

Enzymes of the vitamin K cycle and progression of calcification in the vessel wall

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Introduction and aim:

Vascular calcification is a leading cause of cardio- and cerebrovascular diseases. Vitamin K metabolites, especially K1 and MK-4, are associated with decreased vascular calcification particularly in patients with chronic kidney disease.

We investigate the expression of components of the vitamin K cycle (VKC) and the MK-4 synthesis (MKS) in aorta and bone to identify differences in expression patterns during atherosclerosis (AS) stages in vascular tissue and compare these profiles in both tissue types.

Material and methods:

Gene expression levels of components of the VKC (VKOR, VKORC1L1, GGCX, the chaperone CALU) and the MKS enzymes NQO1 and UBIAD1 were examined with predesigned TaqMan gene expression assays on a LC480 system in aorta and bone of 26 brain dead organ donors. Beta actin was used as a reference gene and relative Cp values were obtained by division.

Calcification stages were determined histologically and were assigned to one of three groups: 1. no changes (unaffected vessels), 2. intima thickening (more than one-fold thickening of the intima without calcification), 3. intima calcification (one or more calcification spots are present).

Enzymes of vitamin K cycle

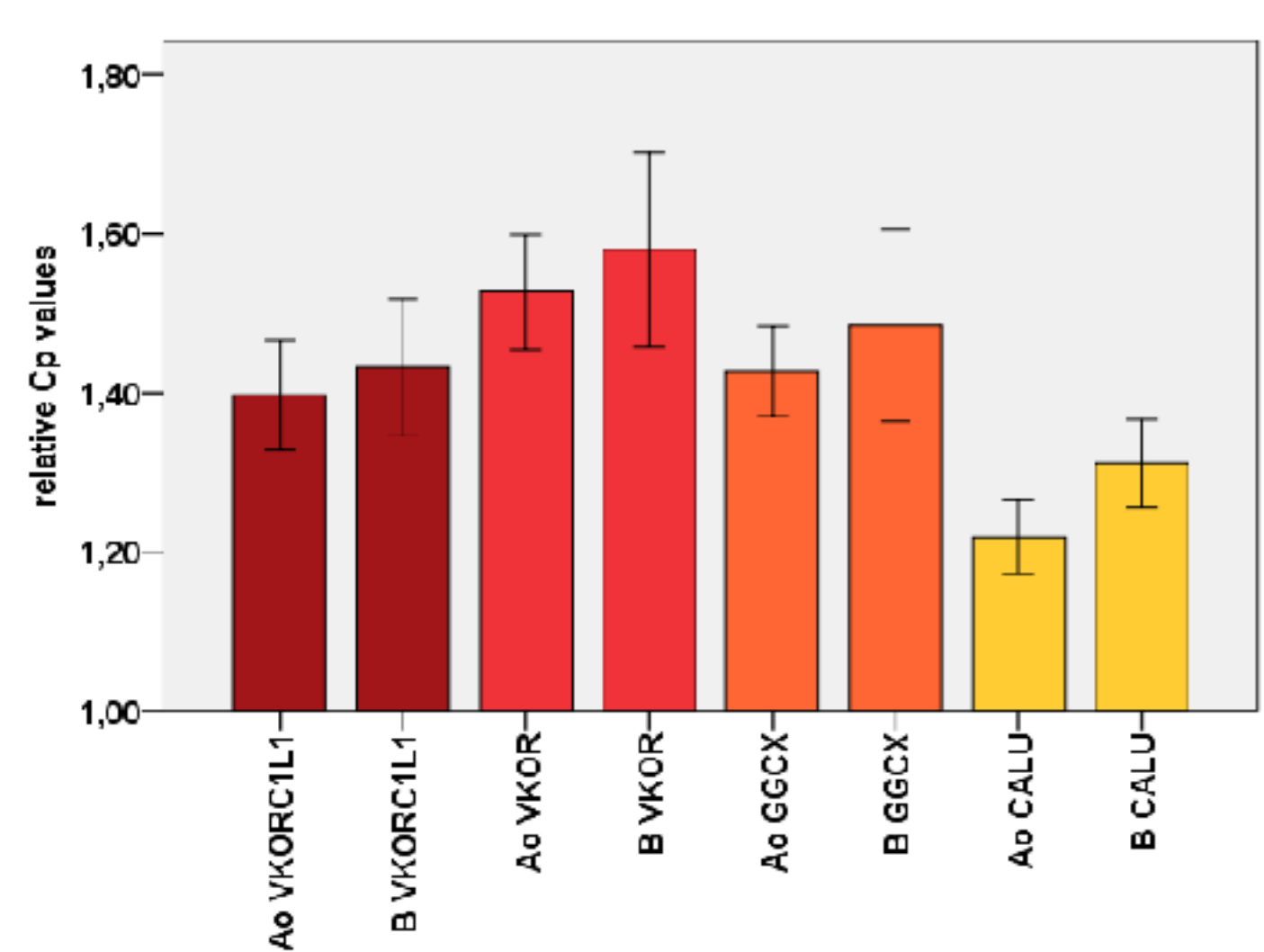


Fig.1: The VKC enzymes VKORC1L1, VKOR, GGCX and the chaperone CALU are expressed in aorta and bone. Ao: aorta; B: bone, error bars: +/- 2 standard deviations.

