SUPPLEMENT

TABLES

14(15)-EET

	SD	JCR	JCR + 20-SOLA	JCR + 20-HEDGE
Aorta	101±6	42±3*	35±7*	43±7*
Carotid artery	98±7	42±12*	44±5*	42±8*
Small mesenteric arteries (~50µM)	345±13	154±12*	148±11*	150±8*
Coronary arteries	302±4	138±3*	135±8*	141±6*

8(9)-EET

SD	JCR	JCR + 20-SOLA	JCR + 20-HEDGE
74±7	75±13	75±8	83±11
72±9	82±8	76±7	74±3
109±3	110±14	110±6	104±3
102±4	102±6	95±8	101±6

* p<0.05 vs. SD

11(12)-EET

	SD	JCR	JCR + 20-SOLA	JCR + 20-HEDGE
Aorta	238±7	92±11*	85±4*	88±11*
Carotid artery	248±11	89±8*	92±8*	91±11*
Small mesenteric arteries (~50µM)	659±13	121±8*	132±14*	129±11*
Coronary arteries	630±14	108±7*	105±8*	101±16*

5(6)-EET

SD	JCR	JCR + 20-SOLA	JCR + 20-HEDGE
149±7	152±14	151±11	149±14
148±11	152±2	145±7	15±5
236±8	213±11	219±13	212±8
240±11	228±7	225±15	231±12

* p<0.05 vs. SD

Table 1. Mass spectrometry (LC/MS/MS) measurements of epoxyeicosatrienoic acids (EETs (pg/mg) in aorta and carotid arteries of SD, JCR and JCR+20-HEDGE-treated rats as indicated.

	SD	JCR	JCR + 20-SOLA	JCR + 20-HEDGE
MABP (mmHg)	94±7	152±4*	135±5*	133±4*
HR (beats/min)	338±11	332±8	335±5	333±4
LVWT,d (mm)	1.66±0.02	2.62±0.02*	2.60±0.03*	2.62±0.03*
IVSWT,d (mm)	1.59±0.03	2.13±0.02*	2.11±0.01*	2.12±0.02*
LVEDD (mm)	7.7±0.02	5.3±0.02*	5.3±0.02*	5.3±0.02*
LVESD (mm)	3.1±0.03	2.6±0.01*	2.6±0.01*	2.6±0.01*
LVEDV (µl)	324±6	216±4*	216±3*	216±3*
LVESV (µl)	40±4	32±2*	32±3*	32±2*
CO (µl/min)	95±8	61±7*	62±2*	61±3*
EF (%)	88±5	85±4	85±3	85±2

* p<0.05 vs. SD

Table 2. Echocardiographic measurements of cardiac function in anesthetized SD, JCR and JCR+20-SOLA- and JCR+20-HEDGE-treated rats as indicated. MABP, mean arterial blood pressure; HR, heart rate, LVWT,d, left ventricular wall thickness in diastole, IVSWT,d, intraventricular septum wall thickness in diastole, LVEDD and LVESD, left ventricular diastolic and systolic diameter; LVEDV and LVESV, left ventricular end diastolic and systolic volume; CO, cardiac output; EF, ejection fraction.

