

# EX VIVO CALCIFICATION OF RAT ARTERIES. EFFECT OF ENDOTHELIAL LESION AND VESSEL SIZE.

NATHALIE GAYRARD<sup>1</sup>, BERNARD JOVER<sup>2</sup>, LAURA JEANSON<sup>3</sup>, ANNE-DOMINIQUE LAJOIX<sup>3</sup>, FLORE DURANTON<sup>1</sup>, ALAIN FICHEUX<sup>1</sup>, ANGEL ARGILES<sup>1</sup>.  
<sup>1</sup>RD-Néphrologie EA7288, BC2M, MONTPELLIER, FRANCE, <sup>2</sup>INSERM, U1046, MONTPELLIER, FRANCE, <sup>3</sup>EA7288, BC2M, MONTPELLIER, FRANCE.



## INTRODUCTION

Vascular calcification is one of the major complications in end-stage renal disease and is associated with an increased risk of cardiovascular events. The culture of arterial rings in high phosphate medium to induce calcification is used to study vascular calcification. With this experimental model, we have compared the ex vivo calcification of arteries of different diameter with or without lesion.

## METHODS

The ability to calcify was assessed ex vivo in artery rings with or without endothelial lesions. Renal and carotid arteries, thoracic and abdominal aortas were dissected from Wistar rats and were cultured 14 days in calcifying (phosphate 3.8mM) or control medium. Calcium content (mg/g of aorta) was determined by the o-cresolphthalein complexone method. Calcium distribution in arterial sections was assessed by von Kossa staining and medial calcification was expressed as percentage of the sectional area of the media.

## RESULTS

Calcification area gradually increased with the decrease in artery size. The calcified area varied from 5% for thoracic aorta to 60% for renal artery (Figure 1).

Injury significantly increased calcification; by 7-fold for thoracic aortic rings, and by 2-fold for carotid aortic rings (Figure 2).