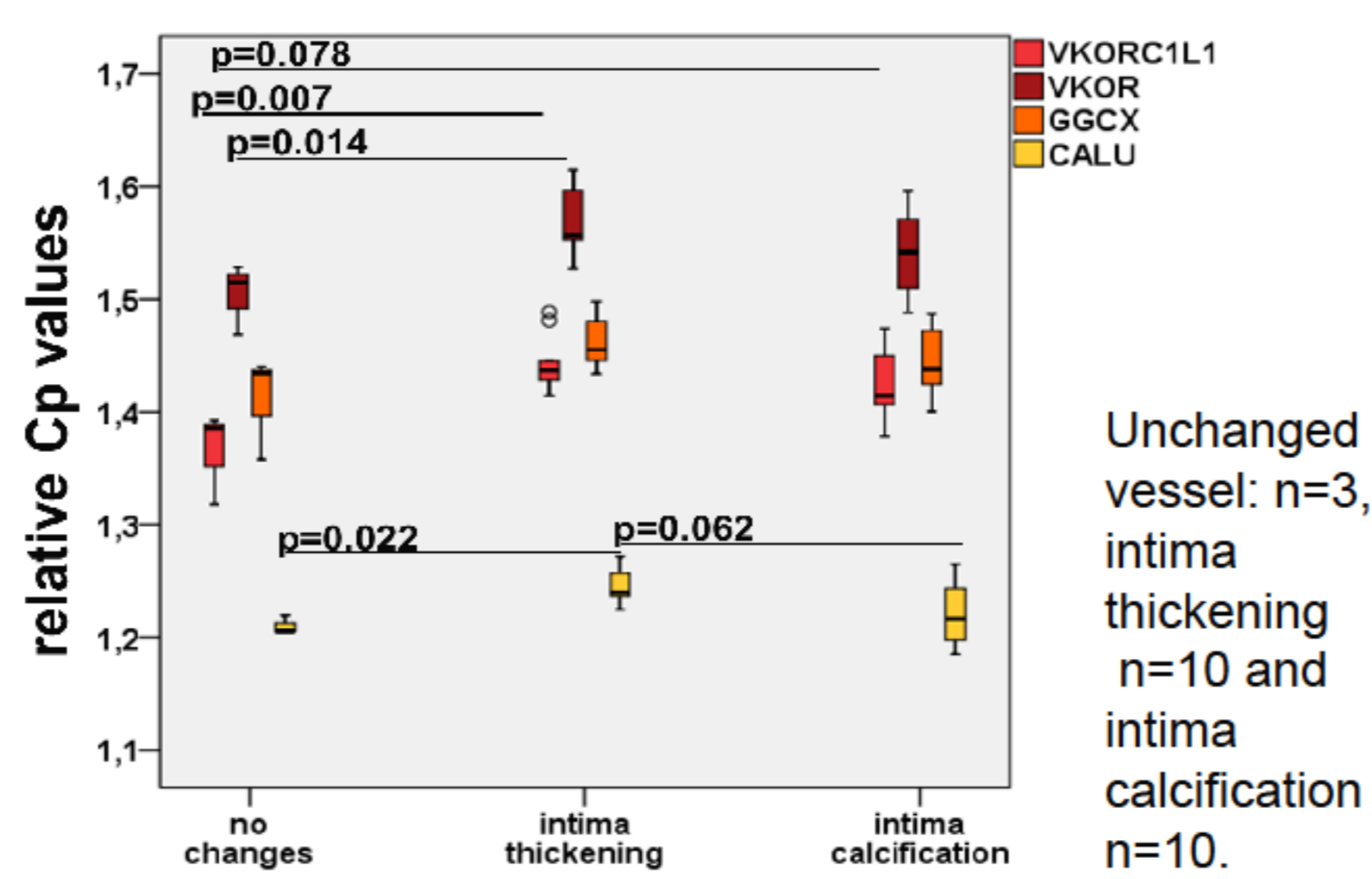


Fig.2: Gene expression of VKC components in aorta in 3 AS stages: Gene expression of VKORC1L1, VKOR and CALU is significantly lower in the stage of intima thickening than in the unchanged vessel. Gene expression of VKORC1L1 and CALU is