FIGURES

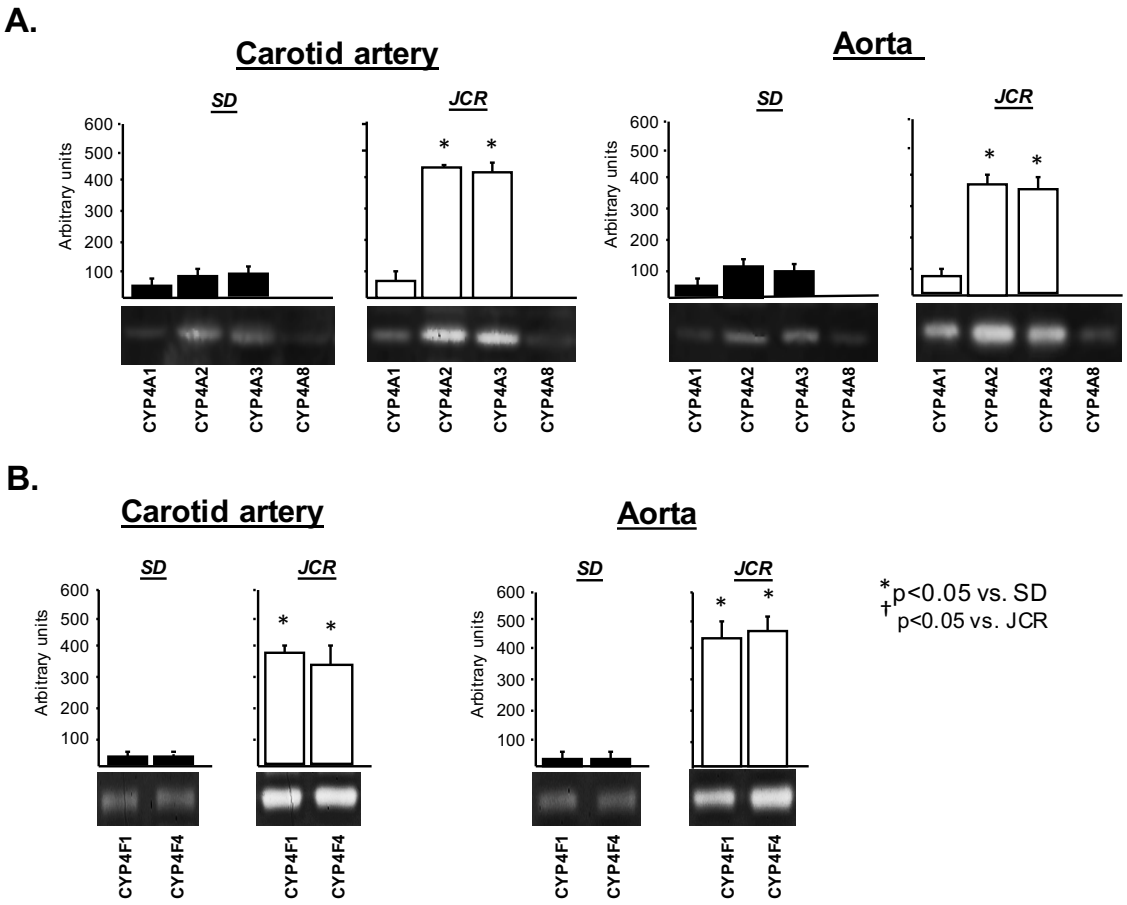


Figure I. SD and JCR rats were sacrificed and arterial tissue collected. CYP4A (**A**) and CYP4F (**B**) isoform expression was analyzed by RT-PCR.

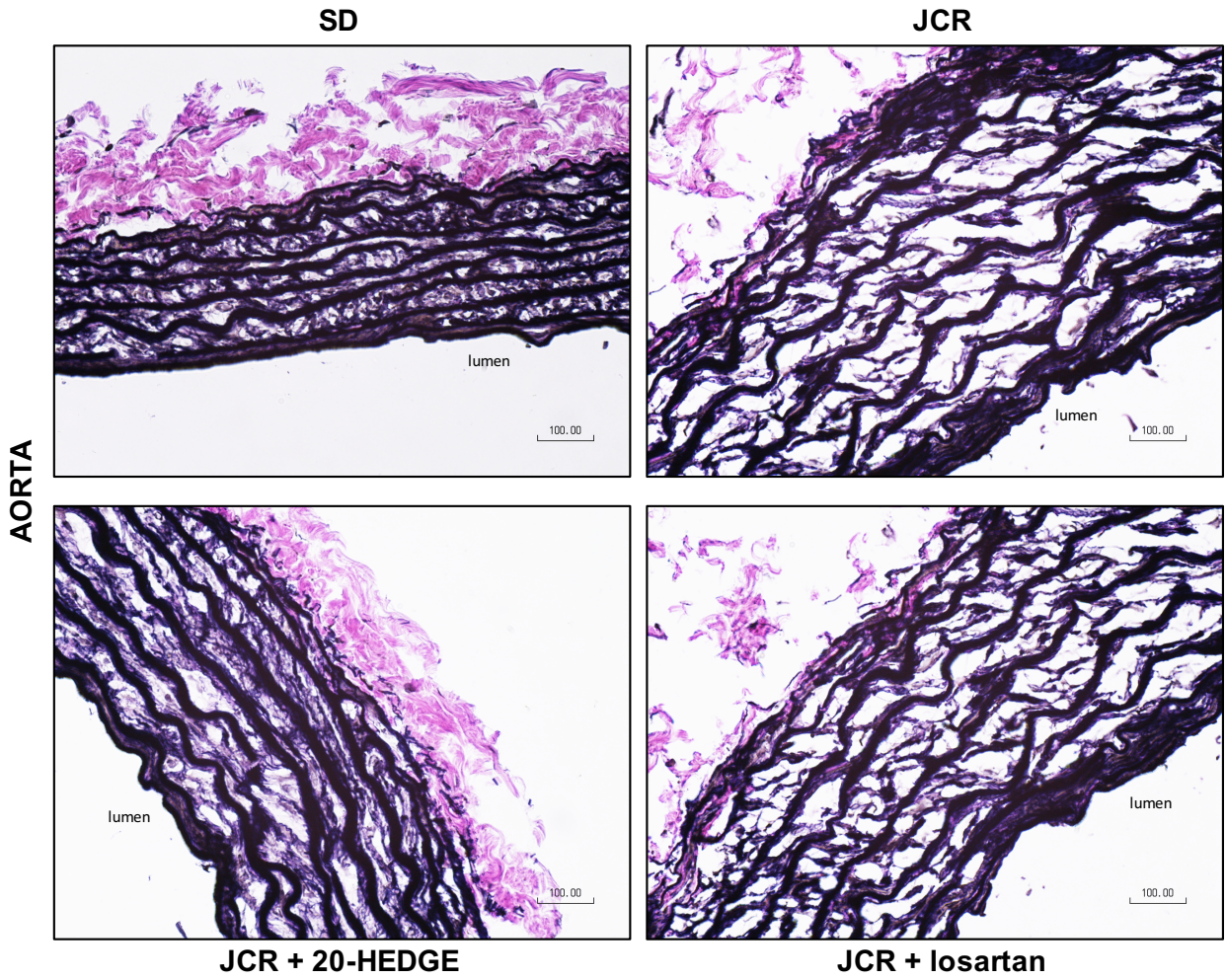
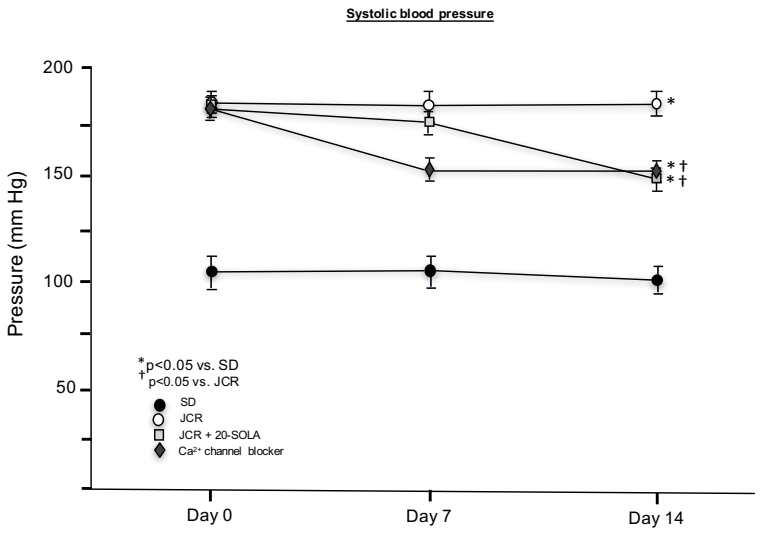
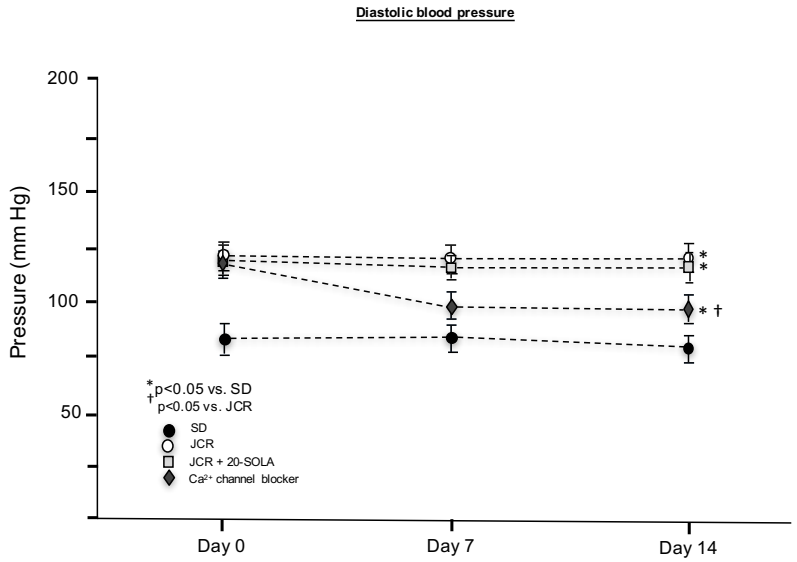


