The regulatory role of coagulation factors on arterial function

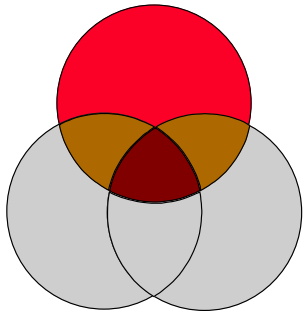
Jérémy Lagrange

ARTERY18

AG Wenzel, Center for Thrombosis and Hemostasis,
University Medical Center Mainz

Virchow's tirad

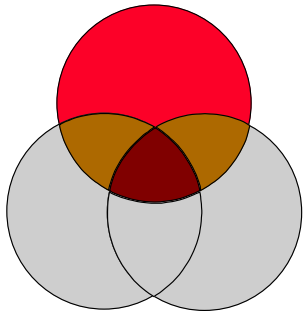
Rudolph Virchow, 1846: Über die Verstopfung der Lungenarterie



- Hypercoagulability
- Low flow
- Vessel wall damage

Virchow's tirad

Rudolph Virchow, 1846: Über die Verstopfung der Lungenarterie



- Hypercoagulability → blood makes too much thrombin
- Low flow → thrombin remains in force locally
- Vessel wall damage → blood is triggered to make thrombin

Common denominator: thrombin

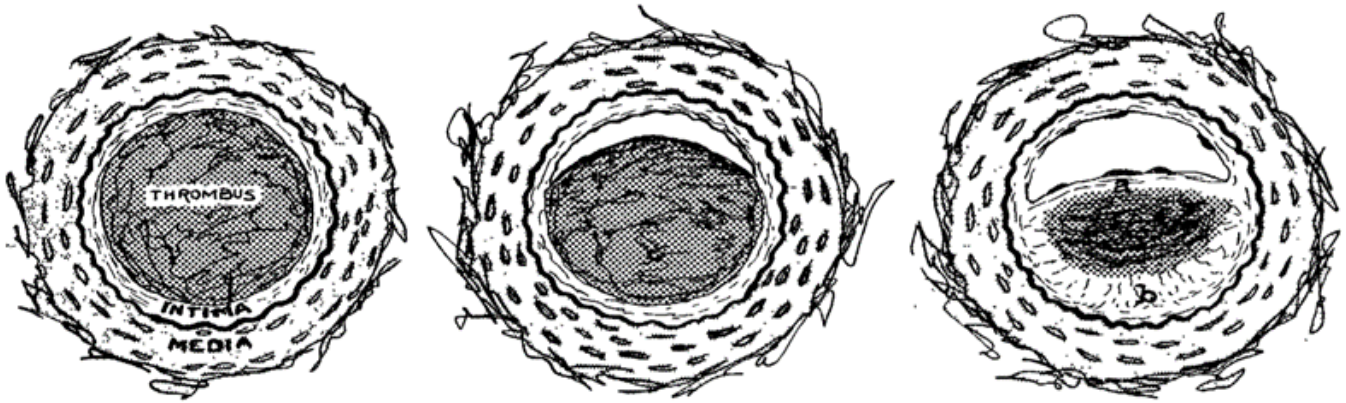
The thrombogenic Theory

THE THROMBOGENIC HYPOTHESIS AND ITS IMPLICATIONS

J. B. DUGUID, M.D.

From the Department of Pathology, Royal Victoria Infirmary, Newcastle upon Tyne

The discovery that mural thrombi in arteries become incorporated in the intima and form fibrous thickenings has raised many new questions, some of which are at present occupying the attention of members of the Pathology Department.



Duguid, *Postgrad Med J.* 1960

Protease Activated Receptors (PARs)

The mechanism by which thrombin activates platelets and other cells is unknown, and, despite considerable effort by a number of laboratories, the functional receptor(s) that mediates thrombin signaling has not been identified.

Protease Activated Receptors (PARs)

The mechanism by which thrombin activates platelets and other cells is unknown, and, despite considerable effort by a number of laboratories, the functional receptor(s) that mediates thrombin signaling has not been identified.

Cell, Vol. 64, 1057–1068, March 22, 1991, Copyright © 1991 by Cell Press

Molecular Cloning of a Functional Thrombin Receptor Reveals a Novel Proteolytic Mechanism of Receptor Activation

Thien-Khai H. Vu,* David T. Hung,*†‡
Virginia I. Wheaton,* and Shaun R. Coughlin*†
*Cardiovascular Research Institute
†Department of Medicine
‡Cancer Research Institute
University of California, San Francisco
San Francisco, California 94143–0524

Vu, *Cell.* 1991

Coagulation factors and vessel development

Role of tissue factor in embryonic blood vessel development

Tissue Factor (TF)

Peter Carmeliet*, Nigel Mackman†, Lieve Moons*, Thomas Luther‡, Pierre Gressens§, Ilse Van Vlaenderen*, Hilde Demunck‖, Michael Kasper‡, Georg Breier¶, Philippe Evrard§, Martin Müller‡, Werner Risau¶, Thomas Edgington† & Désiré Collen*

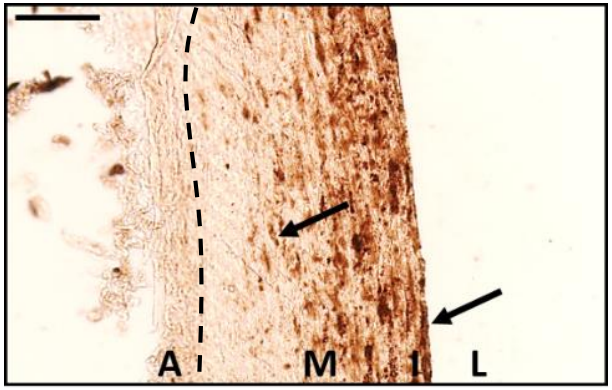
TISSUE factor, a member of the cytokine-receptor superfamily and high-affinity receptor and cofactor for plasma factor VII/VIIa (ref. 1), is the primary cellular initiator of blood coagulation. It is involved in thrombosis and inflammation associated with sepsis, atherosclerosis and cancer[2], and can participate in other cellular processes including intracellular signalling[3], metastasis[4], tumour-associated angiogenesis[5], and embryogenesis[6]. Here we report that inactivation of the tissue factor gene (TF) results in abnormal circulation from yolk sac to embryo beyond embryonic day 8.5, leading to embryo wasting and death. Vitelline vessels from null mice were deficient in smooth-muscle α-actin-expressing mesenchymal cells, which participate in organization of the vessel wall. This implies that tissue factor has a role in blood-vessel development.