increased in the stage of intima calcification compared to intima thickening

Enzymes of MK-4 synthesis

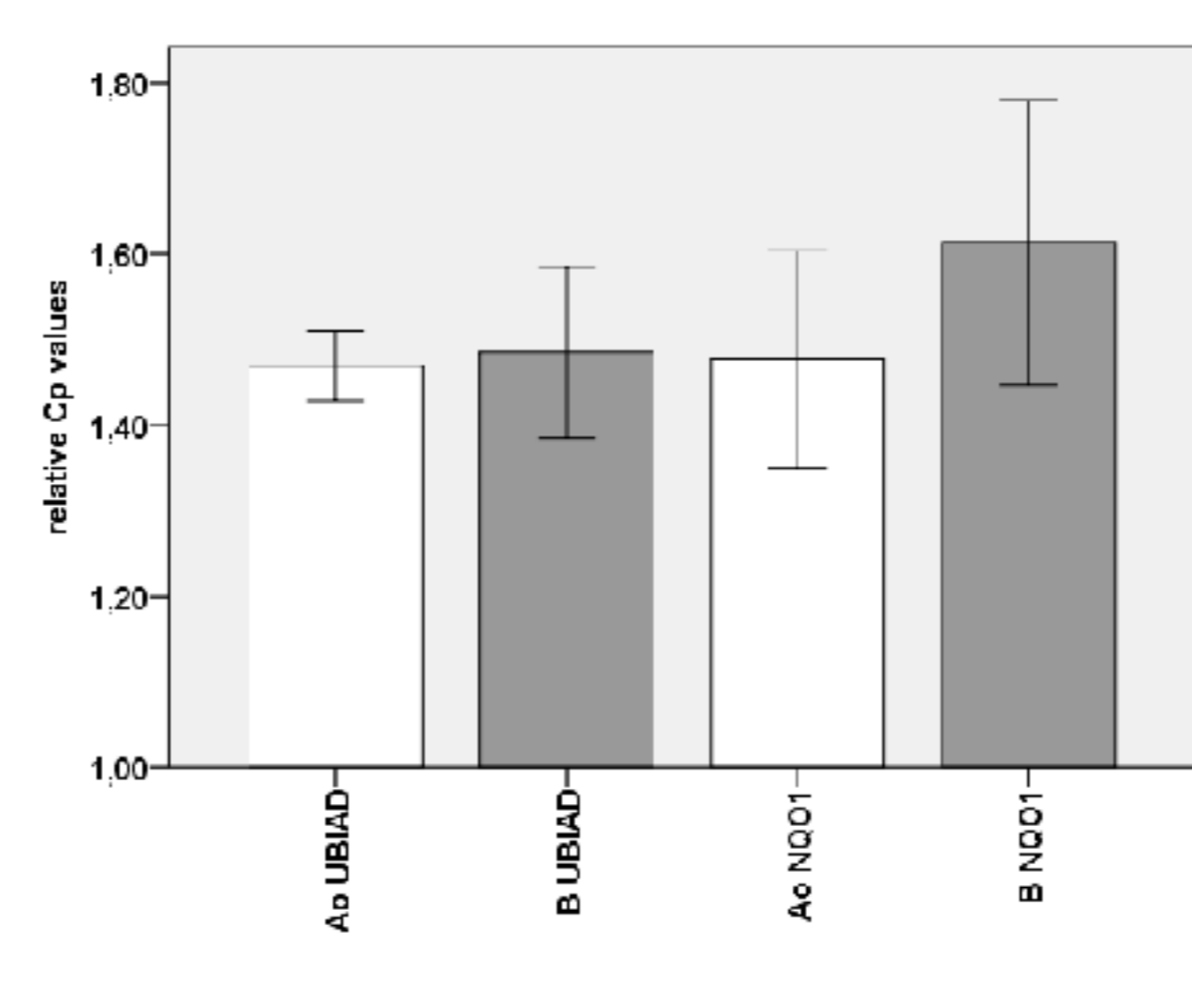


Fig.5: The MKS enzymes NQO1 and UBIAD1 are expressed in aorta and bone. Ao: aorta; B: bone, error bars: +/- 2 standard deviations.