Figure II. SD and JCR rats were treated with 20-HEDGE or losartan for 14 days where indicated, sacrificed and arterial tissue collected. **A.** Representative Verhoeff–Van Gieson (VVG, black) staining of aorta tissue sections at 100X magnification is shown.

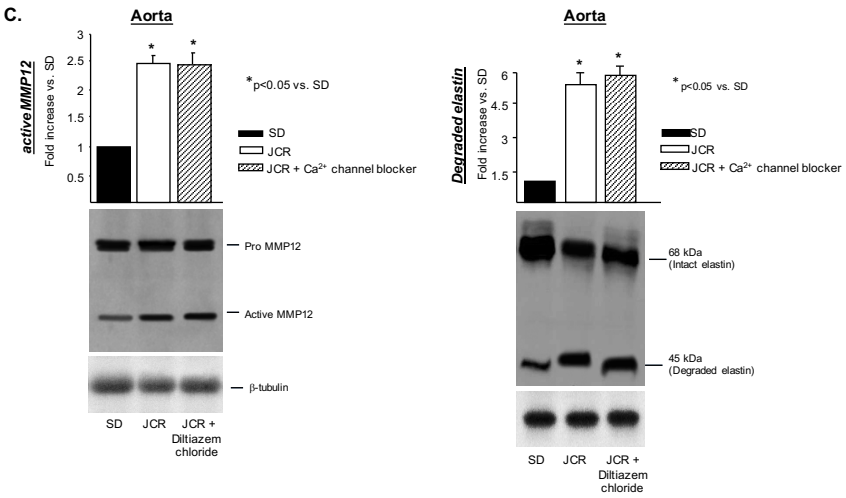
A.


Systolic blood pressure
Pressure (mm Hg)
200
150
100
50
*p<0.05 vs. SD
†p<0.05 vs. JCR
SD
JCR
JCR + 20-SOLA
Ca2+ channel blocker
Day 0
Day 7
Day 14

B.


Diastolic blood pressure
Pressure (mm Hg)
200
150
100
50
*p<0.05 vs. SD
†p<0.05 vs. JCR
SD
JCR
JCR + 20-SOLA
Ca2+ channel blocker
Day 0
Day 7
Day 14

C.


Aorta
activeMMP12
Fold increase vs. SD
*p<0.05 vs. SD
SD
JCR
JCR + Ca2+ channel blocker
Pro MMP12
Active MMP12
β-tubulin
SD
JCR
JCR + Diltiazem chloride
Aorta
Degraded elastin
Fold increase vs. SD
*p<0.05 vs. SD
SD
JCR
JCR + Ca2+ channel blocker
68 kDa (Intact elastin)
45 kDa (Degraded elastin)
SD
JCR
JCR + Diltiazem chloride

