Review of: "Time-dependent Role of Bisphosphonates on Atherosclerotic Plaque Calcification"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The manuscript by Amirala Bakhshian Nik et al. aims to further study the impact of bisphosphonates (BiPs) on atherosclerotic plaque calcification. They previously showed that a BiP treatment modulated microcalcification morphology ex vivo and in vivo (Jessica L. Ruiz *et al.*, PNAS, 2021).

In this new study, the authors treated atherosclerotic mice with BiP for different durations, starting at the onset of microcalcification or later in atherosclerotic development with more advanced calcification. They reported that BiP treatment associated with more calcification in total aorta as in aortic root. These effects were observed both in males and females, but with subtle differences. Females showed overall more calcification for the 10 week-time point, but males seem to develop larger calcifications. This treatment led in parallel to a marked increase in bone mineralization, correlated with treatment durations.

Given the important role of vascular calcification in cardiovascular diseases and the current lack of dedicated treatment, this work addresses an important question, highly relevant in plaque and calcification biology.

-The different effects of BiP between the 5 week- and 15 week- time points are interpreted as a differential effect of BiP on nascent microcalcifications vs more mature calcification. One cannot overrule that these differences originate mostly from the difference of treatment durations. Only the calcification area for the males seems to contradict this hypothesis, but the rest of the results seem to point towards this trend.

-Figure 1: BiP had an impact on vascular calcification but was there an effect on plaque size and inflammation, as it plays a major role in calcification formation? Did the treatment impact mouse weight and lipid profile?

-Figure 2: What about the median/distribution of calcification minerals sizes?

-Figure 2: It is not clear where is the calcification located within the aortic root (plaque, valves?). Serial section with a standard HE and/or VK staining would help.

-Figure 5: Experimental atherosclerosis models can be associated with osteoporosis. Was it the case in this study?

-Figures 1 and 5: symbols do not appear properly on the pdf.

-Figures 3 and 5: format/resolution should be fixed.

-Figure 3: these graphs are the same results as in the Figure 2 but reorganized. This Figure could be removed.

-Figure 1D-G are same results as in the Figure 1B-C. These panels could be removed.