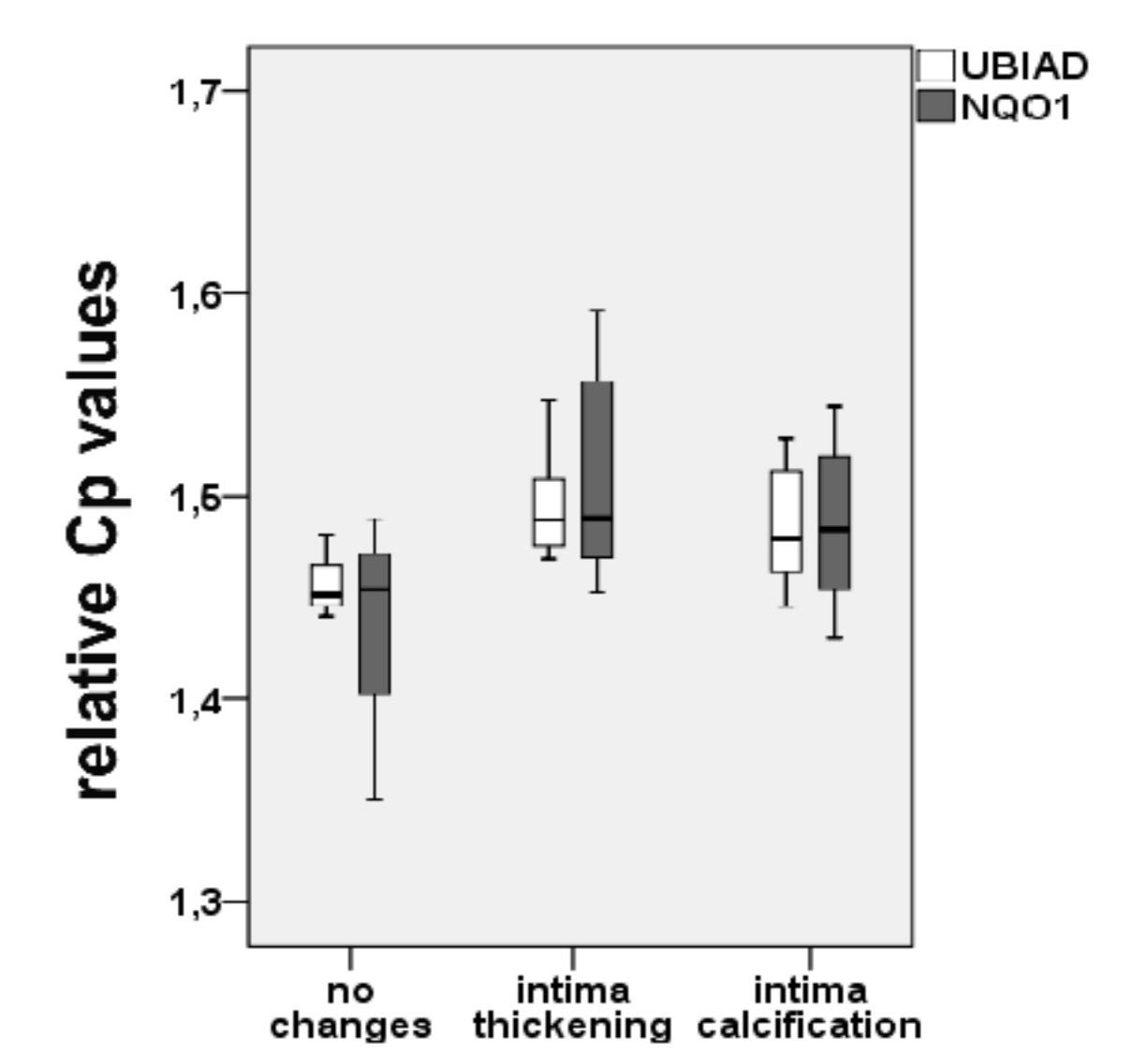


Fig.6: Gene expression of MKS components in aorta in 3 AS stages: Gene expression of UBIAD and NQO1 did not differ in the three AS stages. Unchanged vessel: n=3, intima thickening n=10 and intima calcification n=10.

