiving high-intensity statins compared to those on low-intensity statins. These findings highlight the need for further investigation into the intricate relationship between statin therapy and aortic valve health to identify the optimal lipid-lowering strategy in the management of patients at risk to develop or with AS.

## Funding

This research was funded by the Italian Ministry of Health (Ricerca Corrente to Centro Cardiologico Monzino IRCCS), Italian Ministry of University and Research ('PRIN 2017', project 2017728JPK) and Fondazone Gigi & Pupa Ferrari ONLUS (FPF-14 to P.P.).

## CRediT authorship contribution statement

**Massaiu Ilaria:** Writing – review & editing, Formal analysis, Data curation. **Bertolini Francesca:** Writing – review & editing, Investigation, Data curation. **Rusconi Valentina:** Writing – review & editing, Investigation, Formal analysis, Data curation. **De Giorgi Donato:** Writing – review & editing, Investigation, Data curation. **Franchi Matteo:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Poggio Paolo:** Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Myasoedova Veronika A.:** Writing – original draft, Formal analysis, Data curation, Conceptualization. **Pontone Gianluca:** Writing – review & editing, Investigation, Conceptualization. **Andreani Niccolò:** Writing – review & editing, Formal analysis, Data curation. **Pirola Sergio:** Writing – review & editing, Investigation, Data curation. **Valerio Vincenza:** Writing – review & editing, Investigation, Data curation. **Bonomi Alice:** Writing – review & editing, Formal analysis, Data curation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

None

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phrs.2025.107737.

## Data availability

Data will be made available on request.