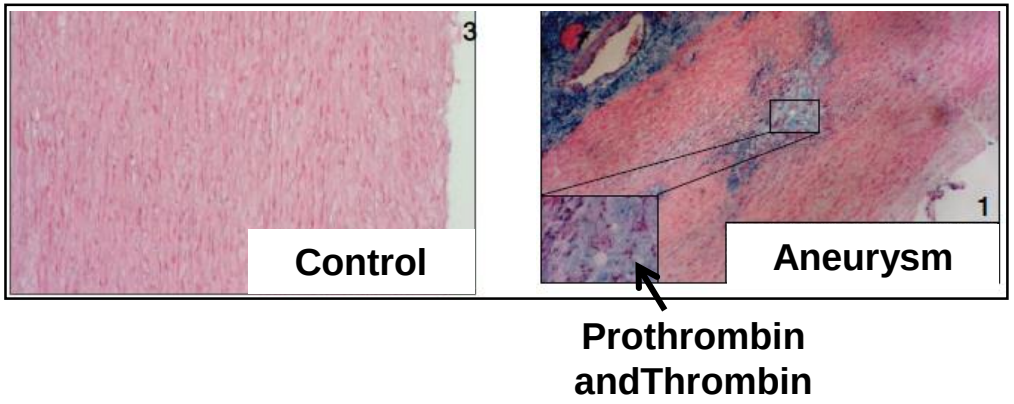
Carmeliet, *Nature.* 1996

Intramural thrombin

- Prothrombin (FII) and thrombin (FIIa) are present in the vascular wall

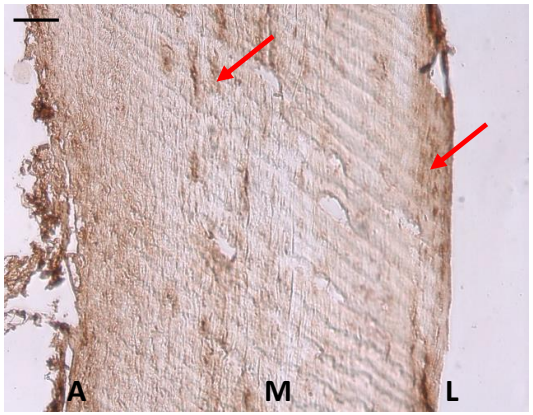
1





Prothrombin andThrombin

Immunostaining for fibrin/fibrinogen



healthy aorta

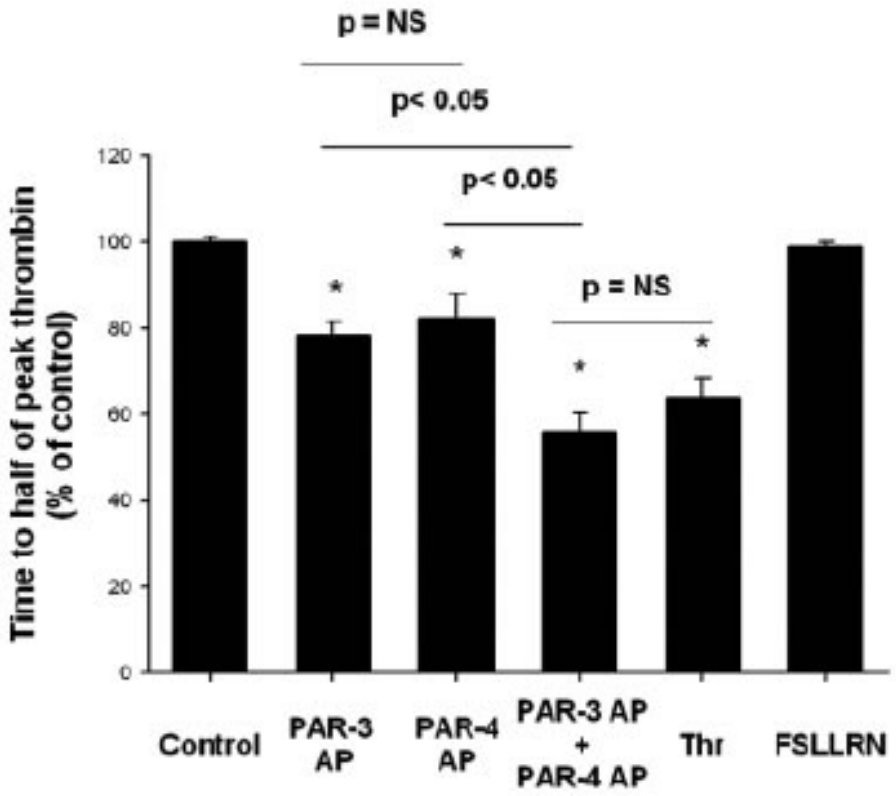
Touat *et al., Arterioscler Thromb Vac Biol*. 2008

Intramural thrombin

- Prothrombin (FII) and thrombin (FIIa) are present in the vascular wall

1

- PAR-3 and -4 activation accelerates TF-induced thrombin generation on the surface of VSMCs



Vidwan *et al., Arterioscler Thromb Vac Biol*. 2010

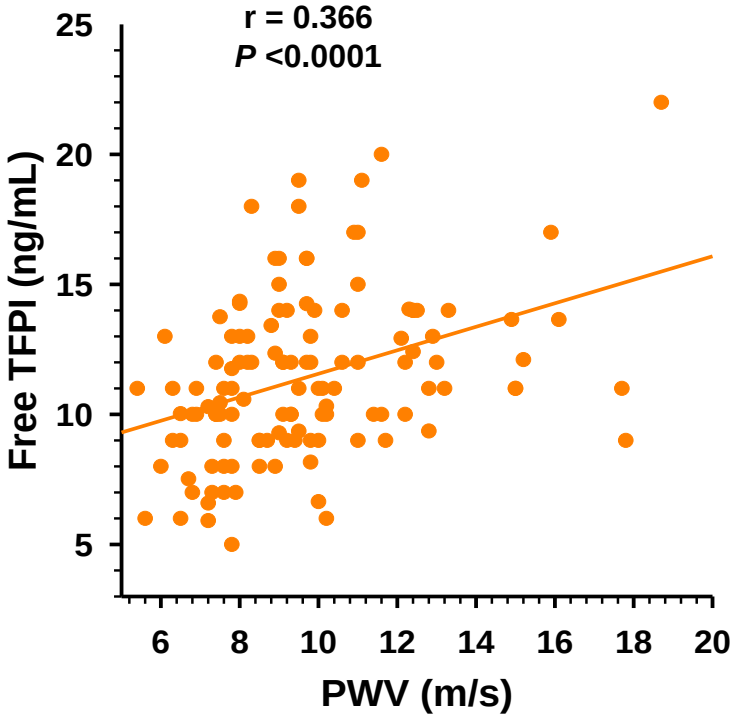
Pulsatile stretch and coagulation factors

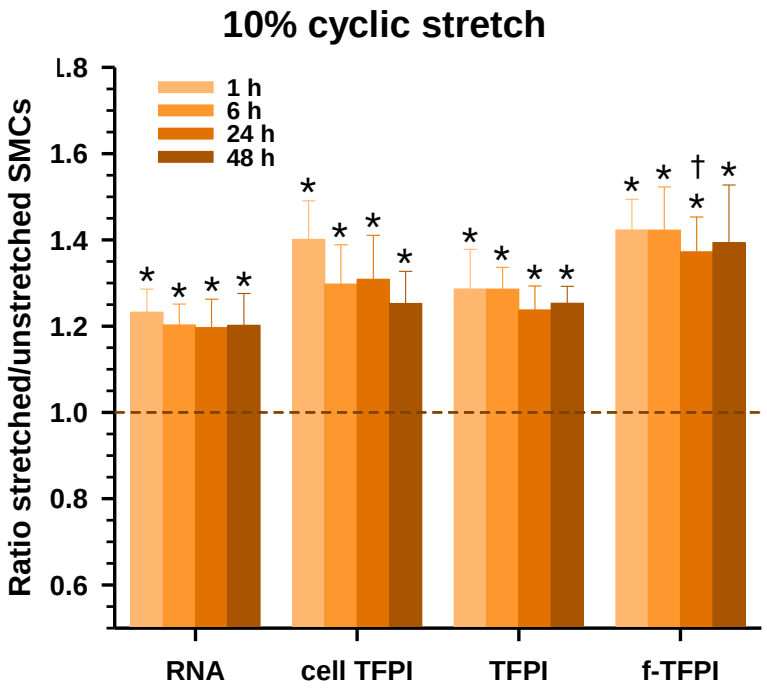
1

- Prothrombin and thrombin are present in the vascular wall
- PAR-3 and -4 activation accelerates TF-induced thrombin generation on the surface of VSMCs

2

- Tissue Factor Pathway Inhibitor (TFPI) increases *in vivo* and *in vitro* with pulsatile stretch





Regnault *et al., Arterioscler Thromb Vac Biol*. 2011

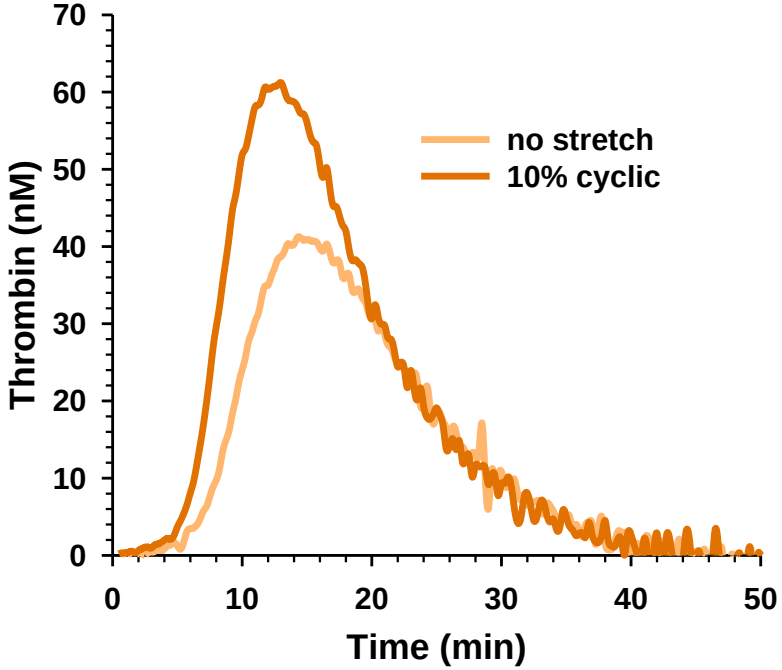
Pulsatile stretch and coagulation factors

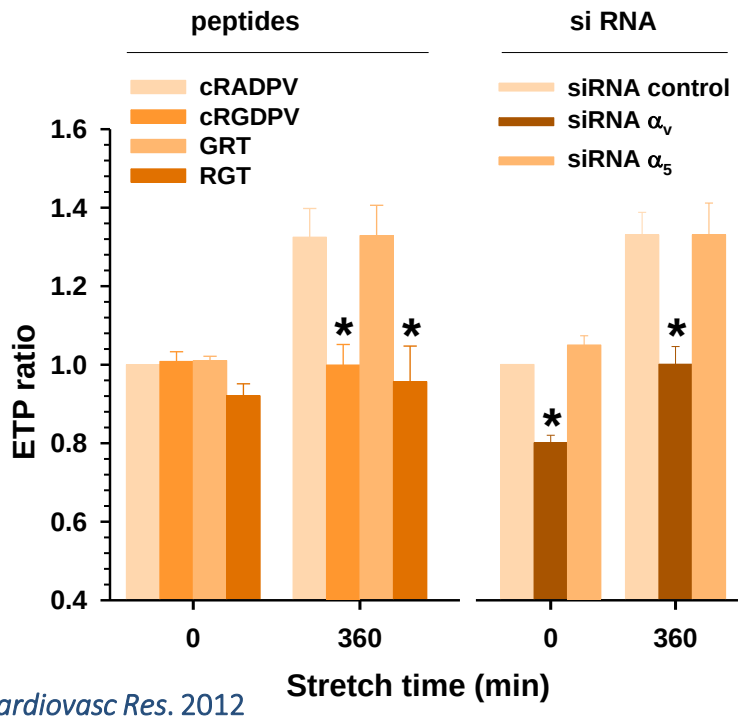
1

- Prothrombin and thrombin are present in the vascular wall
- PAR-3 and -4 activation accelerates TF-induced thrombin generation on the surface of VSMCs

