



# Deletion of Endothelial Estrogen Receptor Alpha Reduces Arterial Stiffness in Angiotensin II infused-Female Mice



Alexander M Lising<sup>1,2</sup>, Francisco Ramirez<sup>3,4</sup>, Samuel W Jenkins<sup>1,2</sup>, Guido Lastra, MD<sup>1,2</sup>, Luis A Martinez-Lemus<sup>3,5</sup> and Camila Manrique Acevedo, MD<sup>1,2</sup>

(1) Department of Medicine, Division of Endocrinology, University of Missouri, Columbia, MO; (2) Research Service, Harry S. Truman Memorial Veterans Hospital, Columbia, MO; (3) Department of Medical Pharmacology and Physiology University of Missouri, Columbia; (4) Department of Biological Engineering, University of Missouri, Columbia, MO; (5) Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO.

## ABSTRACT

**Background:** Vascular stiffness is a naturally occurring phenomenon associated with aging, but conditions such as obesity and type 2 diabetes accelerate its development, particularly in women. The presence of vascular stiffness increases significantly the risk of cardiovascular disease (CVD). Under physiological conditions, estrogen signaling via estrogen receptor alpha (ER $\alpha$ ) increases bioavailable nitric oxide in the endothelium and decreases stiffness. Nevertheless, large clinical trials have failed to demonstrate beneficial cardiovascular effects of estrogen therapy. Our previous work has shown that under conditions of over-nutrition, the lack of ER $\alpha$  ameliorates arterial stiffening in obese and insulin resistant females. Given the central role that activation of the Renin-Angiotensin-System (RAS) has in the pathogenesis of CVD, in the present study we examine the effect of an Angiotensin II (Ang II) infusion in female mice lacking endothelial cell (EC).

## METHODS

**Animals:** Mice harboring a floxed *Esr1* gene were bred to mice expressing a VE-cadherin-Cre to obtain EC restricted deletion of *Esr1* (KO). As control we utilized the doubled floxed mice.

**Ang II infusion:** 24 week old mice were treated with Ang II (500 ng/kg/min) via osmotic minipumps for 4 weeks (n=5 per group).

**Aortic stiffness and vascular reactivity:** Aortic stiffness was evaluated in vivo by ultrasound-based pulse wave velocity (PWV). Endothelial-dependent and -independent vasomotor responses were evaluated in isolated aortic rings.

**Femoral artery stiffness and remodeling:** Arteries were isolated and cannulated onto glass micropipettes, pressurized at 70 mmHg without flow, and warmed to 37°C in commercial myograph chambers. Vessels were then placed in Ca<sup>2+</sup>-free PSS and exposed to consecutive changes in intraluminal pressure from 5 to 120 mmHg while under passive conditions to determine the elastic properties of the arteries.

**Statistics:** Results are presented as means  $\pm$  SE analyzed by Student's T-test.  $p \leq 0.05$  was considered as significant.