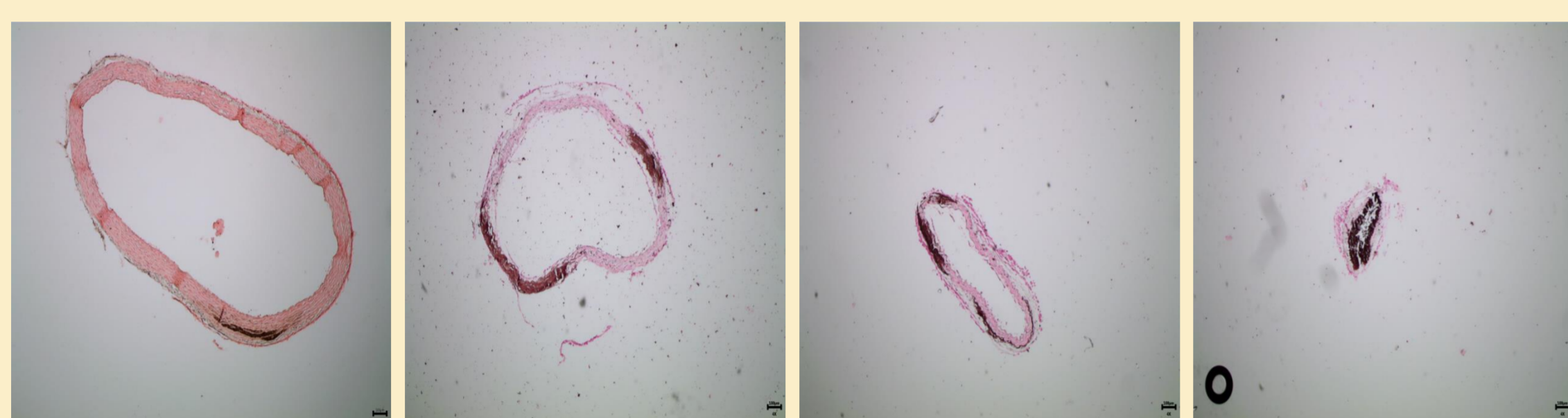
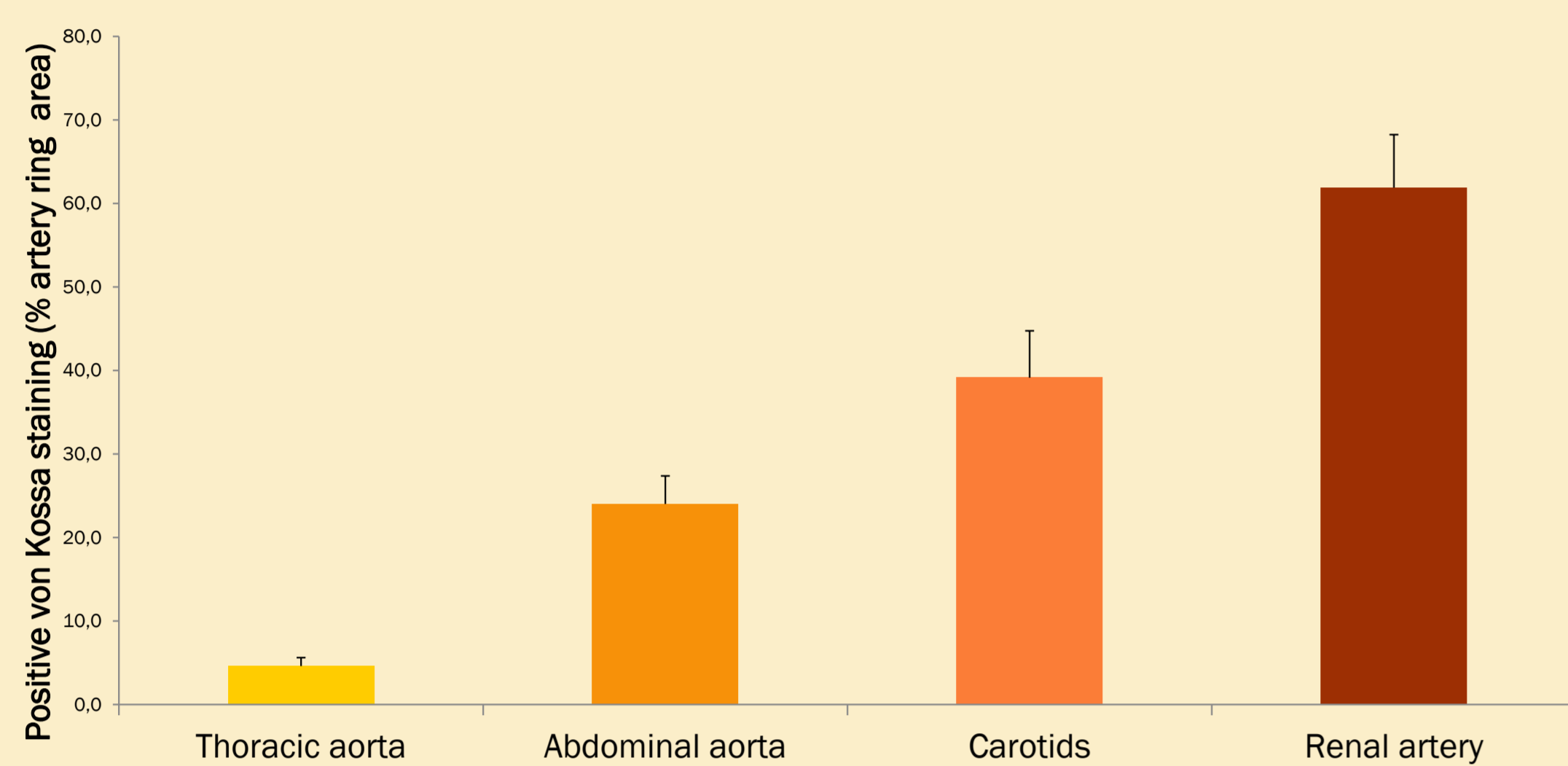