2

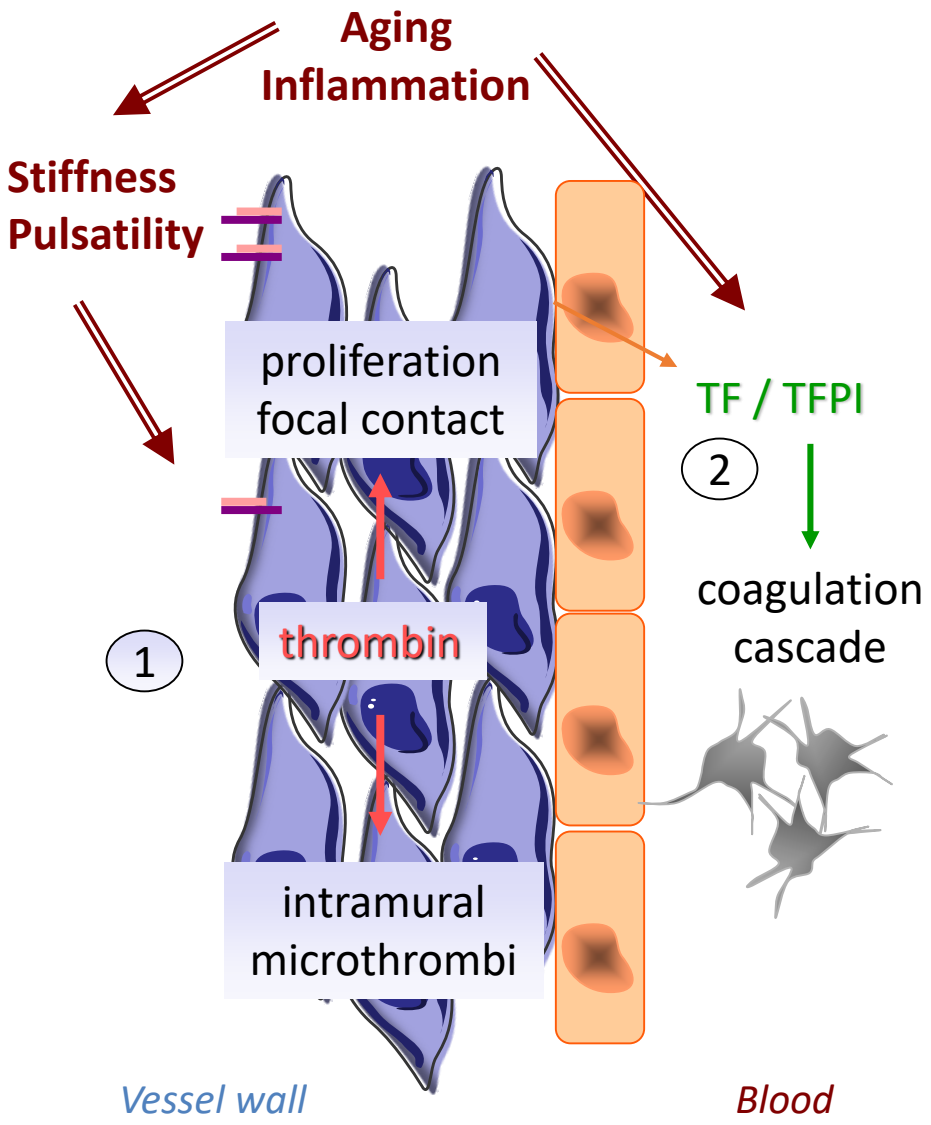
- Tissue Factor Pathway Inhibitor (TFPI) increases *in vivo* and *in vitro* with pulsatile stretch
- Cyclic stretch-induced thrombin generation is mediated by the integrin $\alpha_v\beta_3$ pathway

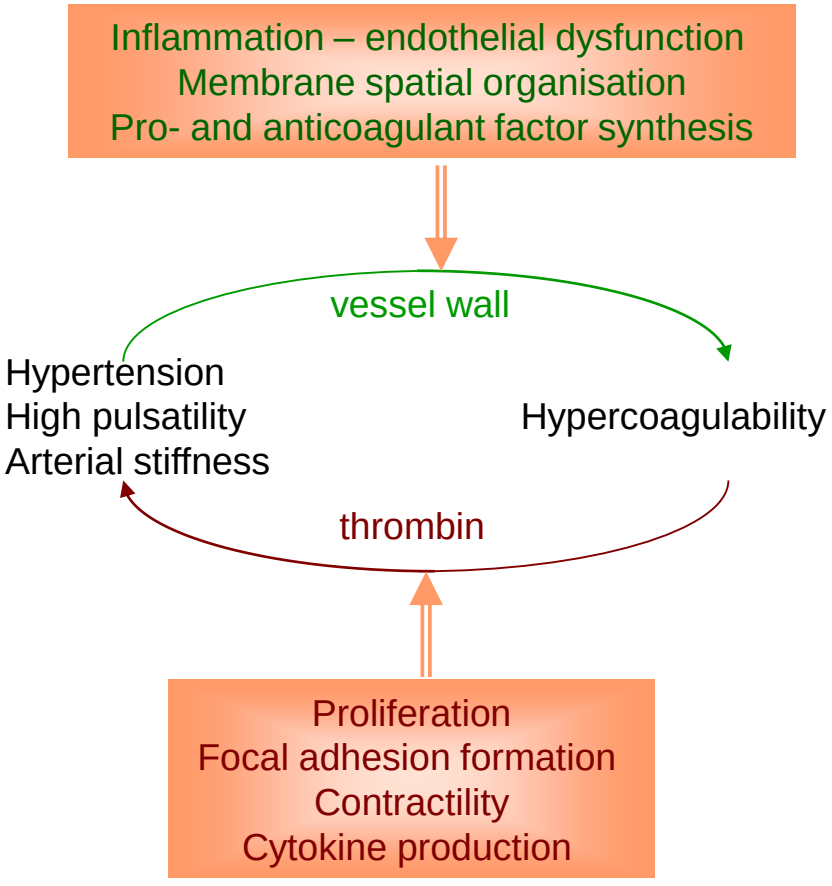




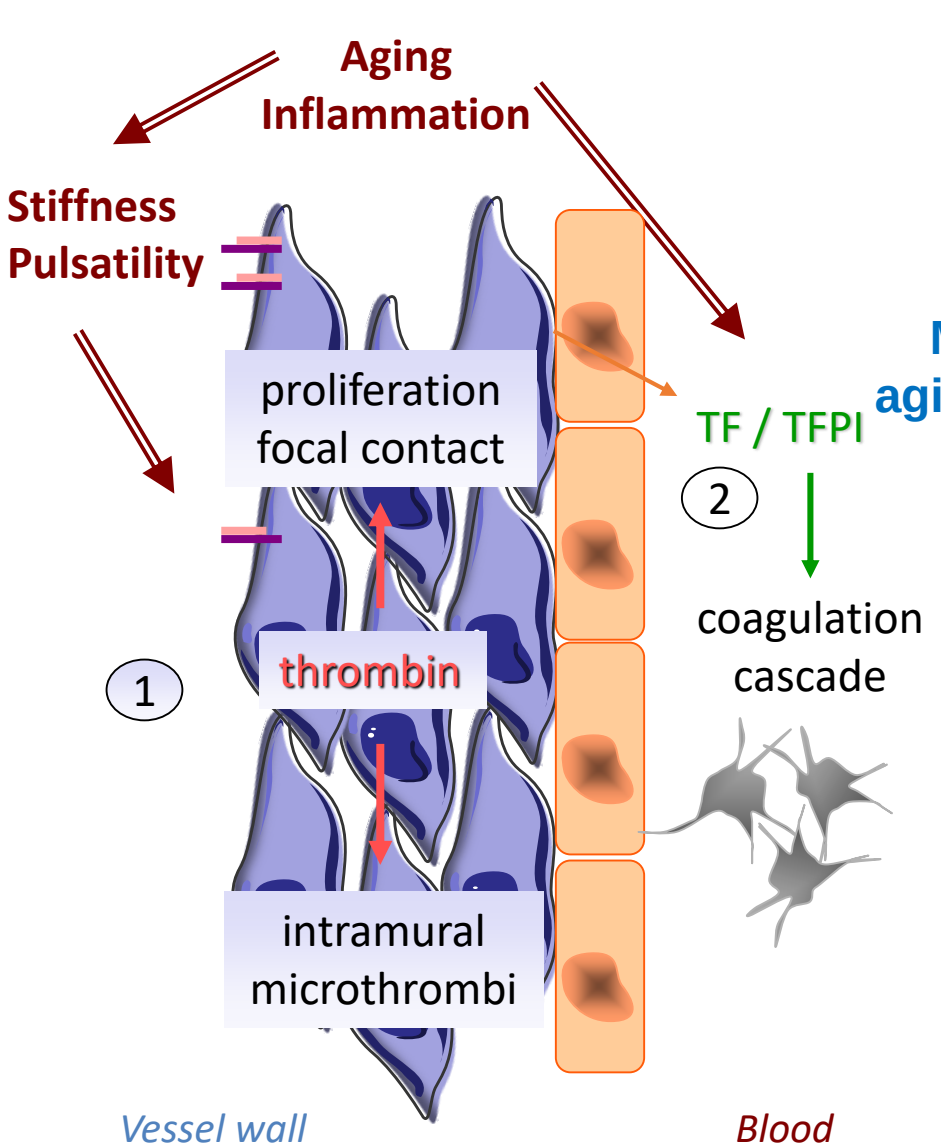
Mao *et al., Cardiovasc Res*. 2012

Introduction





Introduction



Altered VSCMs modulation?

Inflammation – endothelial dysfunction
Membrane spatial organisation
Pro- and anticoagulant factor synthesis

Metabolic and aging modulation?

vessel wall

Hypertension
High pulsatility
Arterial stiffness

Hypercoagulability

thrombin

RAAS?

Proliferation
Focal adhesion formation
Contractility
Cytokine production

Immune cell modulation?

Aims

To explore the role of coagulation factors on the regulation of vascular cells and function and in the development of vascular diseases

Models

Altered VSCM modulation?