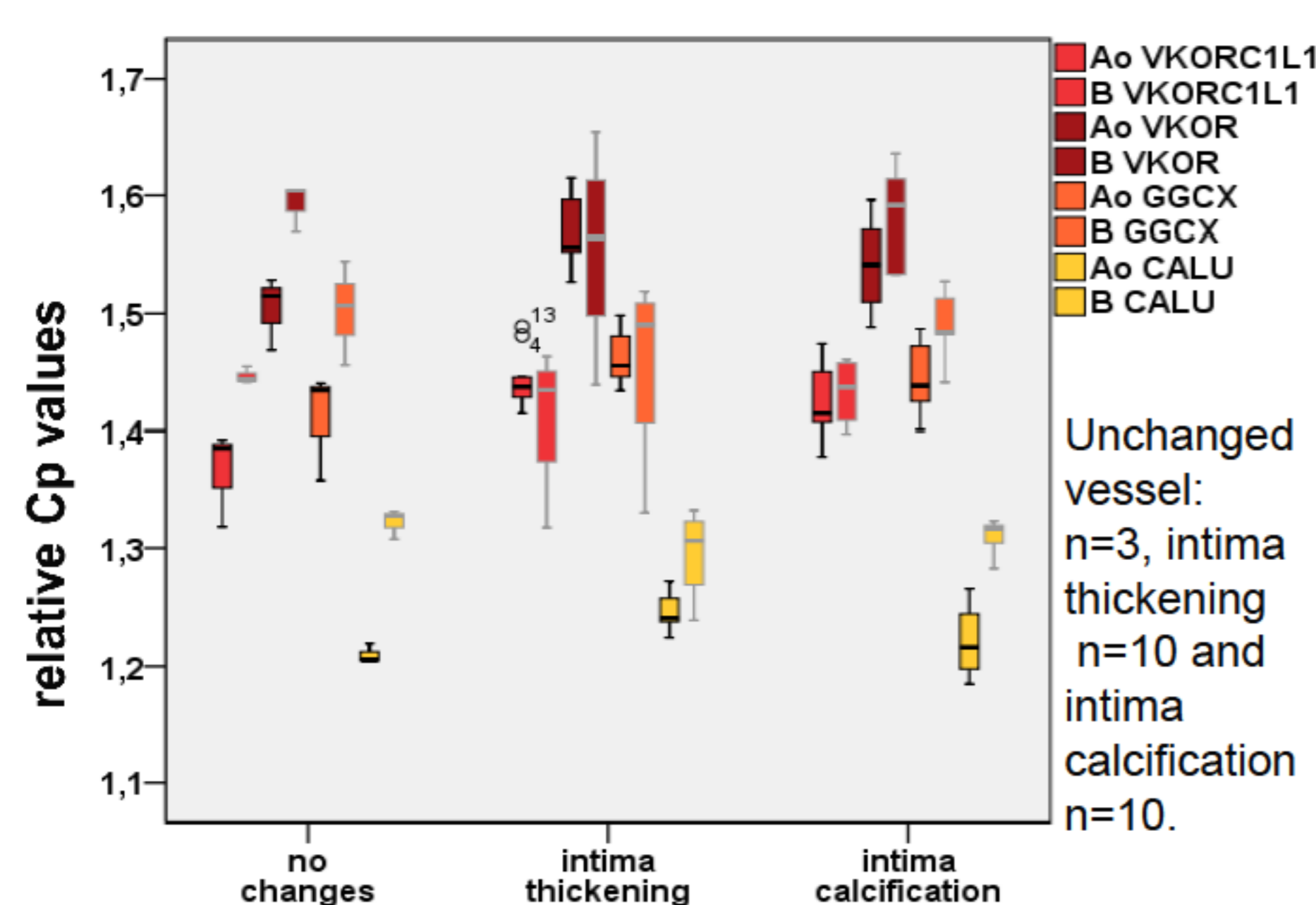


Fig.3: Changes in gene expression in 3 AS stages: There are no changes in gene expression in bone during AS progression. Gene expression of VKOR, VKORC1L1 und CALU (p=0.040, p=0.023, p=0.038) in aorta is significantly and of GGCX (p=0.060) by trend different in the 3 AS stages. Ao: aorta, black frames; B: bone, light grey frames