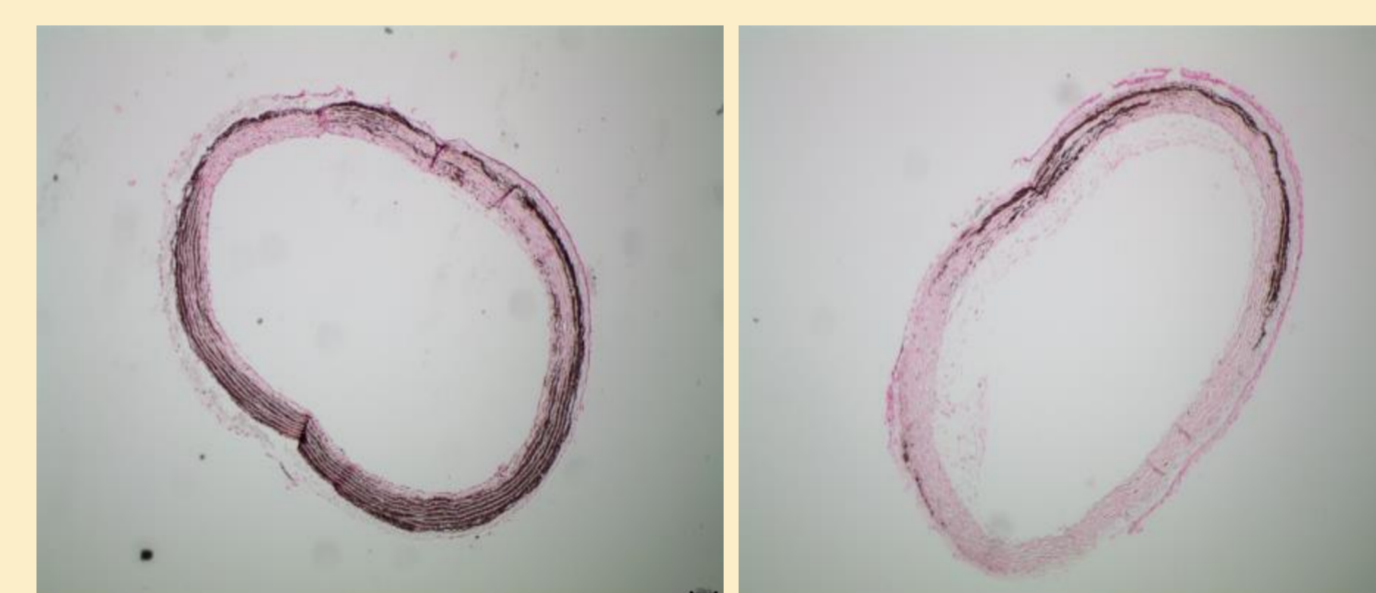
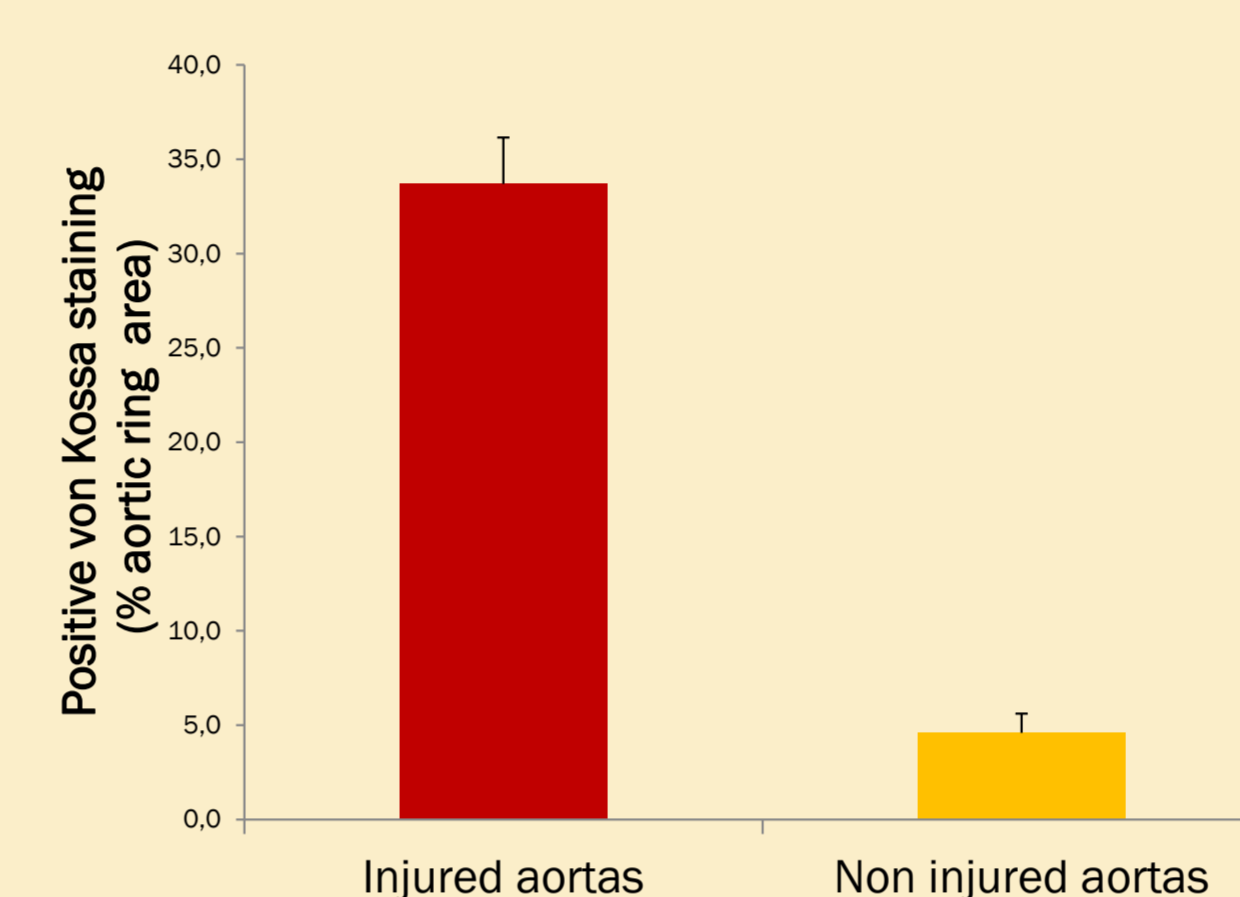