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## ACKNOWLEDGEMENT

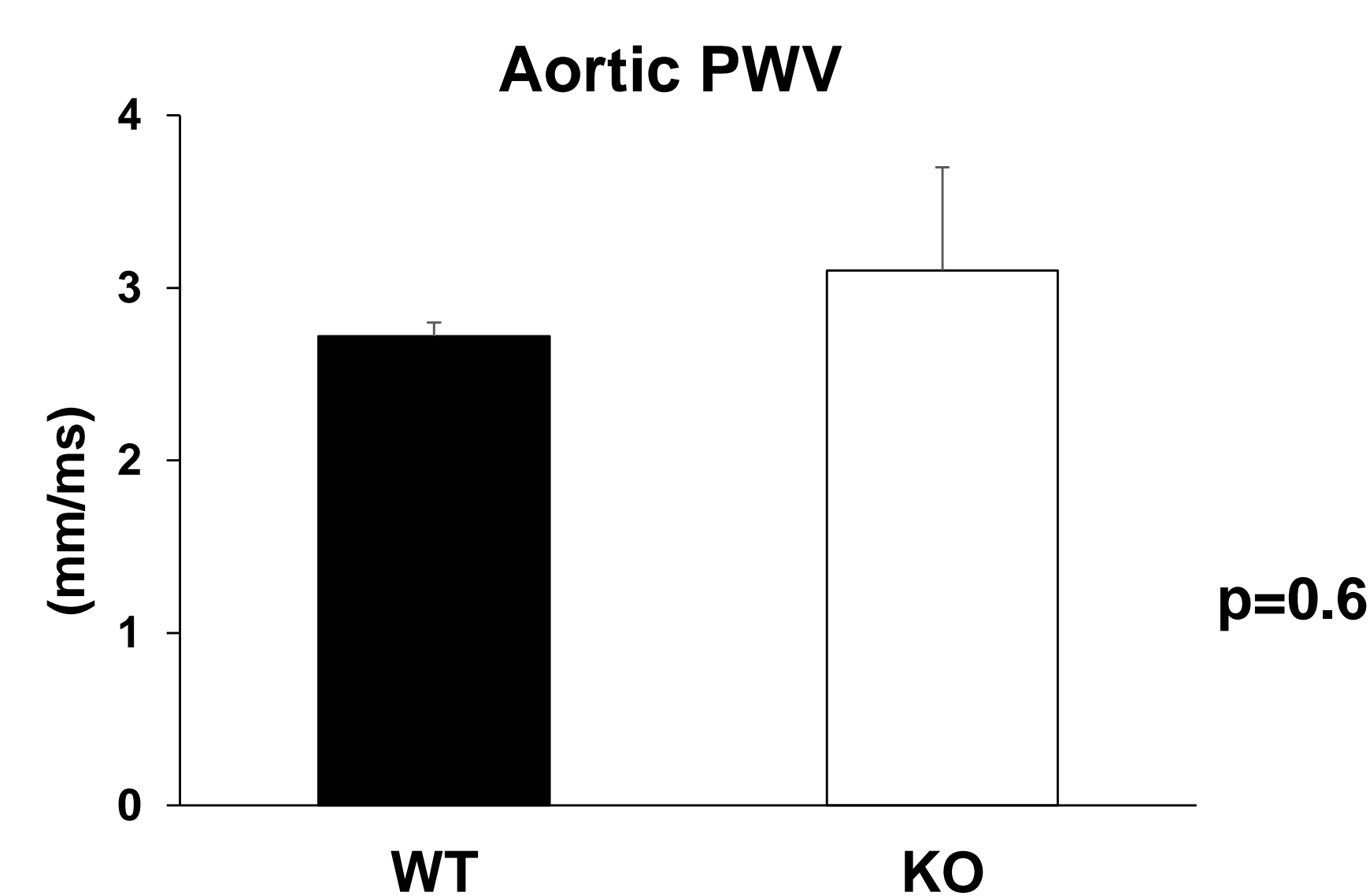
ER $\alpha$  floxed mice (ER $\alpha^{fl/fl}$ ) were provided by Dr. Pierre Chambon (*Institute for Genetics and Cellular and Molecular Biology, University of Strasbourg, France*). This work was supported with resources and facilities at the University of Missouri and the Harry S. Truman Memorial Veterans Hospital in Columbia, MO, including the Small Animal Ultrasound Imaging Center.