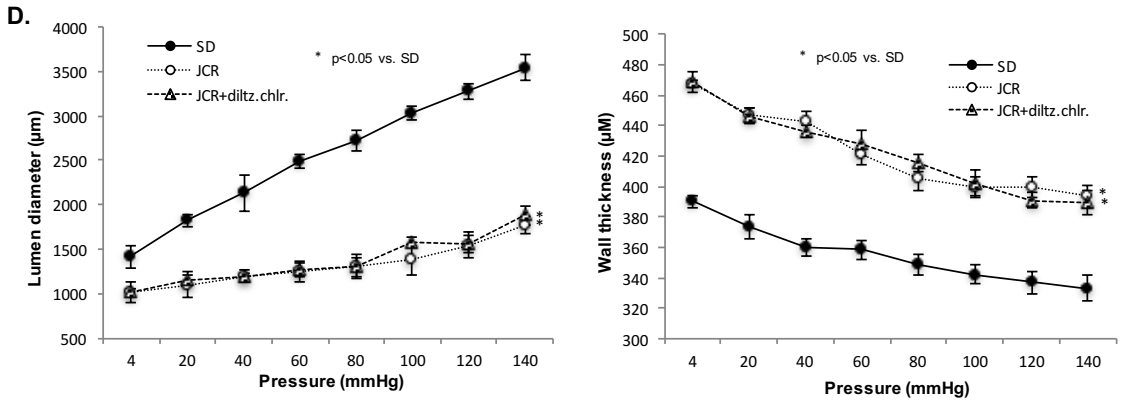
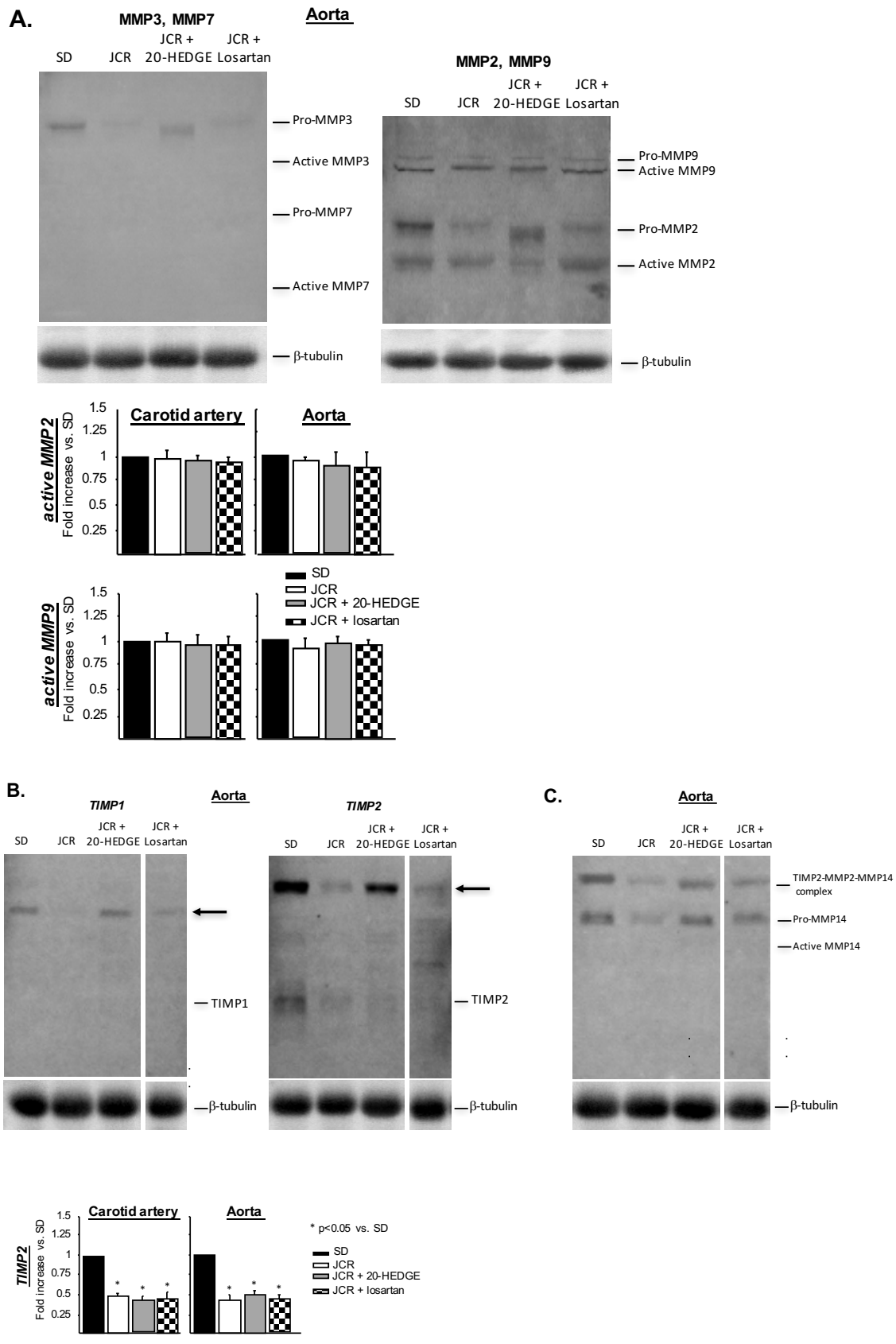


Figure III. JCR rats were treated with 20-SOLA or diltiazem chloride for 14 days where indicated**.** Systolic (**A**) and diastolic (**B**) blood pressure was measured in SD and JCR rats using an aortic catheter connected to a pressure transducer (Millar) on day 0, 7 and 14 of treatment. **C.** Representative Western blots (bottom) and cumulative data (top) showing pro and active (cumulative data) MMP12 expression (**left**) and total and degraded elastin (cumulative data) (**right**) in aorta tissue homogenates are shown. **D.** Lumen diameter and wall thickness of aorta were measured on a pressure myograph. n=8, *, † $p<0.05$ as indicated.