Figure 1. Comparison of different artery calcification by von Kossa staining

### Thoracic aortic rings



### Carotid aortic rings

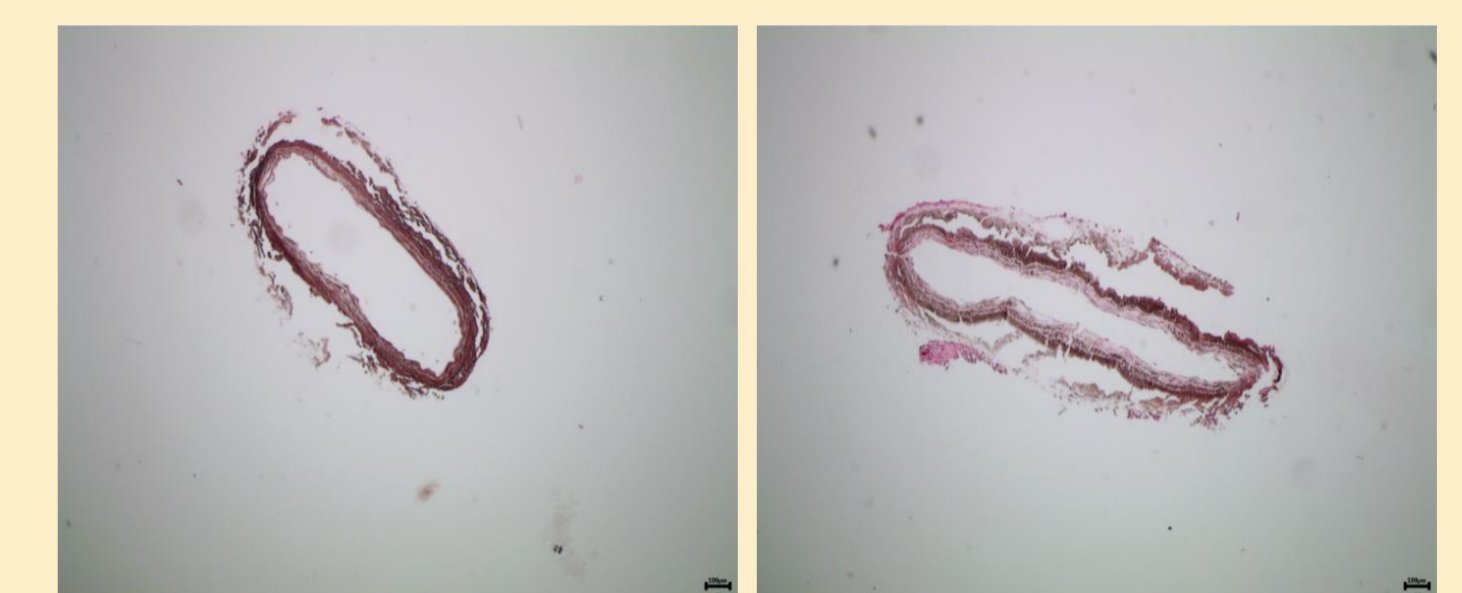
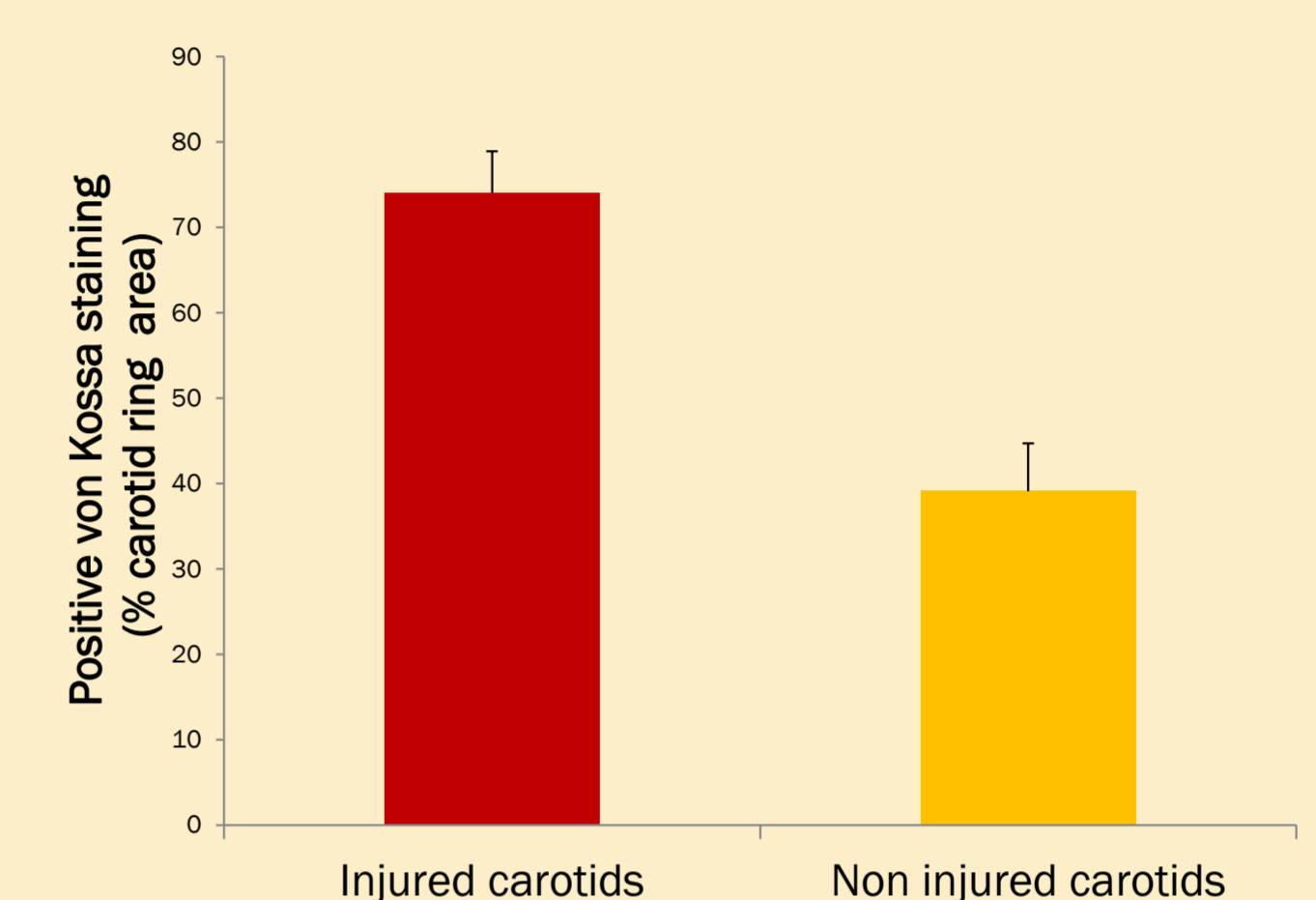