## References

[1] B.R. Lindman, M.A. Clavel, P. Mathieu, B. Iung, P. Lancellotti, C.M. Otto, P. Pibarot, Calcific aortic stenosis, Nat. Rev. Dis. Prim. 2 (2016) 16006.
[2] A.S. Peters, J.P. Duggan, G.D. Trachiotis, J.L. Antevil, Epidemiology of Valvular Heart Disease, Surg. Clin. North Am. 102 (3) (2022) 517–528.
[3] A. Parolari, E. Tremoli, L. Cavallotti, M. Trezzi, S. Kassem, C. Loardi, F. Veglia, G. Ferrari, D. Pacini, F. Alamanni, Do statins improve outcomes and delay the progression of non-rheumatic calcific aortic stenosis? Heart 97 (7) (2011) 523–529.
[4] K.L. Chan, K. Teo, J.G. Dumesnil, A. Ni, J. Tam, Investigators A. Effect of Lipid lowering with rosuvastatin on progression of aortic stenosis: results of the aortic stenosis progression observation: measuring effects of rosuvastatin (ASTRONOMER) trial, Circulation 121 (2) (2010) 306–314.
[5] A.B. Rossebo, T.R. Pedersen, K. Boman, P. Brudi, J.B. Chambers, K. Egstrup, E. Gerdts, C. Gohlke-Barwolf, I. Holme, Y.A. Kesaniemi, W. Malbecq, C. A. Nienaber, S. Ray, T. Skjaerpe, K. Wachtell, R. Willenheimer, S. Investigators, Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis, N. Engl. J. Med 359 (13) (2008) 1343–1356.
[6] S.J. Cowell, D.E. Newby, R.J. Prescott, P. Bloomfield, J. Reid, D.B. Northridge, N. A. Boon, Aortic S. Scottish, Lipid Lowering Trial IoRI. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis, N. Engl. J. Med 352 (23) (2005) 2389–2397.
[7] N.P. Kadoglou, M. Stasinopoulou, N. Velidakis, E. Khattab, E. Christodoulou, E. Gkougkoudi, G. Valsami, The Complex Mechanisms and the Potential Effects of Statins on Vascular Calcification: A Narrative Review, Rev. Cardiovasc Med 25 (2) (2024) 51.
[8] K.S. Ngamdu, D.S. Ghosalkar, H.E. Chung, J.L. Christensen, C. Lee, C.A. Butler, T. Ho, A. Chu, J.R. Heath, M. Baig, W.C. Wu, G. Choudhary, A.R. Morrison, Long-term statin therapy is associated with severe coronary artery calcification, PLoS One 18 (7) (2023) e0289111.
[9] Z. Xinyu, M. Dongxia, H. Yue, J. Xiao, L. Wang, J. Xiaoping, Statins Accelerate Coronary Calcification and Reduce the Risk of Cardiovascular Events, Cardiol. Rev. 31 (6) (2023) 293–298.
[10] M.N. Shahraki, S.M. Jouabadi, D. Bos, B.H. Stricker, F. Ahmadizar, Statin Use and Coronary Artery Calcification: a Systematic Review and Meta-analysis of Observational Studies and Randomized Controlled Trials, Curr. Atheroscler. Rep. 25 (11) (2023) 769–784.
[11] A. Sahebkar, L.E. Simental-Mendia, F. Guerrero-Romero, J. Golledge, G.F. Watts, Effect of statin therapy on plasma proprotein convertase subtilisin kexin 9 (PCSK9) concentrations: a systematic review and meta-analysis of clinical trials, Diabetes Obes. Metab. 17 (11) (2015) 1042–1055.
[12] N. Perrot, V. Valerio, D. Moschetta, S.M. Boekholdt, C. Dina, H.Y. Chen, E. Abner, A. Martinsson, H.D. Manikpurage, S. Rigade, R. Capoulade, E. Mass, M.A. Clavel, T. Le Tourneau, D. Messika-Zeitoun, N.J. Wareham, J.C. Engert, G. Polvani, P. Pibarot, T. Esko, J.G. Smith, P. Mathieu, G. Thanassoulis, J.J. Schott, Y. Bosse, M. Camera, S. Theriault, P. Poggio, B.J. Arsenault, Genetic and In Vitro Inhibition of PCSK9 and Calcific Aortic Valve Stenosis, JACC Basic Transl. Sci. 5 (7) (2020) 649–661.
[13] P. Poggio, P. Songia, L. Cavallotti, S.S. Barbieri, I. Zanotti, B.J. Arsenault, V. Valerio, N. Ferri, R. Capoulade, M. Camera, PCSK9 Involvement in Aortic Valve Calcification, J. Am. Coll. Cardiol. 72 (24) (2018) 3225–3227.
[14] B.A. Bergmark, M.L. O'Donoghue, S.A. Murphy, J.F. Kuder, M.V. Ezhov, R. Ceska, I. Gouni-Berthold, H.K. Jensen, S.L. Tokgozoglu, F. Mach, K. Huber, Z. Gaciong, B. S. Lewis, F. Schiele, J.W. Jukema, T.R. Pedersen, R.P. Giugliano, M.S. Sabatine, An Exploratory Analysis of Proprotein Convertase Subtilisin/Kexin Type 9 Inhibition and Aortic Stenosis in the FOURIER Trial, JAMA Cardiol. 5 (6) (2020) 709–713.
[15] D. Moschetta, E. Di Maria, V. Valerio, I. Massaiu, M. Bozzi, P. Songia, Y. D'Alessandra, V.A. Myasoedova, P. Poggio, Purinergic Receptor P2Y2 Stimulation Averts Aortic Valve Interstitial Cell Calcification and Myofibroblastic Activation, Biomedicines 10 (2) (2022).
[16] A.D. Annoni, D. Andreini, G. Pontone, M.E. Mancini, A. Formenti, S. Mushtaq, A. Baggiano, E. Conte, M. Guglielmo, G. Muscogiuri, M. Muratori, L. Fusini, D. Trabattoni, G. Teruzzi, A.I. Coutinho Santos, M. Agrifoglio, M. Pepi, CT angiography prior to TAVI procedure using third-generation scanner with wide volume coverage: feasibility, renal safety and diagnostic accuracy for coronary tree, Br. J. Radio. 91 (1090) (2018) 20180196.
[17] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, Nat. Methods 9 (7) (2012) 671–675.
[18] V.A. Myasoedova, I. Massaiu, D. Moschetta, M. Chiesa, P. Songia, V. Valerio, V. Alfieri, R. Capoulade, D. Trabattoni, D. Andreini, E. Mass, V. Parisi, P. Poggio, Sex-Specific Cell Types and Molecular Pathways Indicate Fibro-Calcific Aortic Valve Stenosis, Front Immunol. 13 (2022) 747714.