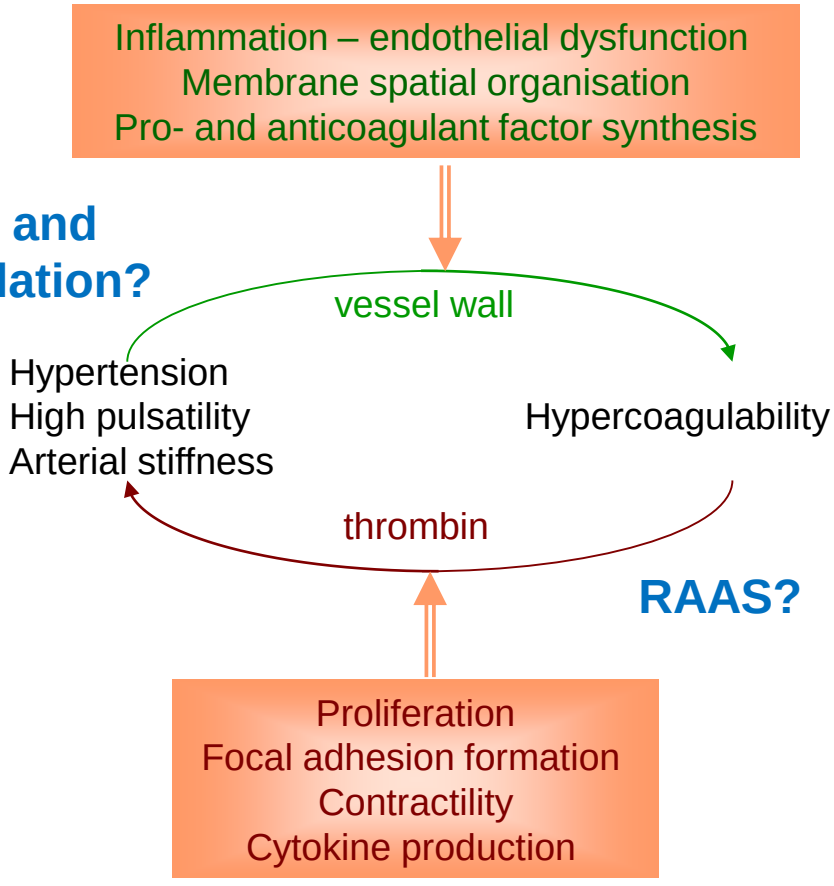
Zucker rats

Spontaneously hypertensive rats (SHR)

Metabolic and aging modulation?

Endothelial mineralocorticoid receptor (MR) mice

Angiotensin II infused animals +/- FXI antisense oligonucleotide

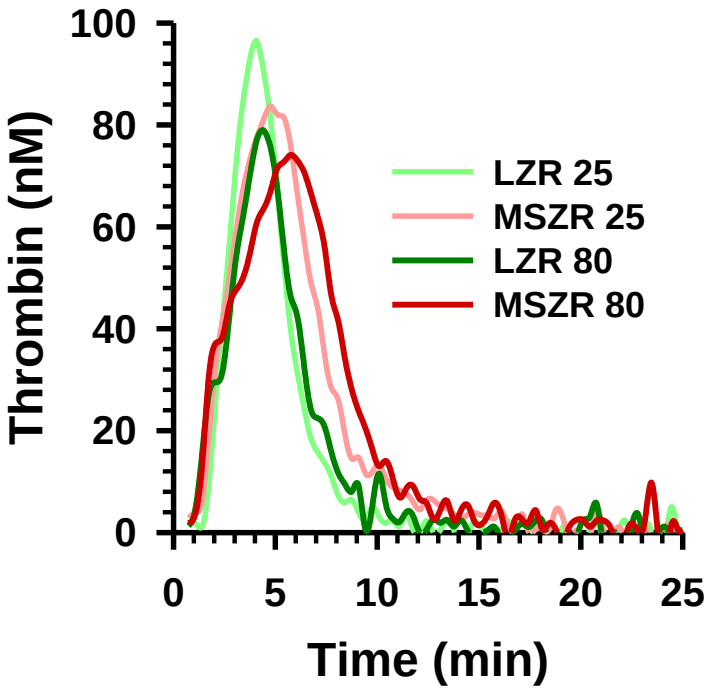


Immune cell modulation?

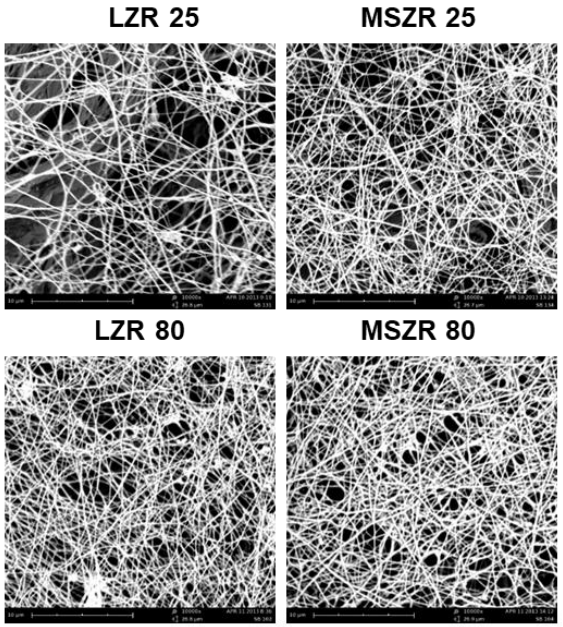
Results

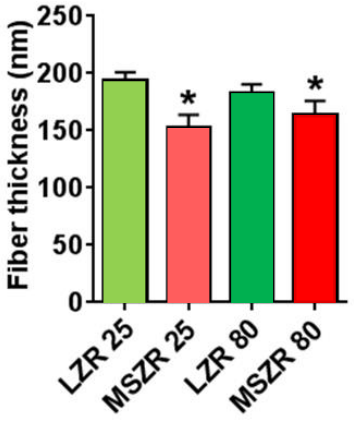
1: Metabolic syndrome, aging and vascular function

MSZR rats display increased thrombin generation



Fibrin clot structure is altered in MSZR





Lean Zucker rat (LZR)
Metabolic Syndrome Zucker Rat (MSZR)

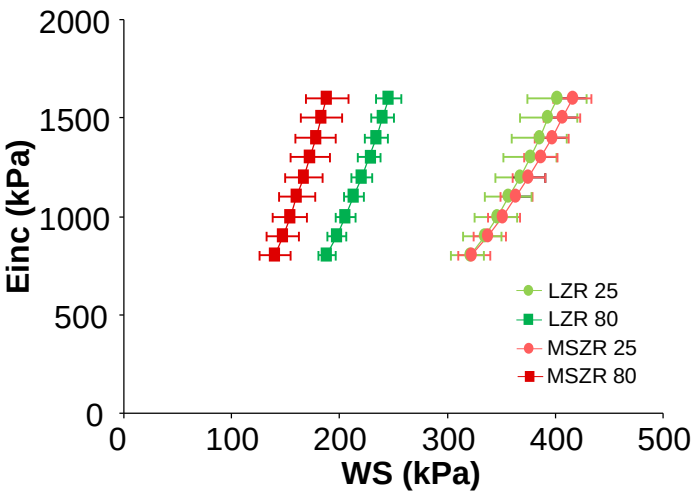
Results are means ± SEM of 7-11 rats per group; * *P*<0.05 *vs* same age LZR

Lagrange *et al*., *Front Physiol*. 2017

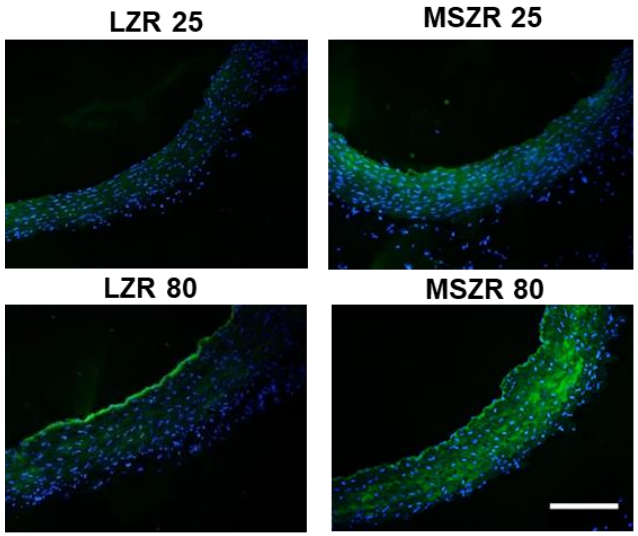
Results

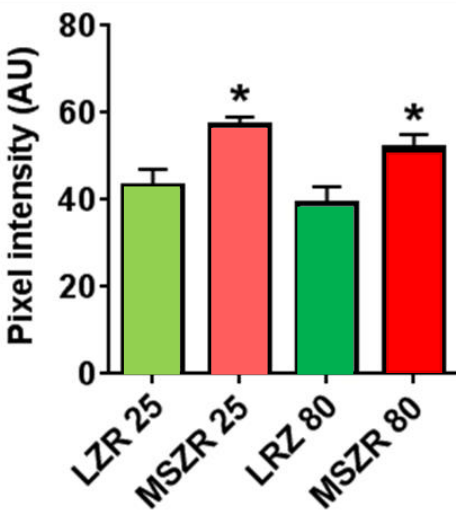
1: Metabolic syndrome, aging and vascular function