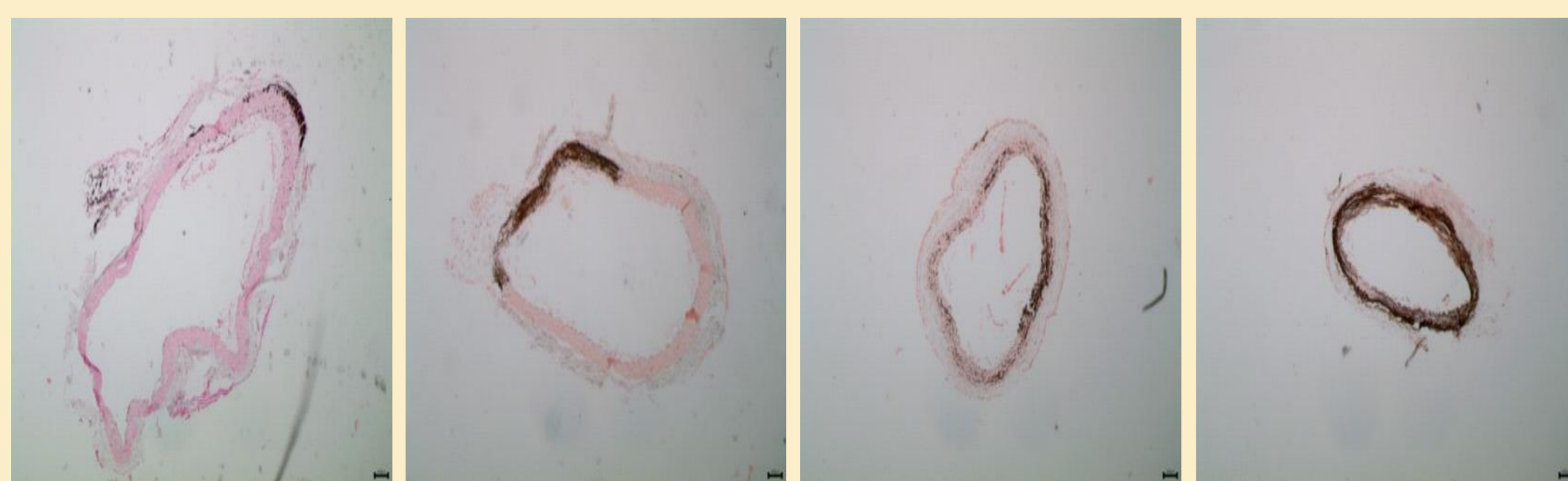
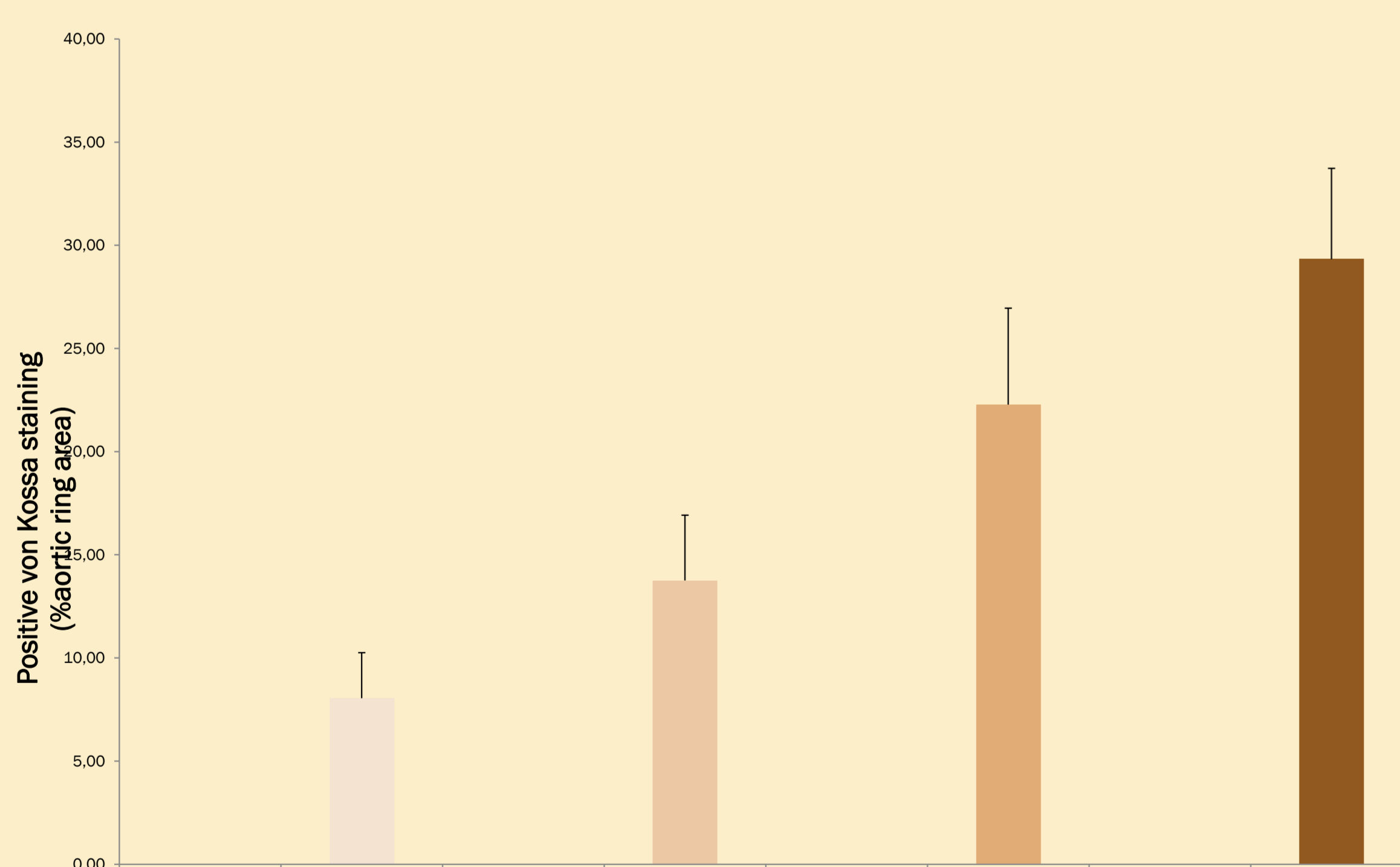