## RESULTS

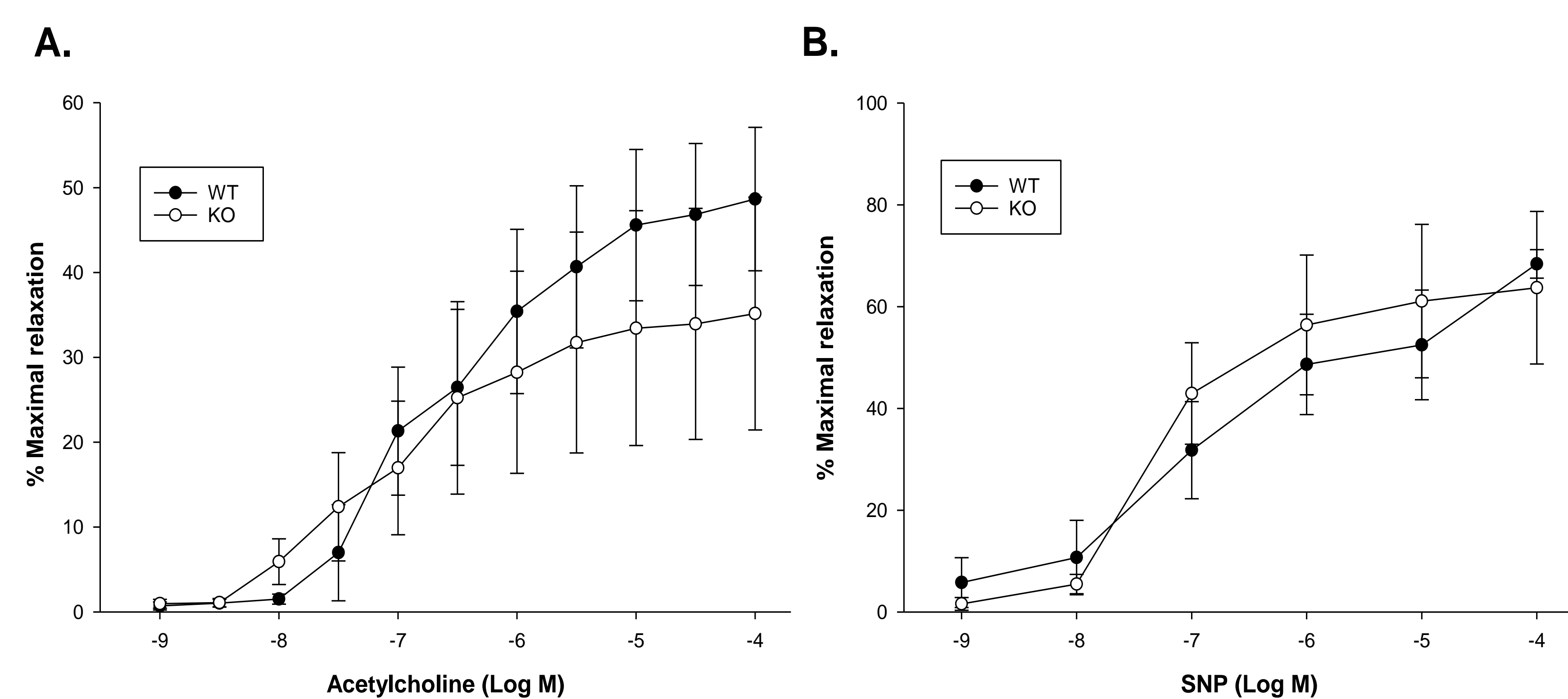
**Table 1. Characteristics at sacrifice of the different cohorts.** Wild type (WT), ER ER $\alpha$  KO (KO). After 4 weeks of Ang II infusion there was a trend toward higher blood glucose in the KO mice. n=5 per group.

	WT + Ang II	KO + Ang II	p
Body Weight (g)	21.96 $\pm$ 0.23	22.2 $\pm$ 0.15	0.4
Glucose (mg/dL)	134.8 $\pm$ 6.33	158 $\pm$ 7.95	<b>*0.05</b>
Heart/tibia length	73.84 $\pm$ 3.02	70.63 $\pm$ 4.42	0.39