Incremental elastic modulus-wall stress is altered with age



MetS increases gelatinolytic metalloproteinase activity in the aortic wall





Results are means ± SEM of 7-11 rats per group; * *P*<0.05 *vs* same age LZR

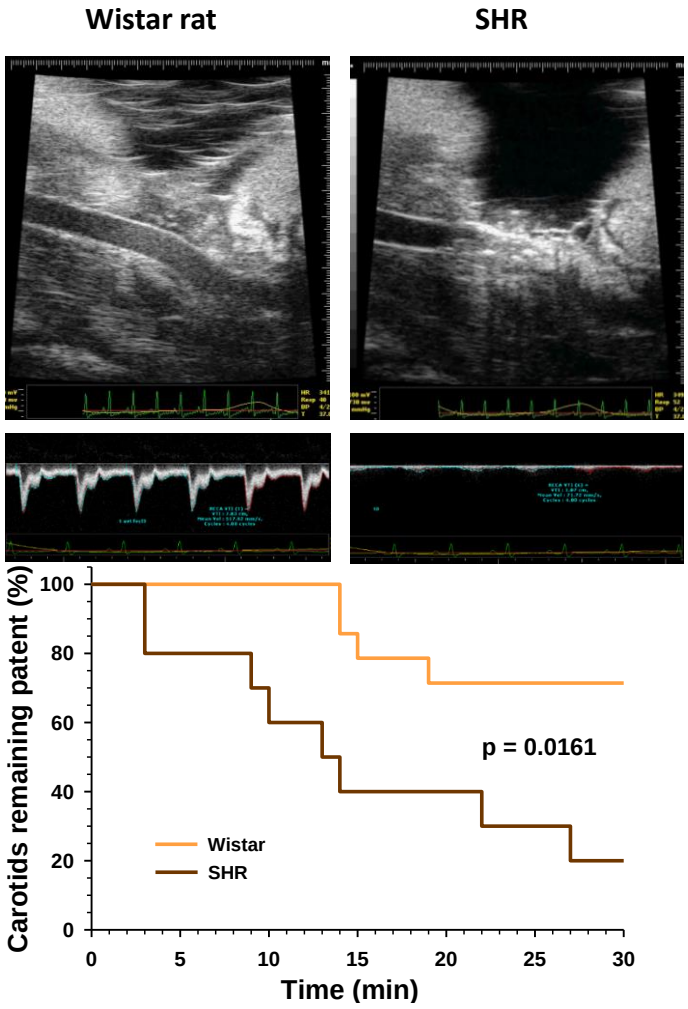
Lagrange *et al*., *Front Physiol*. 2017

Results

2: Tissular thrombin generation in hypertension

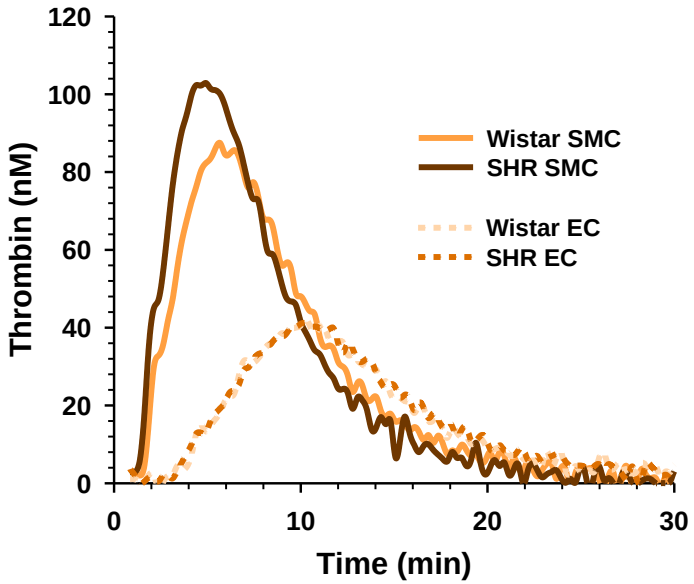
SHR rats display an enlarged thrombosis *in vivo*

$FeCl_3$-induced thrombus formation in carotids



10% $FeCl_3$ application for 3 minutes
n = 10 Wistar , 14 SHR

Thrombin generation on VSMCs

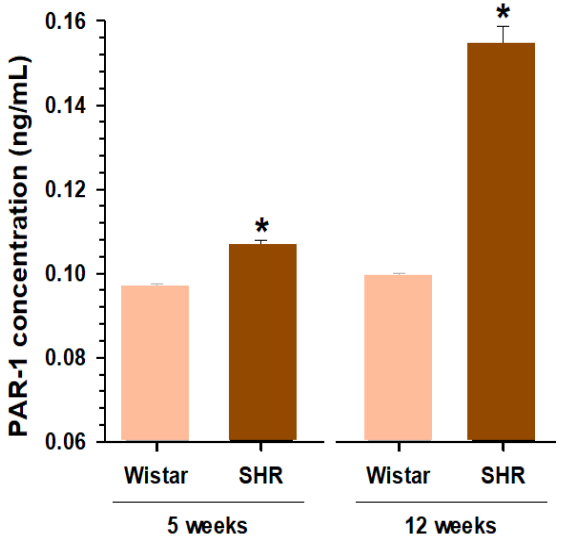


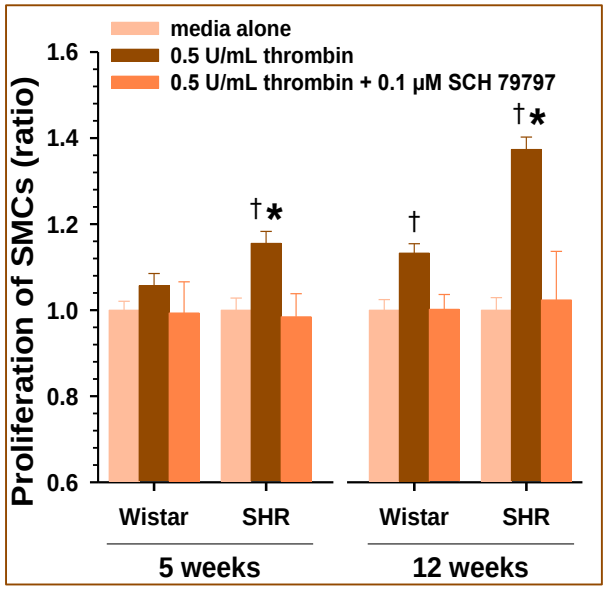
Ait Aissa, Lagrange* et al., ATVB*. 2015

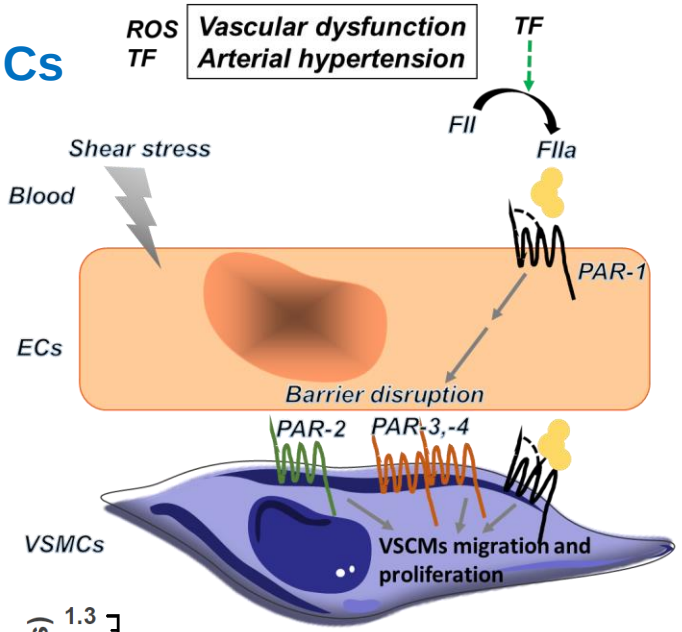
Results

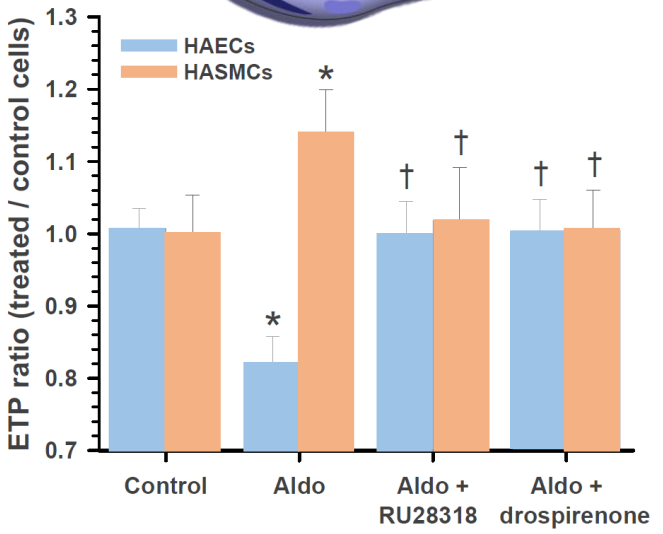
2: Tissular thrombin generation in hypertension