Figure 2. Effect of artery lesion on vascular calcification observed by von Kossa staining

In abdominal aortic rings the calcification increased along the aorta from mesenteric to iliac (Figure 3).

The calcification of cultured thoracic aortic rings is similar regardless of where the ring was taken from the aorta (Figure 4). The proximal and distal distances are references to the location relative to the aortic arch.



From mesenteric to iliac

Figure 3. Representative images of section of dissected ring along abdominal aorta stained by von Kossa

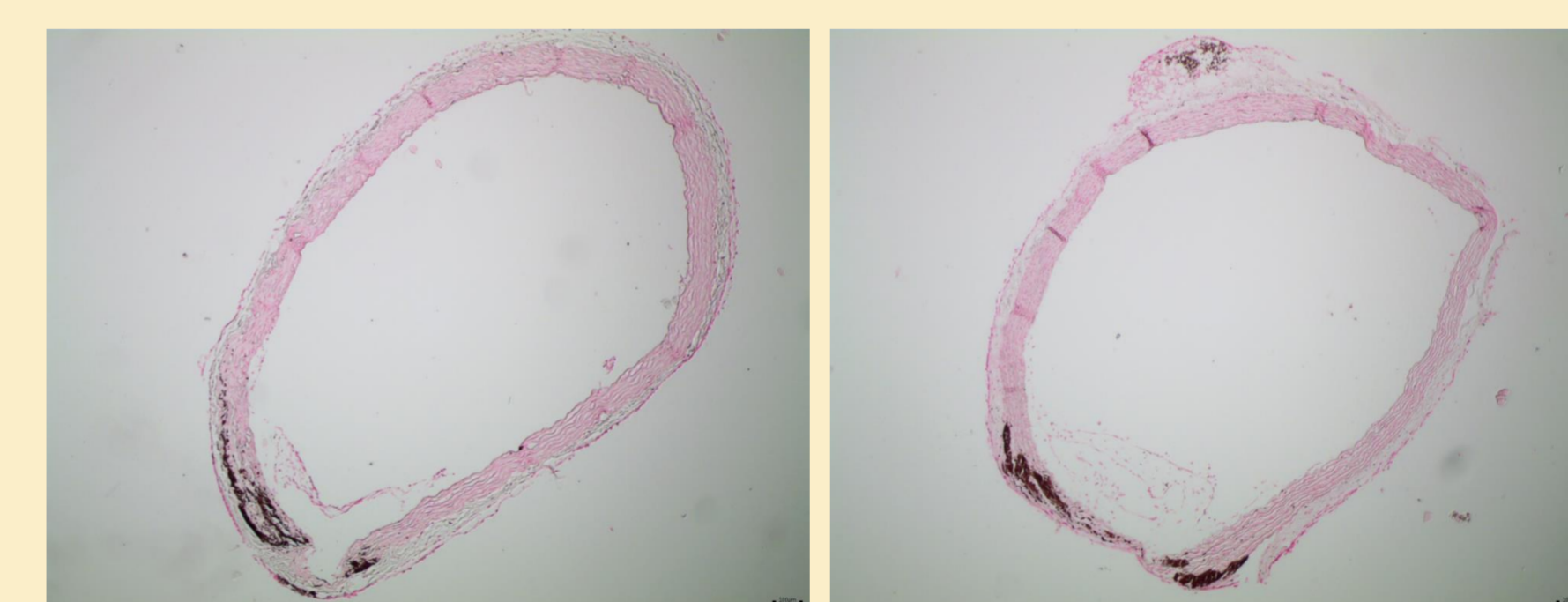
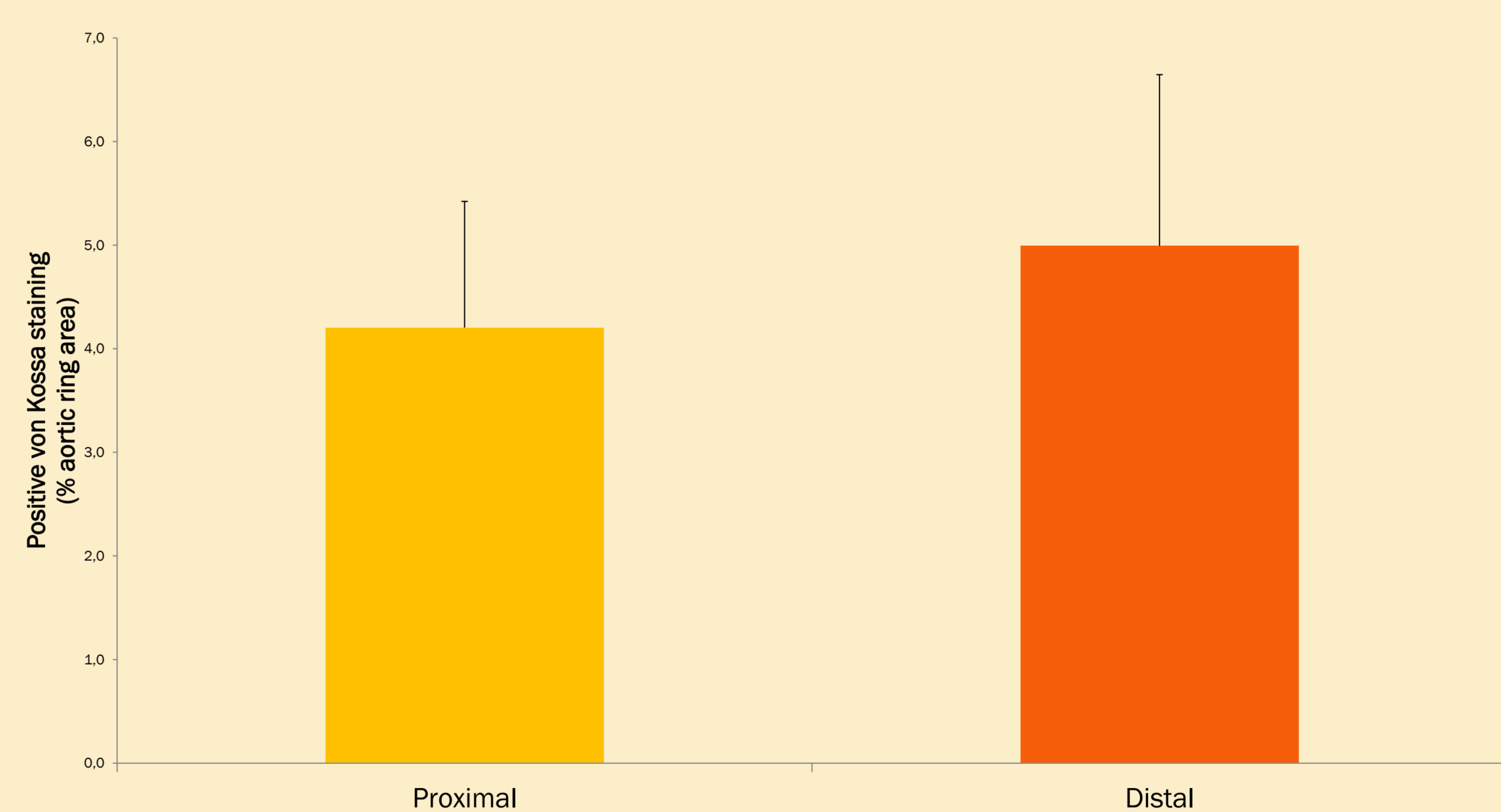


Figure 4. Calcification evaluation of cultured proximal and distal thoracic aorta stained by von Kossa

## CONCLUSION

Vascular media calcification is observed ex-vivo in all types of arteries cultured in high phosphate medium. A gradual increase in calcified surface area is observed with a decrease in arterial diameter, probably influenced by an increase in the ratio of muscle fibers to elastic fibers. The vascular lesion greatly increases the calcification therefore arteries have to be dissected cautiously. Thoracic aorta has a uniform structure allowing a large number of comparable rings for study. Abdominal aorta and the more distal arteries have differential calcification properties, which have to be taken into account in calcification studies.

Contact: RD-Néphrologie, 2 rue des muriers 34090 Montpellier France Mail : nathalie.gaynard@inserm.fr

