40; saline, n = 10), Apoe^{-/}Olr1^{-/-} mice (Ang II, n = 30; saline, n = 9), and Apoe^{-/-}Olr1^{-/-} mice (Ang II, n = 39; saline, n = 9).

Results: Ang II infusion raised systolic blood pressure by tail-cuff methods in all three groups. Serial non-invasive assessment with ultrasound demonstrated that Ang II gradually increased the maximum area of the suprarenal abdominal aortas leading to AAs. Although the incidence and expansion rate of AAA were comparable among genotypes, Orl1 deletion significantly increased the severity and rupture rate of AAs (p = 0.01; incidence of type IV and ruptured AAA was 25% in Apoe^{-/-}Olr1^{-/-} mice vs 5% in Apoe^{-/-}Olr1^{-/-} mice). Since atherosclerosis is considered a major contributor to the development of AAs, the extent of the atheroma burden localized in aneurysmal lesions was evaluated by Oil Red O staining. Orl1 deletion did not decrease atherosclerosis formation in the aneurysmal wall. Interestingly, further histopathological analysis revealed that aneurysmal lesions in LOX-1-deficient mice had fewer fibroblasts and myofibroblasts, as well as thinner adventitial collagen, although the degree of elastin fragmentation or disruption was similar between LOX-1-deficient and control mice. These in vivo data suggested that adventitial fibroblasts play a pivotal role in the increased severity of AAA in LOX-1-deficient mice. To further determine the effects of Orl1 deletion on fibroblast differentiation, fibroblasts were isolated from the adventitia of abdominal aortas harvested from 8-week-old Apoe^{-/-}Olr1^{-/-} and Apoe^{-/-}Olr1^{-/-} mice, and then cultured with Ang II stimulation. An in vitro study confirmed that Orl1 deletion completely abolished the Ang II-induced proliferative activity in the aortic adventitial fibroblasts (p = 0.02).

Conclusions: Orl1 deletion may not mitigate aneurysm development but rather increase the vulnerability to rupture by suppressing adventitial fibroblast proliferation and collagen.

### Abstracts

**PS-BPB01-7 ANGIOTENSIN II INDUCES AORTIC RUPTURE AND DISESECTION IN OSTEOPROTEGERIN-DEFICIENT MICE**

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**Background:** The biological mechanism of action for osteoprotegerin (OPG), a soluble decoy receptor for the receptor activator of nuclear factor kappa-B ligand (RANKL) in the vascular structure, has not been elucidated. The study aim was to determine if OPG affects aortic structural integrity in angiotensin II-induced hypertension.

**Methods and Results:** Mortality was higher (P < 0.001 by Log-rank test) in 8-week-old male homozygotes of OPG gene knockout (OPG^{-/-}) mice given subcutaneous administration of angiotensin II (1,000 ng/kg/min) for 28 days, with an incidence of 21% fatal aortic rupture (Chi-square, 8.024, P = 0.005), and 23% aortic dissection (Chi-square, 4.258, P = 0.038), than in age-matched wild type mice. The absence of OPG was associated with decreased medial and adventitial thickness and increased numbers of elastin breaks as well as with 2.5-fold increased peristin expression and soluble RANKL concentrations. PG-Ylated human recombinant OPG administration decreased all-causes mortality (22%, P < 0.001 by Log-rank test), the incidence of fatal aortic rupture (-13%, P = 0.08), and aortic dissection (-23%, P < 0.001) with decreasing numbers of elastin breaks (P = 0.0539) at the supra-renal aorta, peristin expressions (P = 0.0011), and soluble RANKL concentrations (P = 0.0012) in angiotensin II-infused OPG^{-/-} mice.

**Conclusions:** These data suggest that OPG protects against aortic rupture and dissection in Ang II-induced hypertension by inhibiting RANKL activity and periostin expression.

### Abstracts

**PS-BPB01-8 IMPACT OF DIFFERENT DIURETIC TYPES ON ARTERIAL STIFFNESS AND CALCIFICATION IN A RAT MODEL OF CHRONIC KIDNEY DISEASE**

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**Introduction:** We reported that antihypertensive therapy with a hydrochlorothiazide (HCTZ)-based regimen aggravates arterial stiffness and vascular calcification in a rat model of chronic kidney disease (CKD) with mineral and bone disorder (MBD). In this study, we investigated whether the aggravation of arterial stiffness and vascular calcification in CKD-MBD rats is induced by HCTZ and whether these abnormalities could also occur with other types of diuretic currently used in clinic.

**Methods:** In rats with renal mass ablation-induced CKD, MBD was generated by a Ca/P-rich diet and calcitriol supplementation. The animals were divided into 4 groups: 1) CKD-MBD control; 2) CKD-MBD + HCTZ (thiazide diuretic, 5 mg/kg/d); 3) CKD-MBD + Chlorthalidone (thiazide-like diuretic, 5 mg/kg/d); and 4) CKD-MBD + Furosemide (loop diuretics, 10 mg/kg/d). At week 6, hemodynamic parameters including systolic and mean blood pressure (SBP and MBP), pulse pressure (PP) and pulse wave velocity (PWV) were determined. The thoracic aorta was harvested to assess vascular calcification.

**Conclusions:** We defined a novel molecular pathway in endothelial cells, where Nox4-induced H2O2 production activates PARP/TRPM2 signaling followed by Ca2+ influx, eNOS activation and NO release. The absence of Nox4 impairs Ca2+ homeostasis leading to endothelial dysfunction, an effect exacerbated in hypertension. The protective role of Nox4 corroborates the idea that the use of antioxidants based on the sole evidence that decreased oxidative stress is cardio-protective, it is not effective.

**PS-BPB01-9 IMPACT OF OPG IN AN OPTIMAL ANGII HYPERTENSION MODEL ON ARTERIAL STIFFNESS, DIASTOLIC FUNCTION, AND CALCIFICATION**

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**Introduction:** We reported that antihypertensive therapy with a hydrochlorothiazide (HCTZ)-based regimen aggravates arterial stiffness and vascular calcification in a rat model of chronic kidney disease (CKD) with mineral and bone disorder (MBD). In this study, we investigated whether the aggravation of arterial stiffness and vascular calcification in CKD-MBD rats is induced by HCTZ and whether these abnormalities could also occur with other types of diuretic currently used in clinic.

**Methods:** In rats with renal mass ablation-induced CKD, MBD was generated by a Ca/P-rich diet and calcitriol supplementation. The animals were divided into 4 groups: 1) CKD-MBD control; 2) CKD-MBD + HCTZ (thiazide diuretic, 5 mg/kg/d); 3) CKD-MBD + Chlorthalidone (thiazide-like diuretic, 5 mg/kg/d); and 4) CKD-MBD + Furosemide (loop diuretics, 10 mg/kg/d). At week 6, hemodynamic parameters including systolic and mean blood pressure (SBP and MBP), pulse pressure (PP) and pulse wave velocity (PWV) were determined. The thoracic aorta was harvested to assess vascular calcification.