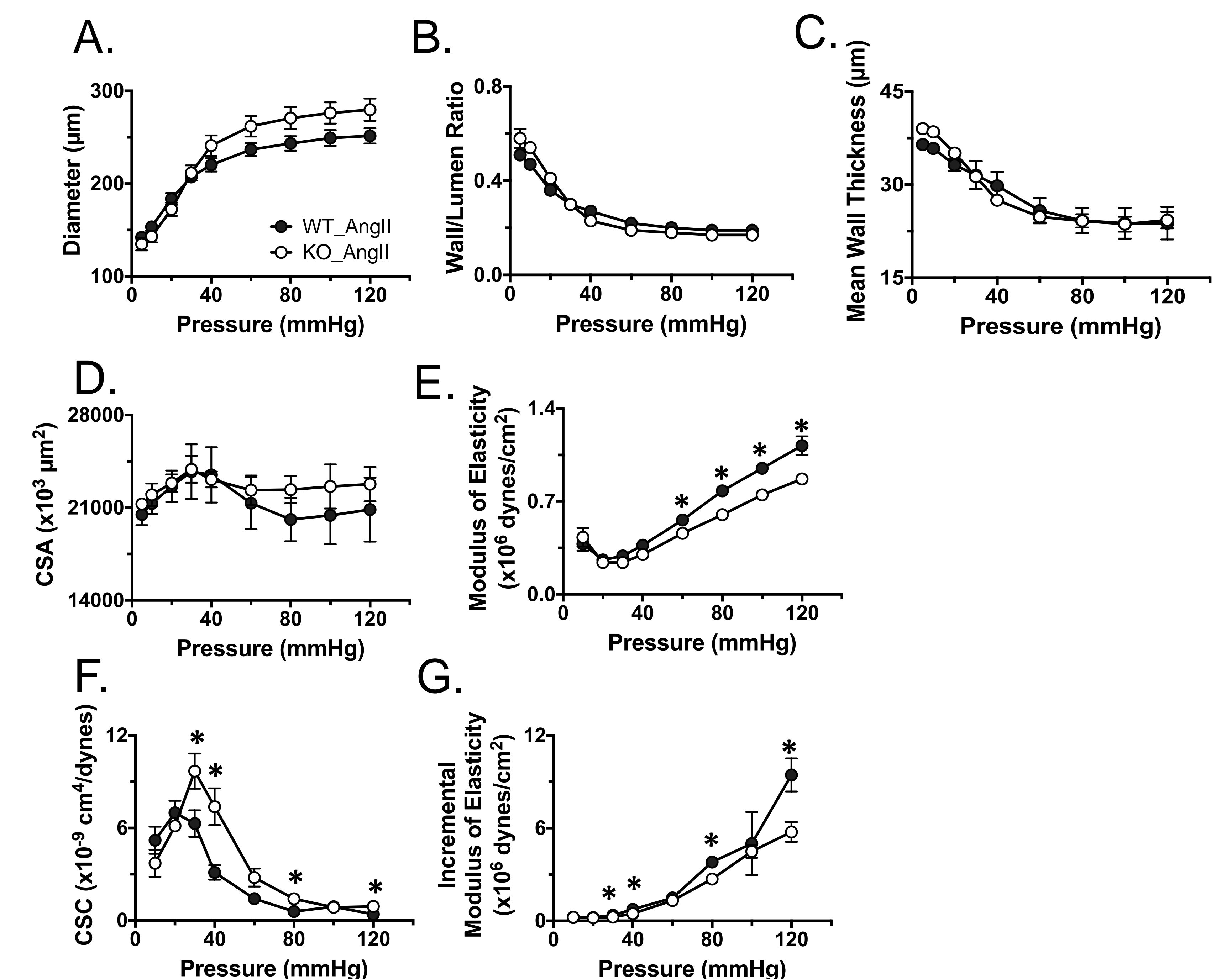
**Figure 2. Absence of EC ER $\alpha$  does not result in significant differences in arterial stiffness assessed by PWV.** n=5 per group.



**Figure 3. Deletion of ER $\alpha$  does not result in significant differences in aortic endothelial dependent or independent vasodilatory responses.** (A) Acetylcholine dose-response curve (B) Sodium nitroprusside (SNP) dose-response curve. n=5 per group



**Figure 4. Endothelial ER $\alpha$  knockout results in decreased stiffness of femoral arteries after 4 weeks of Ang II infusion.** Femoral arteries remodeling and mechanical parameters shown below. (A) Vascular diameter-pressure relationships; (B) vascular wall lumen ratio-pressure relationships; (C) mean wall thickness-relationships; (D) vascular wall cross-sectional area (CSA)-pressure relationships; (E) moduli of elasticity-pressure curves. Panel (F) shows cross sectional compliance-pressure relationships and (G) incremental moduli of elasticity-pressure curves. N=4 per group.; \*  $p \leq 0.05$  WT vs. KO



## CONCLUSIONS

- ✓ Deletion of ER $\alpha$  in endothelial cells in the setting of RAS activation promotes hyperglycemia.
- ✓ In conditions RAS activation, EC absence of ER $\alpha$  does not result in significant impairment in endothelial-dependent or -independent aortic dilatation.
- ✓ Deletion of ER $\alpha$  in EC decreases femoral artery stiffness (decreased modulus of elasticity) and increases compliance, which might precede the development of aortic stiffness
- ✓ Future studies are needed to elucidate the mechanisms underlying the relationship between estrogen signaling and vascular dysfunction