A.
MMP3, MMP7
Aorta
SD
JCR
JCR + 20-HEDGE
JCR + Losartan
Pro-MMP3
Active MMP3
Pro-MMP7
Active MMP7
β-tubulin
MMP2, MMP9
Pro-MMP9
Active MMP9
Pro-MMP2
Active MMP2
β-tubulin
Carotid artery
Aorta
active MMP2
Fold increase vs. SD
1.5
1.25
1
0.75
0.5
0.25
active MMP9
SD
JCR
JCR + 20-HEDGE
JCR + losartan
B.
TIMP1
TIMP2
Aorta
TIMP1
TIMP2
β-tubulin
Carotid artery
Aorta
* p<0.05 vs. SD
C.
Aorta
TIMP2-MMP2-MMP14 complex
Pro-MMP14
Active MMP14
β-tubulin

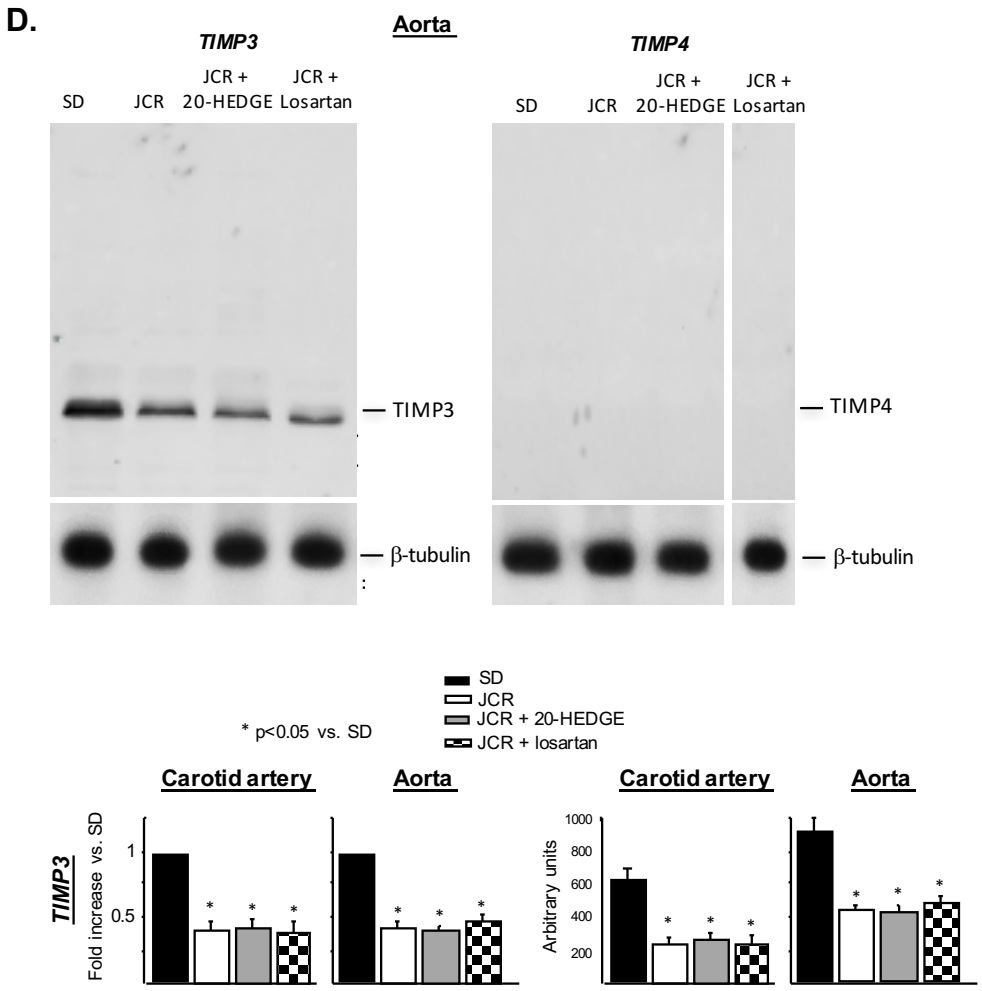


Figure IV. SD and JCR rats were treated with 20-HEDGE or losartan for 14 days where indicated, sacrificed and aortic tissue collected. Representative Western blots (top) and cumulative data (bottom) showing pro and active (cumulative data) MMP3, MMP7, MMP2 and MMP9 expression (**A**), TIMP1 and TIMP2 expression (**B**), pro and active MMP14 expression (**C**), and TIMP3 and TIMP4 expression (**D**) in aorta tissue homogenates are shown. n=8, *, † $p<0.05$ as indicated. *Note:* Where cumulative data for active MMPs or TIMPs are missing, no protein expression was detected even with high amount of protein (100 µg) at long (5-10 min) exposures.