PAR-1-dependent increased proliferation of SHR VSMCs





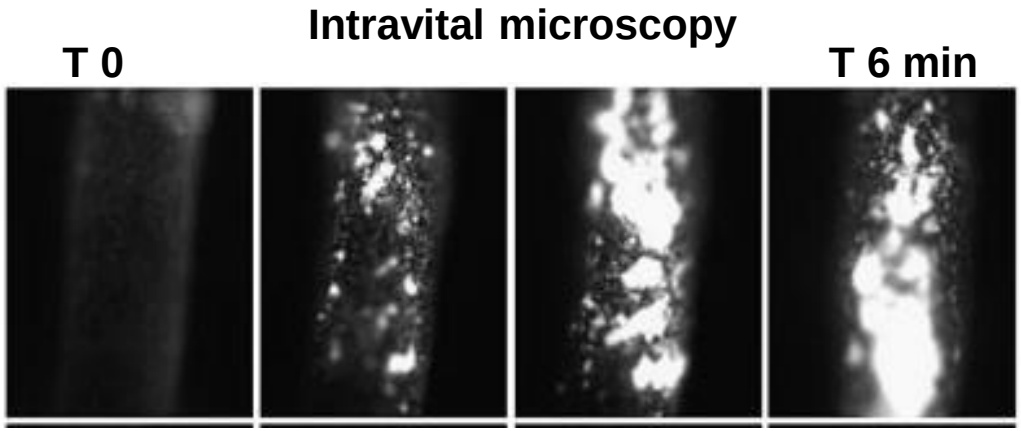




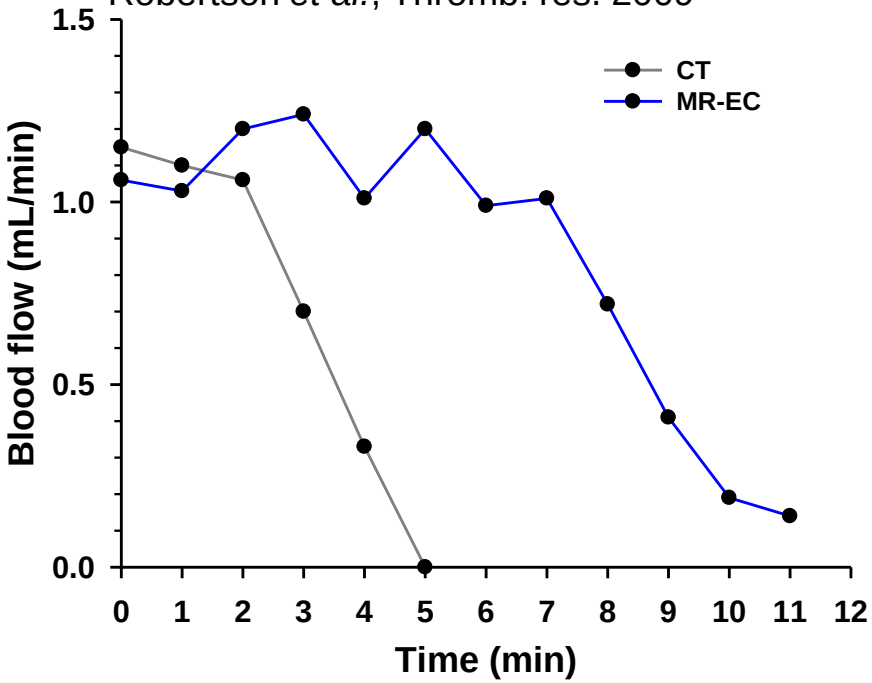
* P<0.05 versus SMCs from Wistar rats at the same age; † P<0.05 vs media alone
Results are means ± SEM, n=5 per group

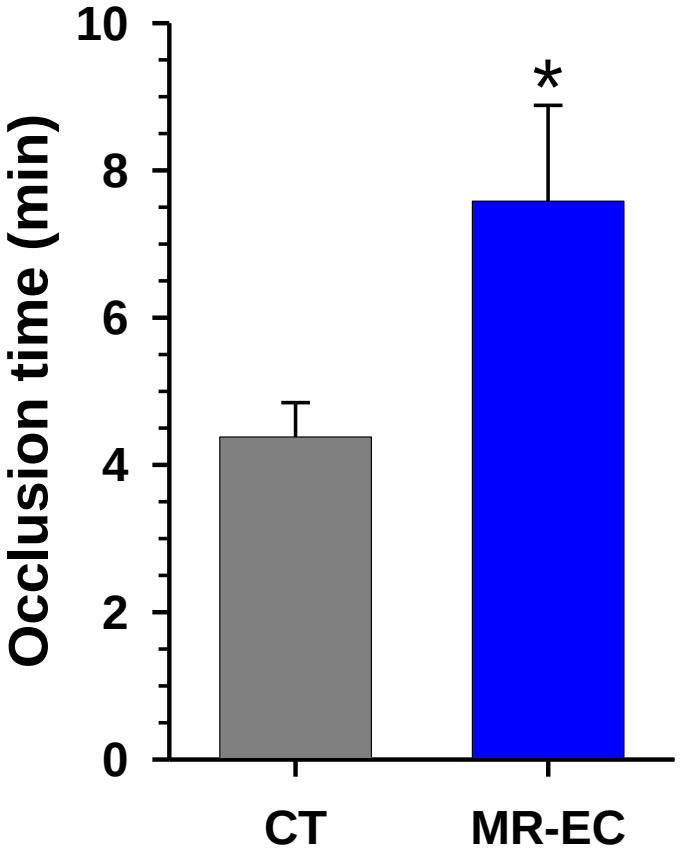
Ait Aissa, Lagrange* et al., ATVB*. 2015

3: Endothelial MR protects against thrombosis



Robertson *et al.*, Thromb. res. 2009





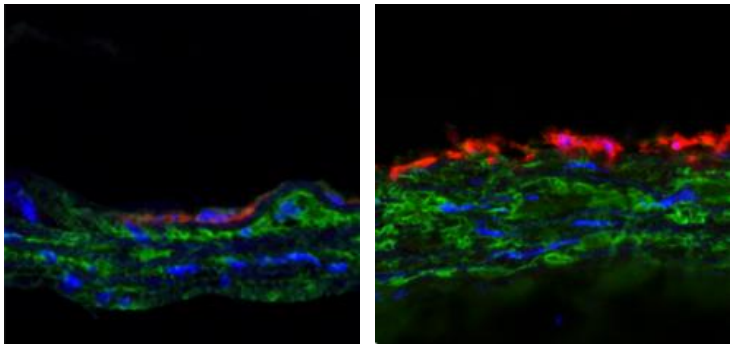
* $p < 0.05$ versus control

Results are mean ± SEM

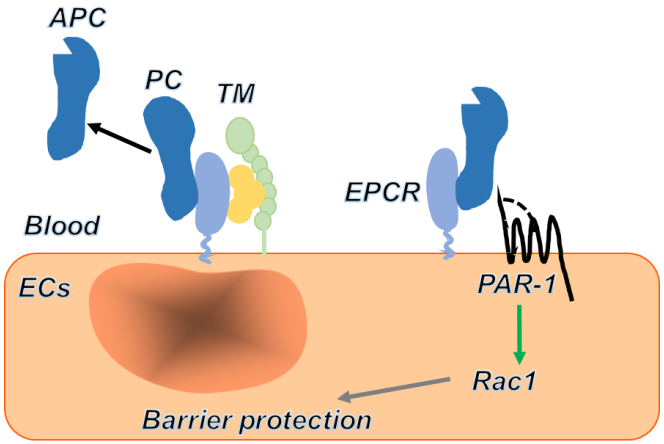
Lagrange *et al.*, *FASEB*. 2014

3: Endothelial MR protects against thrombosis

CT MR-EC



Expression of EPCR (**red**) in aorta



Endothelial Protein C receptor (EPCR) is increased in MR-EC mice endothelium

Parameters	CT	MR-EC
Aorta levels		
TM, ng/ml	*2.1 ± 0.2*	*2.3 ± 0.4*
EPCR, ng/ml	***13.5 ± 0.4***	***15.7 ± 0.8*** *
ADAM17, ng/ml	*1.2 ± 0.2*	*1.1 ± 0.1*
Plasma levels		
sEPCR, ng/ml	*22 ± 5*	*17 ± 3*