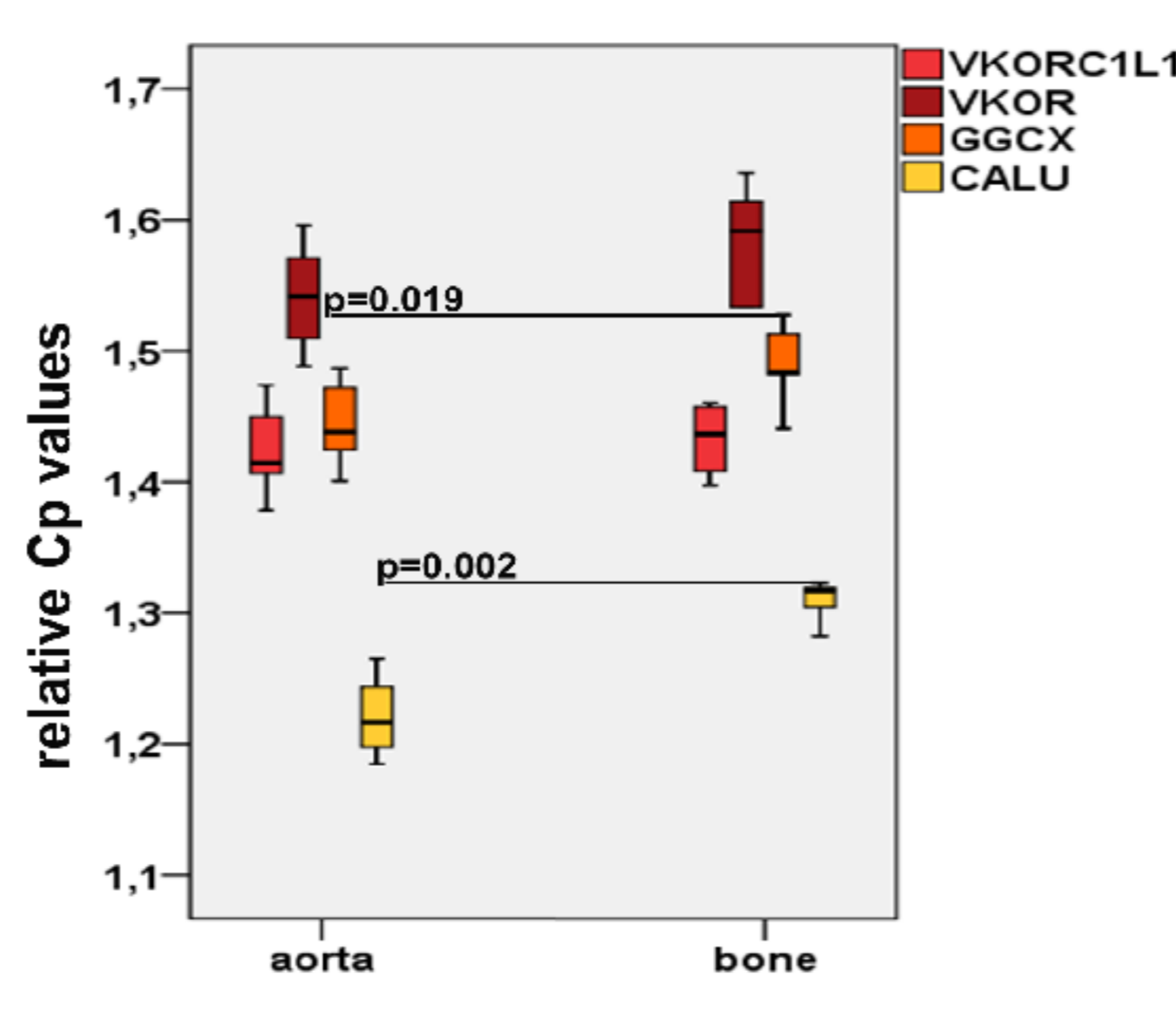


Fig.4: Gene expression of VKC components in aorta and bone during intima calcification: Gene expression of GGCX and CALU is in bone significantly decreased compared to calcified aorta. aorta n=8; bone n=5.