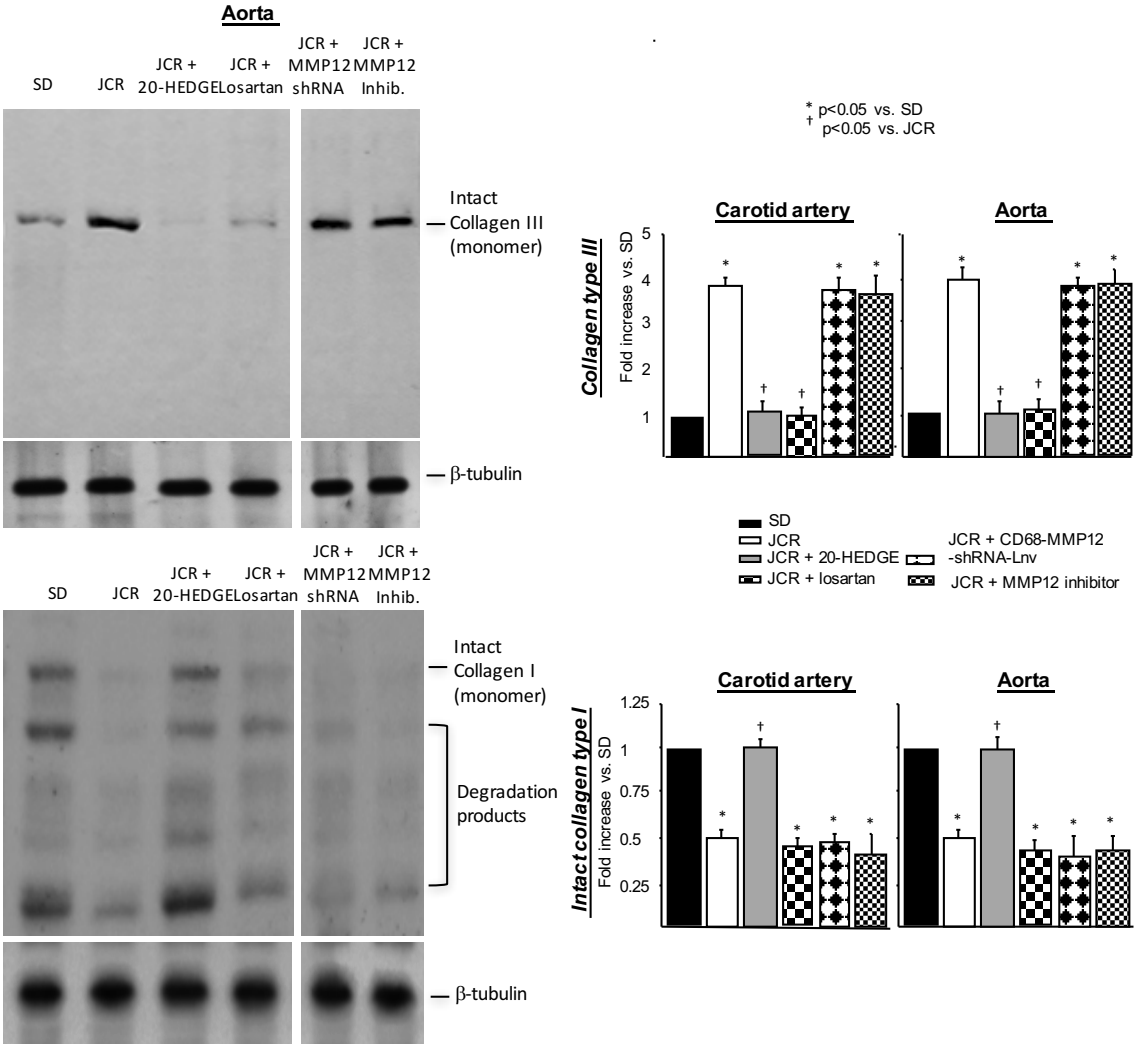
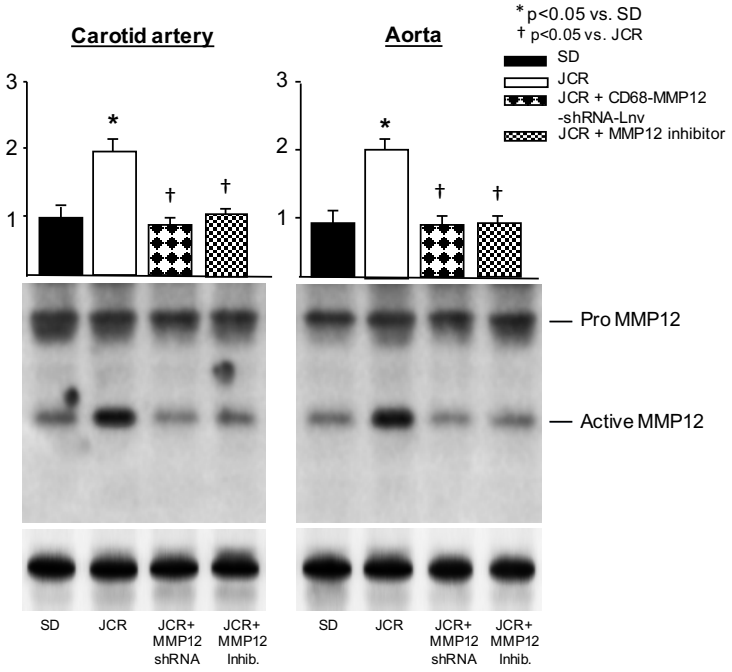


Figure V. SD and JCR rats were treated with 20-HEDGE, losartan, the MMP12-shRNA-Lnv or the pharmacological MMP12 inhibitor for 14 days where indicated, sacrificed and aortic tissue collected. **A.** Representative Western blots (left) and cumulative data (right, intact collagen) showing type III (top) and type I (bottom) collagen expression are shown. n=8, *, † $p<0.05$ as indicated.



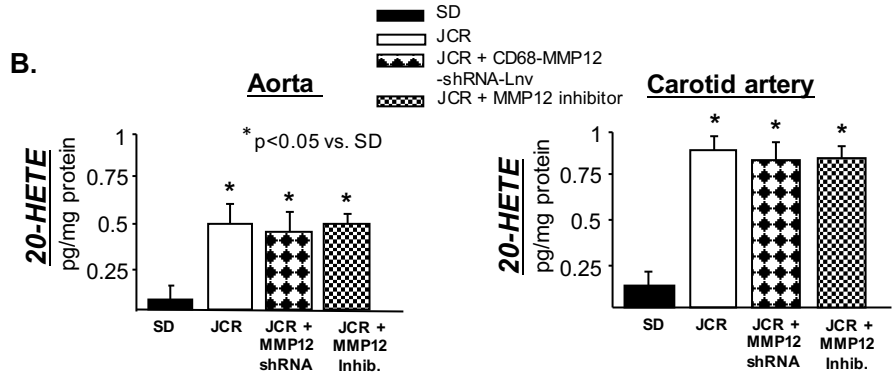


FIGURE VI. SD and JCR rats were treated with the MMP12-shRNA-Lnv or the pharmacological MMP12 inhibitor for 14 days where indicated, sacrificed and arterial tissue collected. **A.** Representative Western blots (bottom) and cumulative data (top) showing pro and active (cumulative data) MMP12 expression in carotid artery and aorta tissue homogenates are shown. **B.** LC-MS quantitation of 20-HETE production represented in pg/mg protein. n=8, *, † $p<0.05$ as indicated.