[19] G. Mancia, F. Rea, M. Ludergnani, G. Apolone, G. Corrao, Renin-Angiotensin-Aldosterone System Blockers and the Risk of Covid-19, N. Engl. J. Med 382 (25) (2020) 2431–2440.
[20] S. Schneeweiss, J.A. Rassen, R.J. Glynn, J. Avorn, H. Mogun, M.A. Brookhart, High-dimensional propensity score adjustment in studies of treatment effects using health care claims data, Epidemiology 20 (4) (2009) 512–522.
[21] C. Andrade, The numbers needed to treat and harm (NNT, NNH) statistics: what they tell us and what they do not, J. Clin. Psychiatry 76 (3) (2015) e330–333.
[22] B. Cariou, K. Si-Tayeb, C. Le May, Role of PCSK9 beyond liver involvement, Curr. Opin. Lipido 26 (3) (2015) 155–161.
[23] M. Chen, S. Liu, Atorvastatin reduces calcification in valve interstitial cells via the NF-kappaB signalling pathway by promoting Atg5-mediated autophagy, Eur. J. Histochem 68 (2) (2024).
[24] M.J. Jarrett, Q. Yao, N. Venardos, M.J. Weyant, T.B. Reece, X. Meng, D. A. Fullerton, Simvastatin down-regulates osteogenic response in cultured human aortic valve interstitial cells, J. Thorac. Cardiovasc Surg. 161 (4) (2021) e261–e271.
[25] J.A. Benton, H.B. Kern, L.A. Leinwand, P.D. Mariner, K.S. Anseth, Statins block calcific nodule formation of valvular interstitial cells by inhibiting alpha-smooth muscle actin expression, Arterioscler. Thromb. Vasc. Biol. 29 (11) (2009) 1950–1957.
[26] E.L. Monzack, X. Gu, K.S. Masters, Efficacy of simvastatin treatment of valvular interstitial cells varies with the extracellular environment, Arterioscler. Thromb. Vasc. Biol. 29 (2) (2009) 246–253.
[27] T. Maeda, A. Matsunuma, I. Kurahashi, T. Yanagawa, H. Yoshida, N. Horiuchi, Induction of osteoblast differentiation indices by statins in MC3T3-E1 cells, J. Cell Biochem 92 (3) (2004) 458–471.
[28] F. Taylor, M.D. Huffman, A.F. Macedo, T.H. Moore, M. Burke, G. Davey Smith, K. Ward, S. Ebrahim, Statins for the primary prevention of cardiovascular disease, Cochrane Database Syst. Rev. 2013 (1) (2013) CD004816.
[29] T. Nozue, Lipid Lowering Therapy and Circulating PCSK9 Concentration, J. Atheroscler. Thromb. 24 (9) (2017) 895–907.
[30] A.V. Khera, A. Qamar, M.P. Reilly, R.L. Dunbar, D.J. Rader, Effects of niacin, statin, and fenofibrate on circulating proprotein convertase subtilisin/kexin type 9 levels in patients with dyslipidemia, Am. J. Cardiol. 115 (2) (2015) 178–182.
[31] H.J. Jeong, H.S. Lee, K.S. Kim, Y.K. Kim, D. Yoon, S.W. Park, Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2, J. Lipid Res 49 (2) (2008) 399–409.
[32] J.C. Godoy, I.R. Niesman, A.R. Busija, A. Kassan, J.M. Schilling, A. Schwarz, E. A. Alvarez, N.D. Dalton, J.C. Drummond, D.M. Roth, G. Karanigas, H.H. Patel, A. E. Zemljic-Harpf, Atorvastatin, but not pravastatin, inhibits cardiac Akt/mTOR signaling and disturbs mitochondrial ultrastructure in cardiac myocytes, FASEB J. 33 (1) (2019) 1209–1225.
[33] W.G. Wang, Y.F. He, Y.L. Chen, F.M. Zhao, Y.Q. Song, H. Zhang, Y.H. Ma, X. Guan, W.Y. Zhang, X.L. Chen, C. Liu, H.L. Cong, Proprotein convertase subtilisin/kexin type 9 levels and aortic valve calcification: A prospective, cross sectional study, J. Int Med Res 44 (4) (2016) 865–874.
[34] J. Ljungberg, M. Janiec, I.A. Bergdahl, A. Holmgren, J. Hultdin, B. Johansson, U. Naslund, A. Siegbahn, T. Fall, S. Soderberg, Proteomic Biomarkers for Incident Aortic Stenosis Requiring Valvular Replacement, Circulation 138 (6) (2018) 590–599.
[35] A. Langsted, B.G. Nordestgaard, M. Benn, A. Tybjaerg-Hansen, P.R. Kamstrup, PCSK9 R46L Loss-of-Function Mutation Reduces Lipoprotein(a), LDL Cholesterol, and Risk of Aortic Valve Stenosis, J. Clin. Endocrinol. Metab. 101 (9) (2016) 3281–3287.
[36] J.L. Christensen, S. Tan, H.E. Chung, D.S. Ghosalkar, R. Qureshi, A. Chu, W. Yu, J. Berus, N.R. Shah, W.C. Wu, H. Chun, E. Aikawa, G. Choudhary, A.R. Morrison, Aortic valve calcification predicts all-cause mortality independent of coronary calcification and severe stenosis, Atherosclerosis 307 (2020) 16–20.
[37] P. Raggi, M.C. Gongora, A. Gopal, T.Q. Callister, M. Budoff, L.J. Shaw, Coronary artery calcium to predict all-cause mortality in elderly men and women, J. Am. Coll. Cardiol. 52 (1) (2008) 17–23.
[38] R. Detrano, A.D. Guerci, J.J. Carr, D.E. Bild, G. Burke, A.R. Folsom, K. Liu, S. Shea, M. Szklo, D.A. Bluemke, D.H. O'Leary, R. Tracy, K. Watson, N.D. Wong, R. A. Kronmal, Coronary calcium as a predictor of coronary events in four racial or ethnic groups, N. Engl. J. Med 358 (13) (2008) 1336–1345.
[39] Y. Hata, J. Mochizuki, S. Okamoto, H. Matsumi, K. Hashimoto, Aortic calcification is associated with coronary artery calcification and is a potential surrogate marker for ischemic heart disease risk: A cross-sectional study, Med. (Baltim. ) 101 (29) (2022) e29875.
[40] M.H. Criqui, J.O. Denenberg, J.H. Ix, R.L. McClelland, C.L. Wassel, D.E. Rifkin, J. J. Carr, M.J. Budoff, M.A. Allison, Calcium density of coronary artery plaque and risk of incident cardiovascular events, JAMA 311 (3) (2014) 271–278.