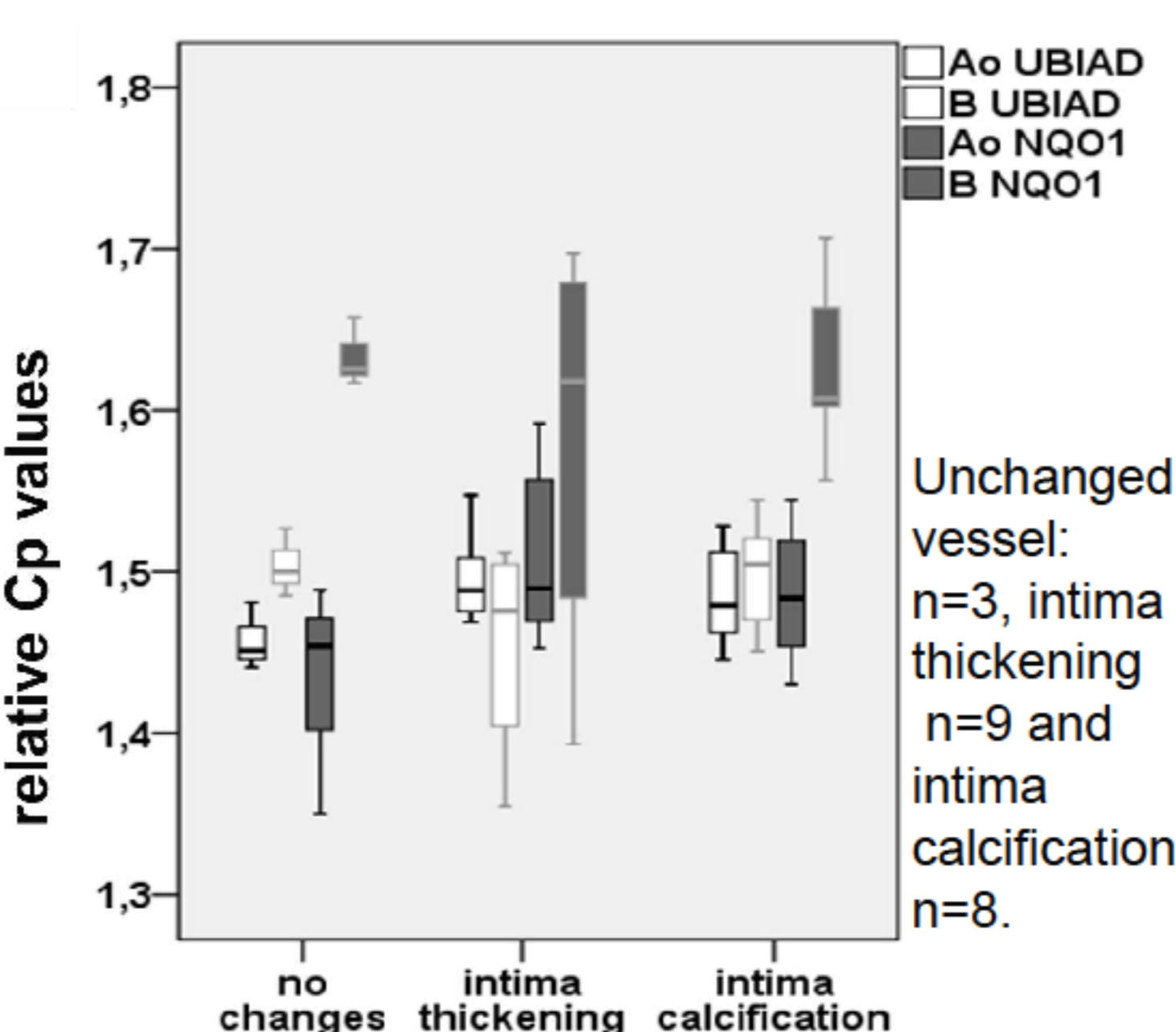


Fig.7: Changes in gene expression in 3 AS stages: There are no changes in gene expression in bone during AS progression. Gene expression of UBIAD and NQO1 is not different in bone and aorta in the 3 AS stages. Ao: aorta, black frames; B: bone, light grey frames