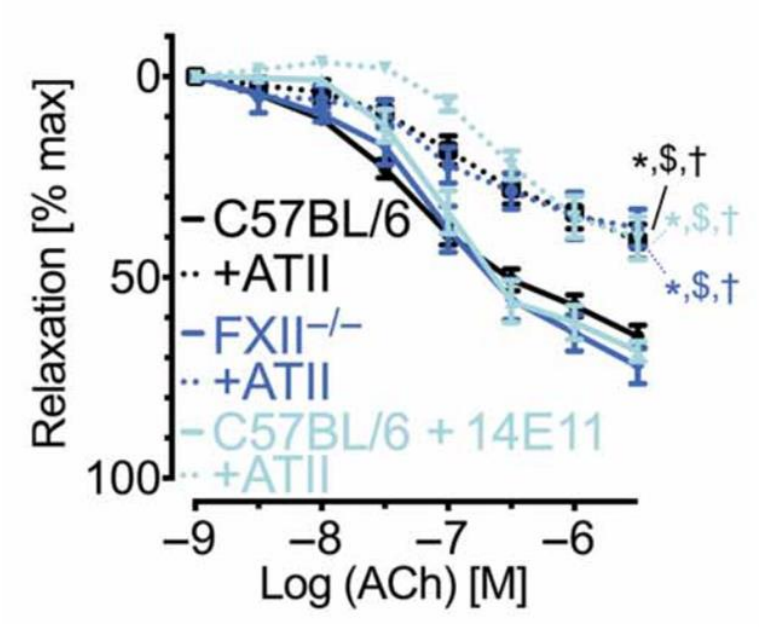
* $p < 0.05$ versus control

Results are mean ± SEM

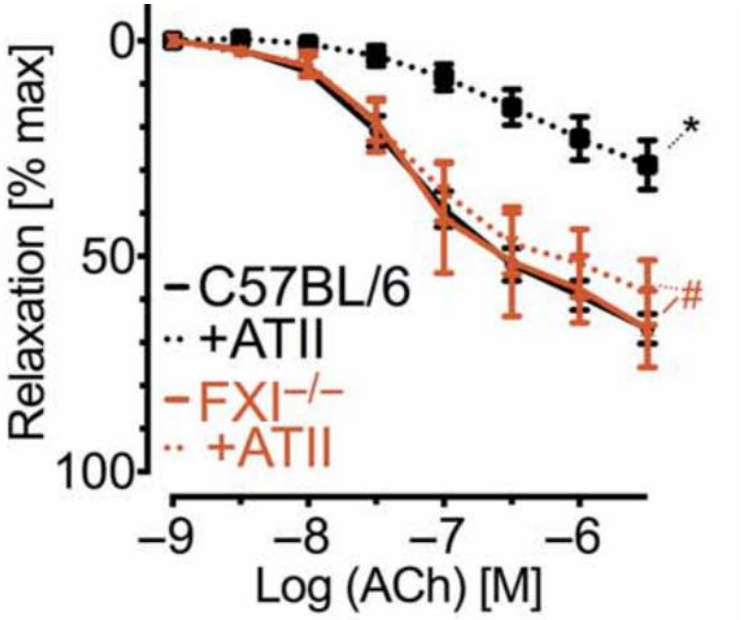
Lagrange *et al.*, *FASEB*. 2014

4: FXI implication in vascular dysfunction development

FXII impact on vascular relaxation



FXI impact on vascular relaxation

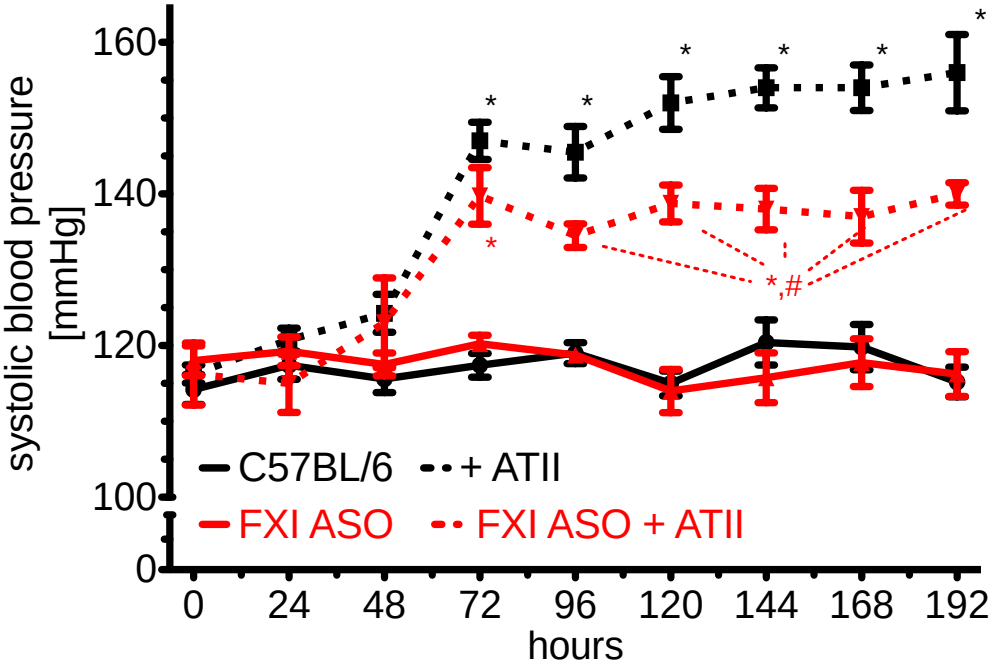


Results are mean ± SEM
4-12 animal per group

Kossmann, Lagrange* al., Science Translational Medicine*. 2017

4: FXI implication in vascular dysfunction development

Inhibition of FXI synthesis attenuates blood pressure increase in response to angiotensin II infusion

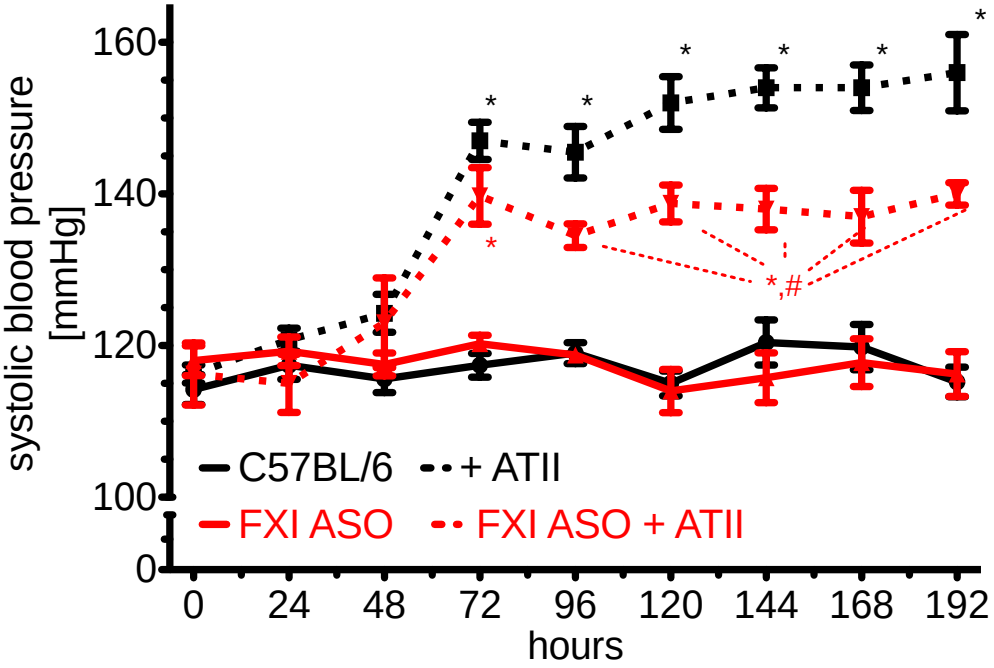


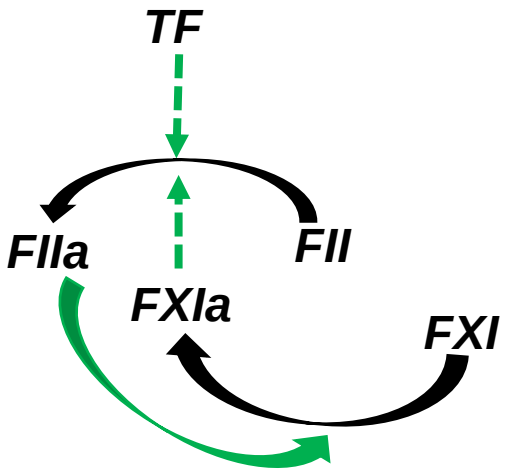
Means ± SEM of 4-5 animal per group
*, p<0.05 vs. C57BL/6 and C57BL/6 +FXI ASO; #, p<0.05 vs. C57BL/6 +ATII.

Kossmann, Lagrange* al., Science Translational Medicine*. 2017

4: FXI implication in vascular dysfunction development

Inhibition of FXI synthesis attenuates blood pressure increase in response to angiotensin II infusion





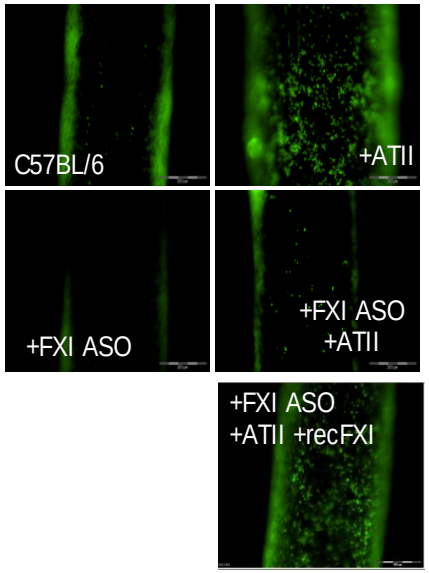
Means ± SEM of 4-5 animal per group
*, p<0.05 vs. C57BL/6 and C57BL/6 +FXI ASO; #, p<0.05 vs. C57BL/6 +ATII.

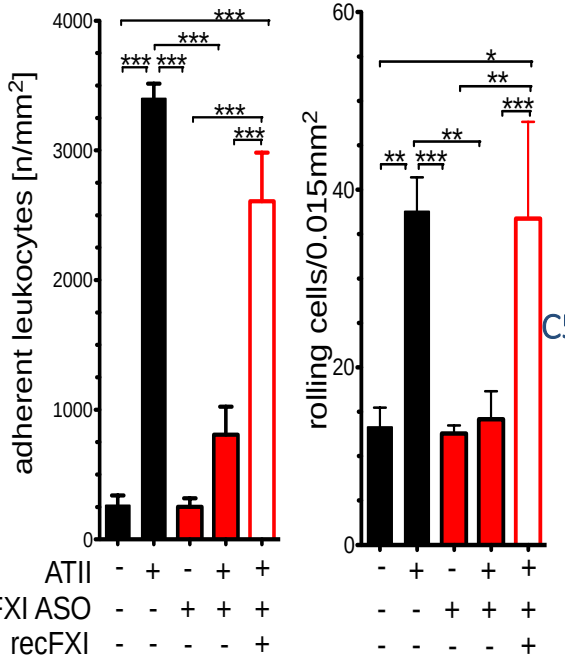
Kossmann, Lagrange* al., Science Translational Medicine*. 2017

Results

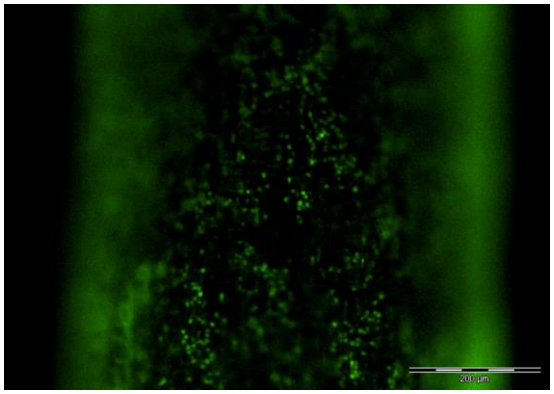
4: FXI implication in vascular dysfunction development