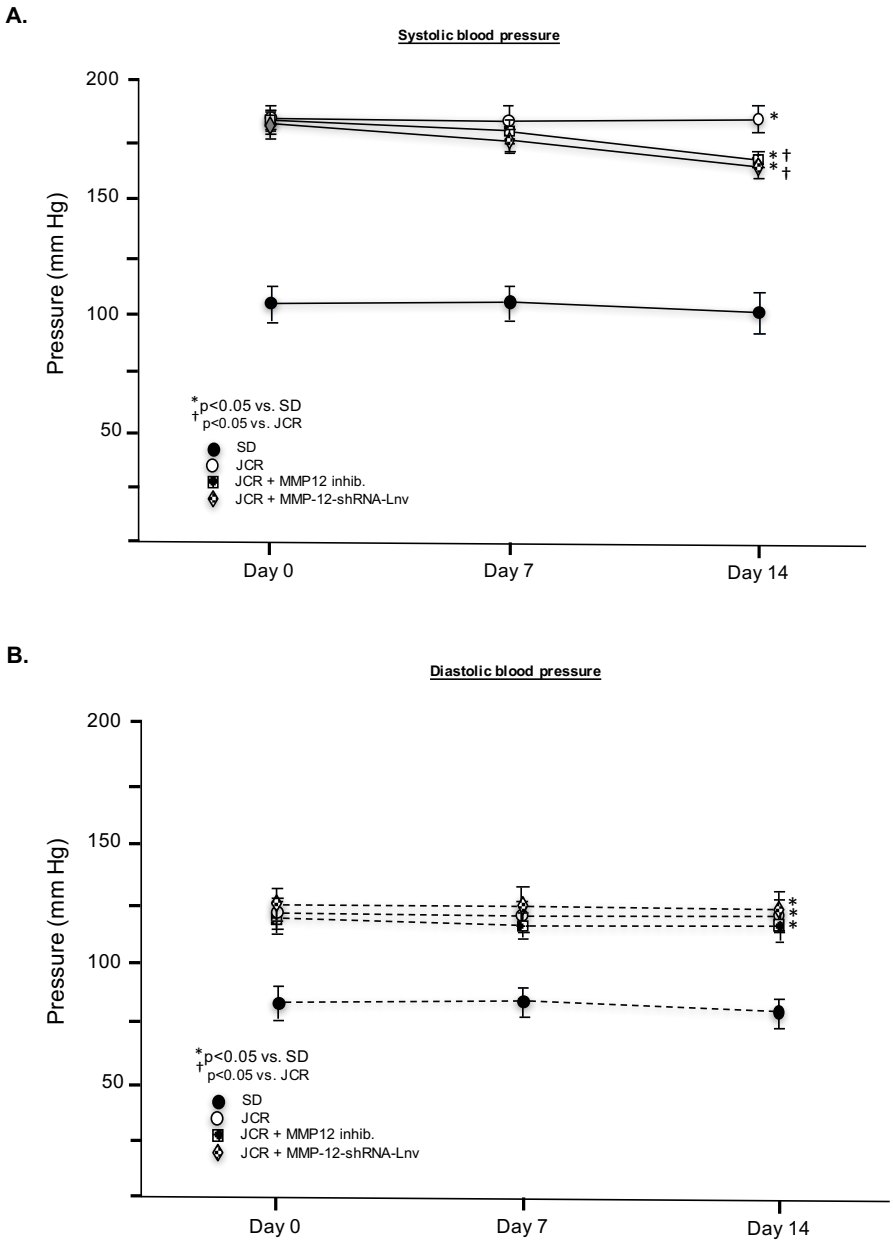
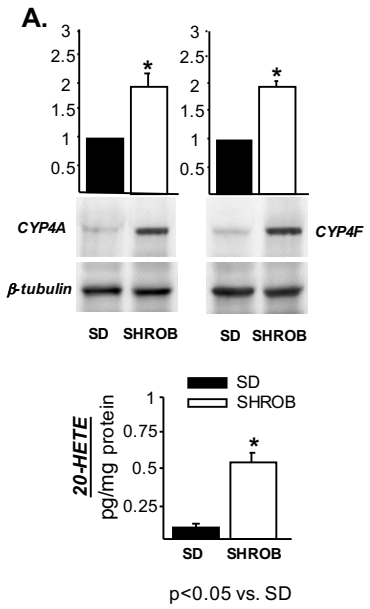
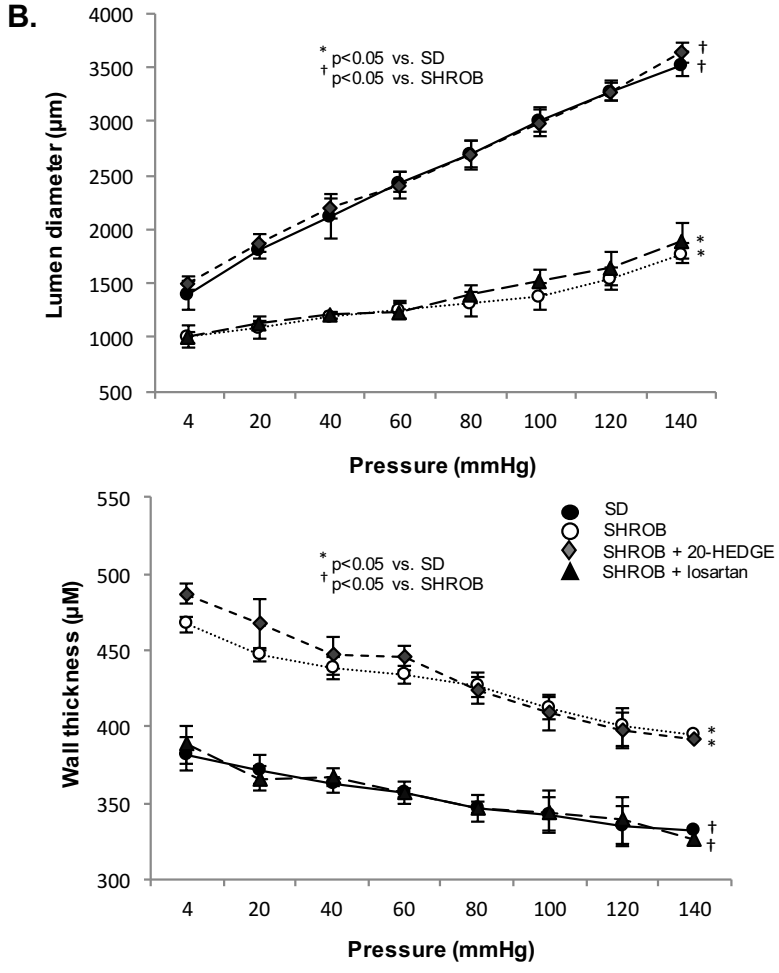