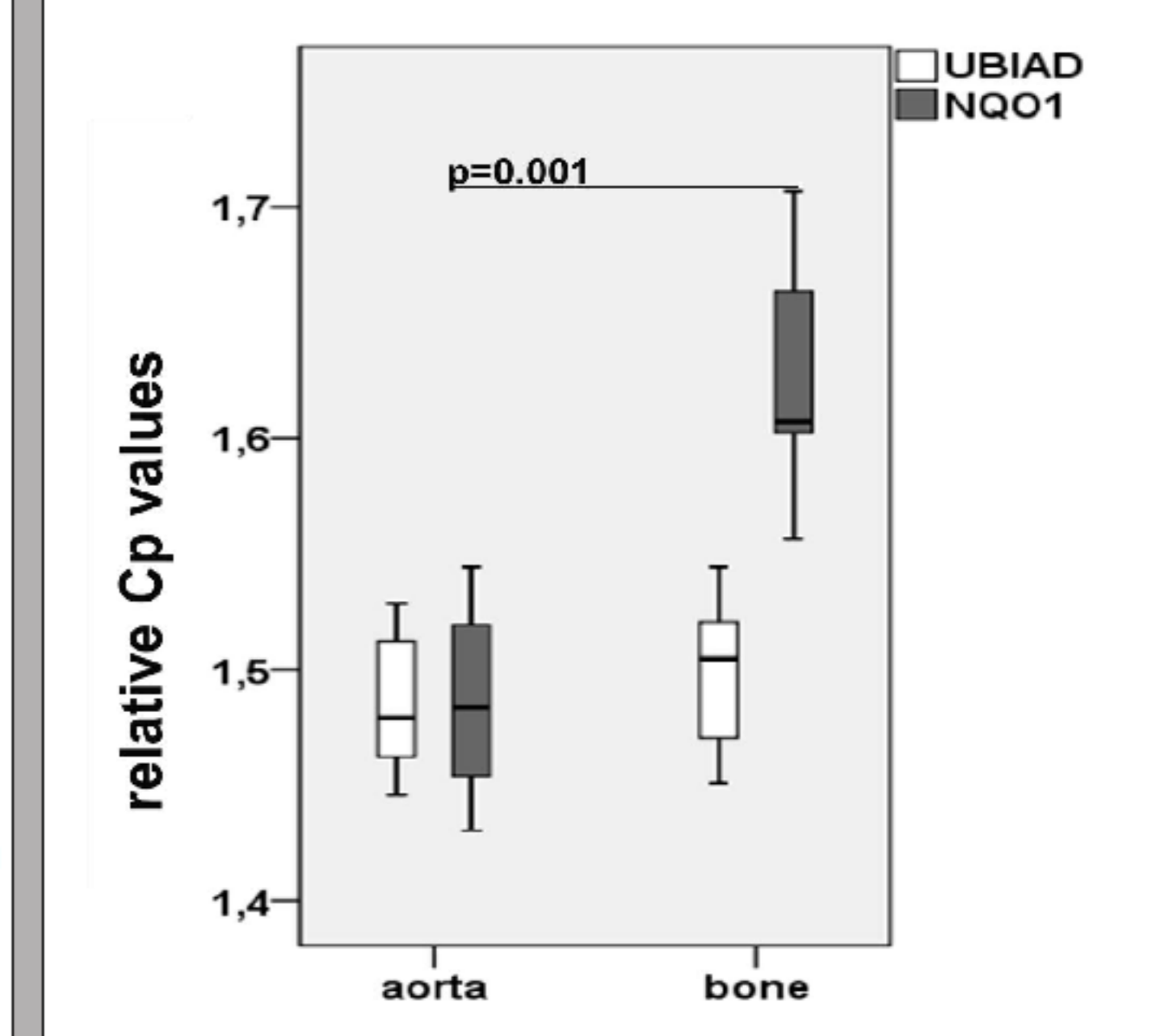


Fig.8: Gene expression of MKS enzymes in aorta and bone during intima calcification: Gene expression of NQO1 is in bone significantly lower than in the calcified aorta. aorta n=8; bone n=5.

Summary:

- We show that bone and aorta express the components of the vitamin K cycle.
- Bone and aorta express the enzymes necessary to synthesize MK-4.
- We demonstrate that different gene expression patterns exist in AS progression in bone and aorta.
- During AS progression gene expression patterns change in the aorta but not in bone.
- Gene expression of components of the VKC and MKS differ between bone and aorta only in the (later) stage of vessel calcification.

Conclusion:

Gene expression of enzymes of vitamin K metabolism changes during calcification of the vessel wall. These data might implicate a more complex role of vitamin K metabolizing enzymes in vascular calcification than previously known.

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