[41] R. Puri, S.J. Nicholls, M. Shao, Y. Kataoka, K. Uno, S.R. Kapadia, E.M. Tuzcu, S. E. Nissen, Impact of statins on serial coronary calcification during atheroma progression and regression, J. Am. Coll. Cardiol. 65 (13) (2015) 1273–1282.
[42] Heart Protection Study Collaborative G. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial, Lancet 360 (9326) (2002) 7–22.
[43] G.G. Schwartz, A.G. Olsson, M.D. Ezekowitz, P. Ganz, M.F. Oliver, D. Waters, A. Zeiher, B.R. Chaitman, S. Leslie, T. Stern, Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering Study I. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial, JAMA 285 (13) (2001) 1711–1718.
[44] H.S. Hecht, S.M. Harman, Relation of aggressiveness of lipid-lowering treatment to changes in calcified plaque burden by electron beam tomography, Am. J. Cardiol. 92 (3) (2003) 334–336.
[45] A. Healy, J.M. Berus, J.L. Christensen, C. Lee, C. Mantsounga, W. Dong, J. P. Watts Jr., M. Assali, N. Ceneri, R. Nilson, J. Neverson, W.C. Wu, G. Choudhary, A.R. Morrison, Statins Disrupt Macrophage Rac1 Regulation Leading to Increased Atherosclerotic Plaque Calcification, Arterioscler. Thromb. Vasc. Biol. 40 (3) (2020) 714–732.
[46] S.E. Lee, J.M. Sung, D. Andreini, M.J. Budoff, F. Cademartiri, K. Chinnaiyan, J. H. Choi, E.J. Chun, E. Conte, I. Gottlieb, M. Hadamitzky, Y.J. Kim, A. Kumar, B. K. Lee, J.A. Leipsic, E. Maffei, H. Marques, G. Pontone, G. Raff, S. Shin, P.H. Stone, H. Samady, R. Virmani, J. Narula, D.S. Berman, L.J. Shaw, J.J. Bax, F.Y. Lin, J. K. Min, H.J. Chang, Differential association between the progression of coronary artery calcium score and coronary plaque volume progression according to statins: the Progression of AtheRosclerotic PlAque DetermIned by Computed TomoGraphic Angiography Imaging (PARADIGM) study, Eur. Heart J. Cardiovasc Imaging 20 (11) (2019) 1307–1314.
[47] M. Henein, G. Granasen, U. Wiklund, A. Schmermund, A. Guerci, R. Erbel, P. Raggi, High dose and long-term statin therapy accelerate coronary artery calcification, Int J. Cardiol. 184 (2015) 581–586.
[48] Y. Zhao, R. Nicoll, Y.H. He, M.Y. Henein, The effect of statins on valve function and calcification in aortic stenosis: A meta-analysis, Atherosclerosis 246 (2016) 318–324.
[49] A. Jeppsson, B. Rocca, E.C. Hansson, T. Gudbjartsson, S. James, J.K. Kaski, U. Landmesser, G. Landoni, P. Magro, E. Pan, H.B. Ravn, S. Sandner, E. Sandoval, M.S. Uva, M. Milojevic, E.S.D. Group, EACTS Guidelines on perioperative medication in adult cardiac surgery, Eur. J. Cardiothorac. Surg. 2024 (2024).