FIGURE VII. JCR rats were treated with the MMP12-shRNA-Lnv or the pharmacological MMP12 inhibitor for 14 days where indicated**.** Systolic (**A**) and diastolic (**B**) blood pressure was measured in SD and JCR rats using an aortic catheter connected to a pressure transducer (Millar) on day 0, 7 and 14 of treatment. n=8, *, † $p<0.05$ as indicated.


A.
CYP4A
CYP4F
β-tubulin
SD SHROB
SD SHROB
SD
SHROB
20-HETE
pg/mg protein
SD SHROB
p<0.05 vs. SD


B.
Lumen diameter (μm)
* p<0.05 vs. SD
† p<0.05 vs. SHROB
Pressure (mmHg)
Wall thickness (μM)
SD
SHROB
SHROB + 20-HEDGE
SHROB + losartan
Pressure (mmHg)

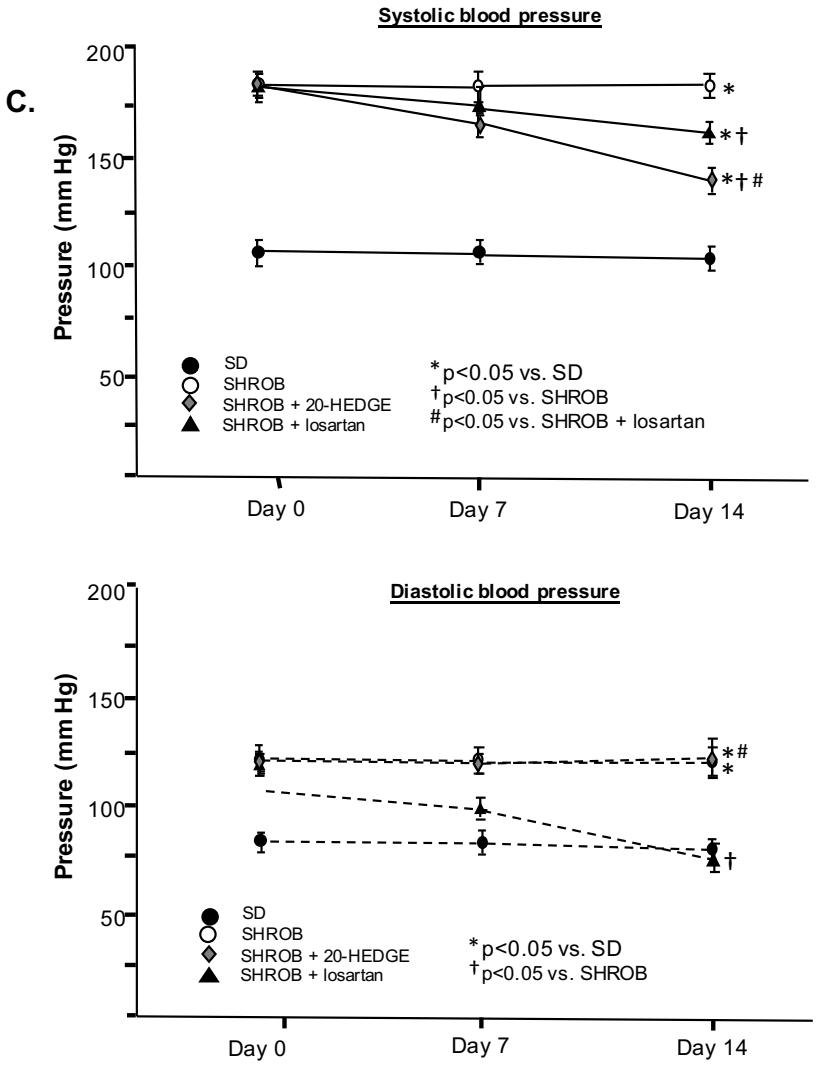


FIGURE VIII. SD and SHROB rats were treated with 20-HEDGE or losartan for 14 days where indicated, sacrificed and aortic tissue collected. **A.** Representative Western blots and cumulative data (top) showing CYP4A and CYP4F expression and LC-MS quantitation of 20-HETE production represented in pg/mg protein (bottom) in aorta tissue homogenates. n=8, * $p<0.05$ as indicated. **B.** Lumen diameter and wall thickness of aorta were measured on a pressure myograph. n=8, *, † $p<0.05$ as indicated. **C.** Systolic (top) and diastolic (bottom) blood pressure was measured in SD and SHROB rats using an aortic catheter connected to a pressure transducer (Millar) on day 0, 7 and 14 of treatment. n=8, *, †, # $p<0.05$ as indicated.