FXI is crucial for leukocytes adhesion and rolling

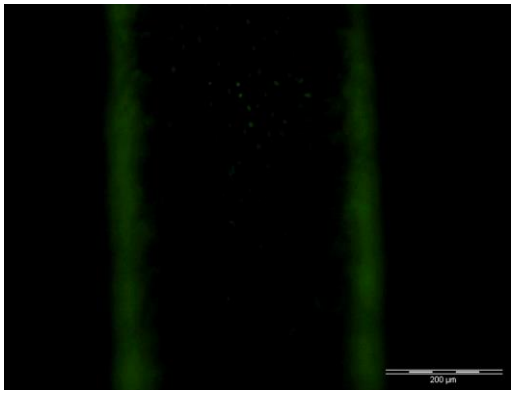




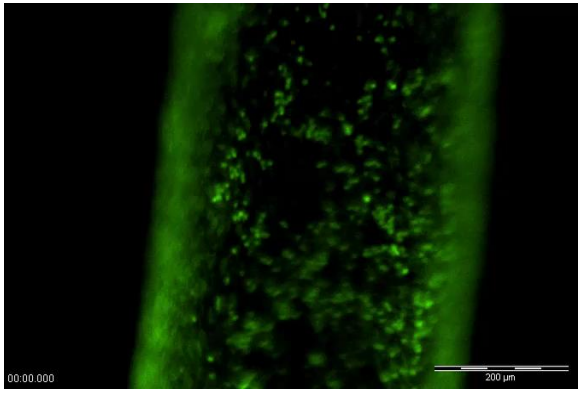
C57BL/6 + Angiotensin II



C57BL/6 + Angiotensin II + FXI ASO



C57BL/6 + Angiotensin II + FXI ASO + recFXI

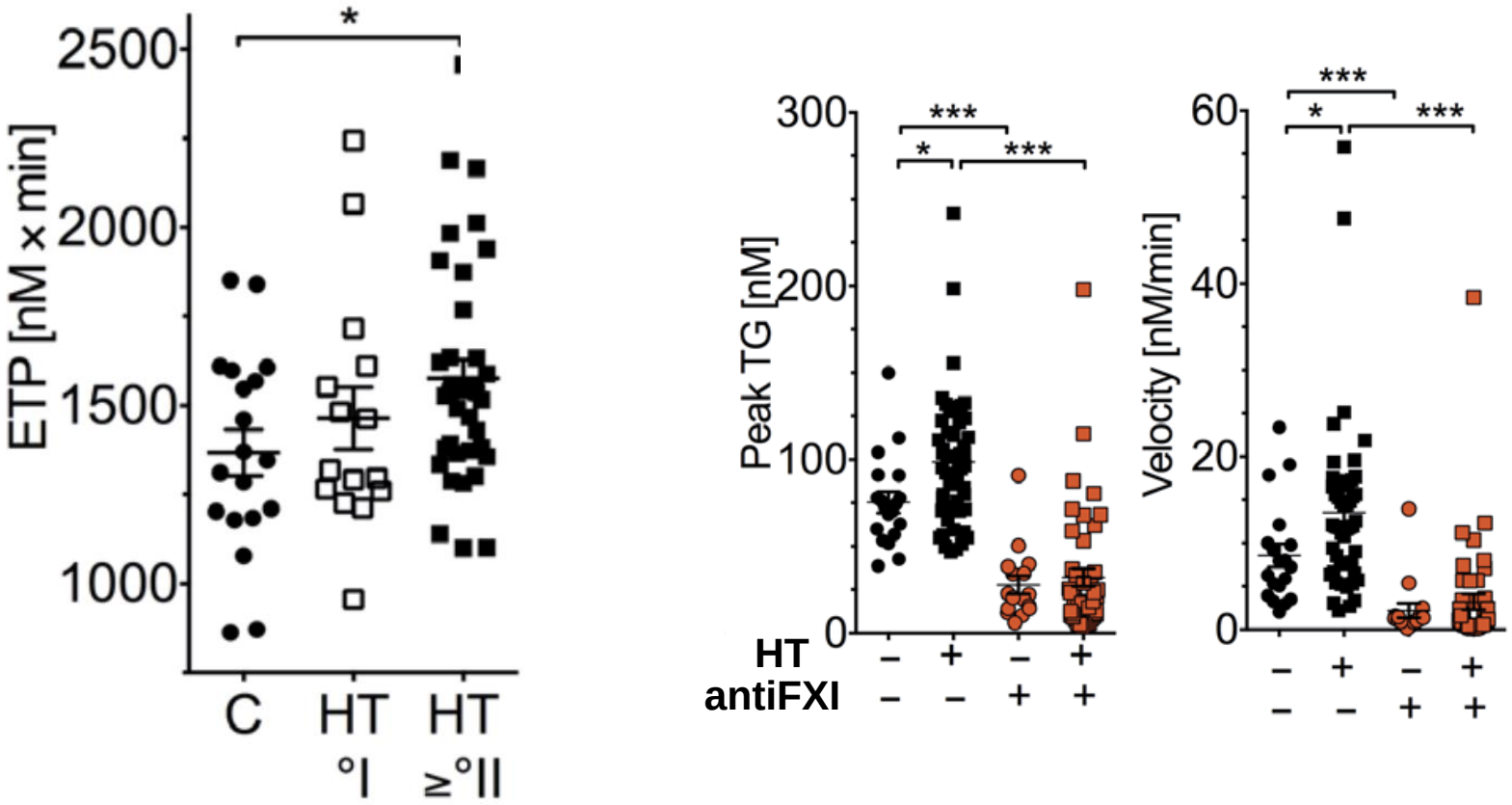


Means ± SEM of 3-5 animal per group *, p<0.05, **, p<0.01, ***, p<0.001 vs. control

Kossmann, Lagrange* al., Science Translational Medicine*. 2017

4: FXI implication in vascular dysfunction development

Thrombin generation is increased in hypertensive patients and FXI inhibition normalized thrombin generation parameters



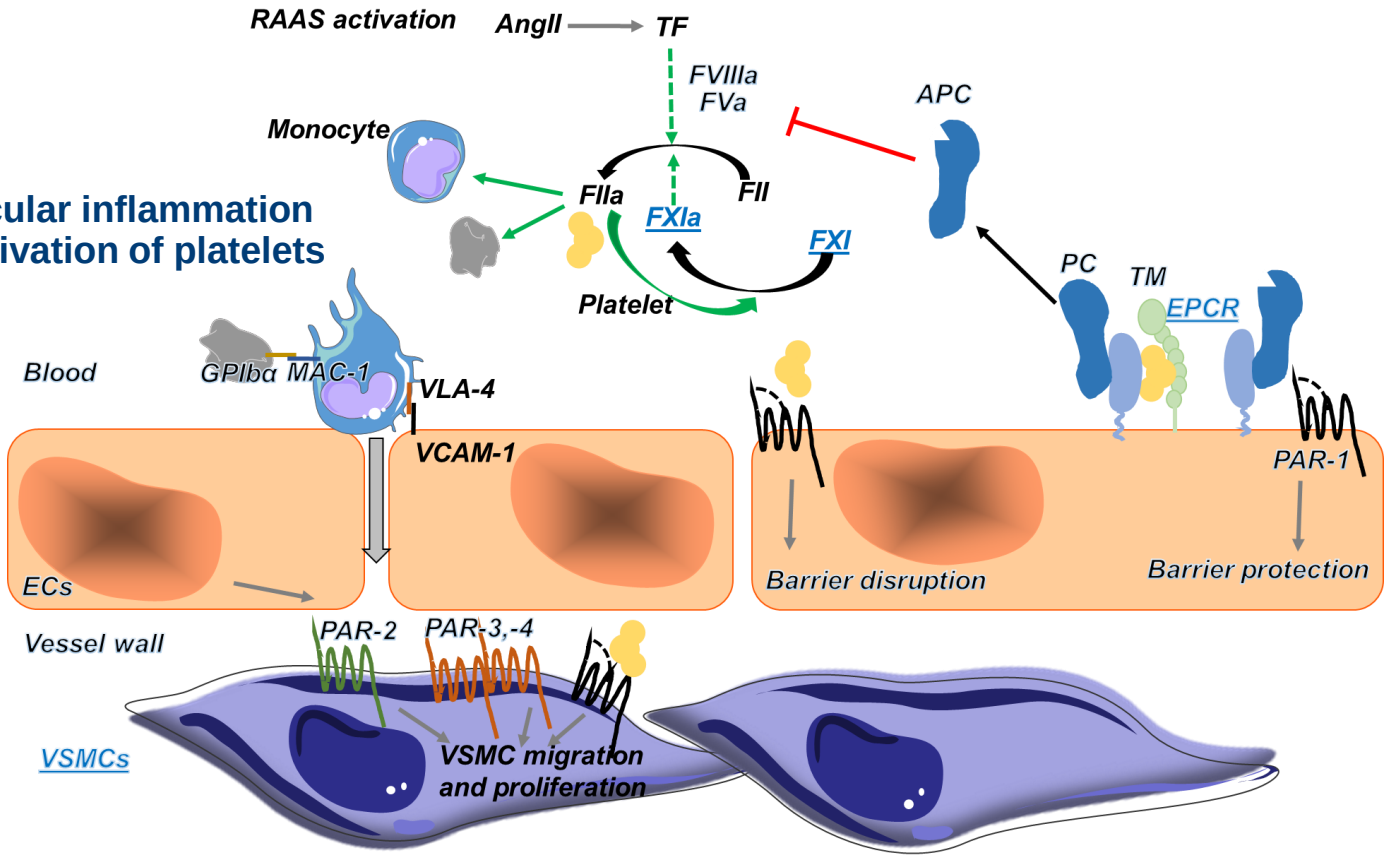
controlled hypertension (control, n = 19), arterial hypertension grade I (HT°I; n = 16), and arterial hypertension grade II or higher (HT≥°II; n = 36)

Data are means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001

Kossmann, Lagrange* al., Science Translational Medicine*. 2017

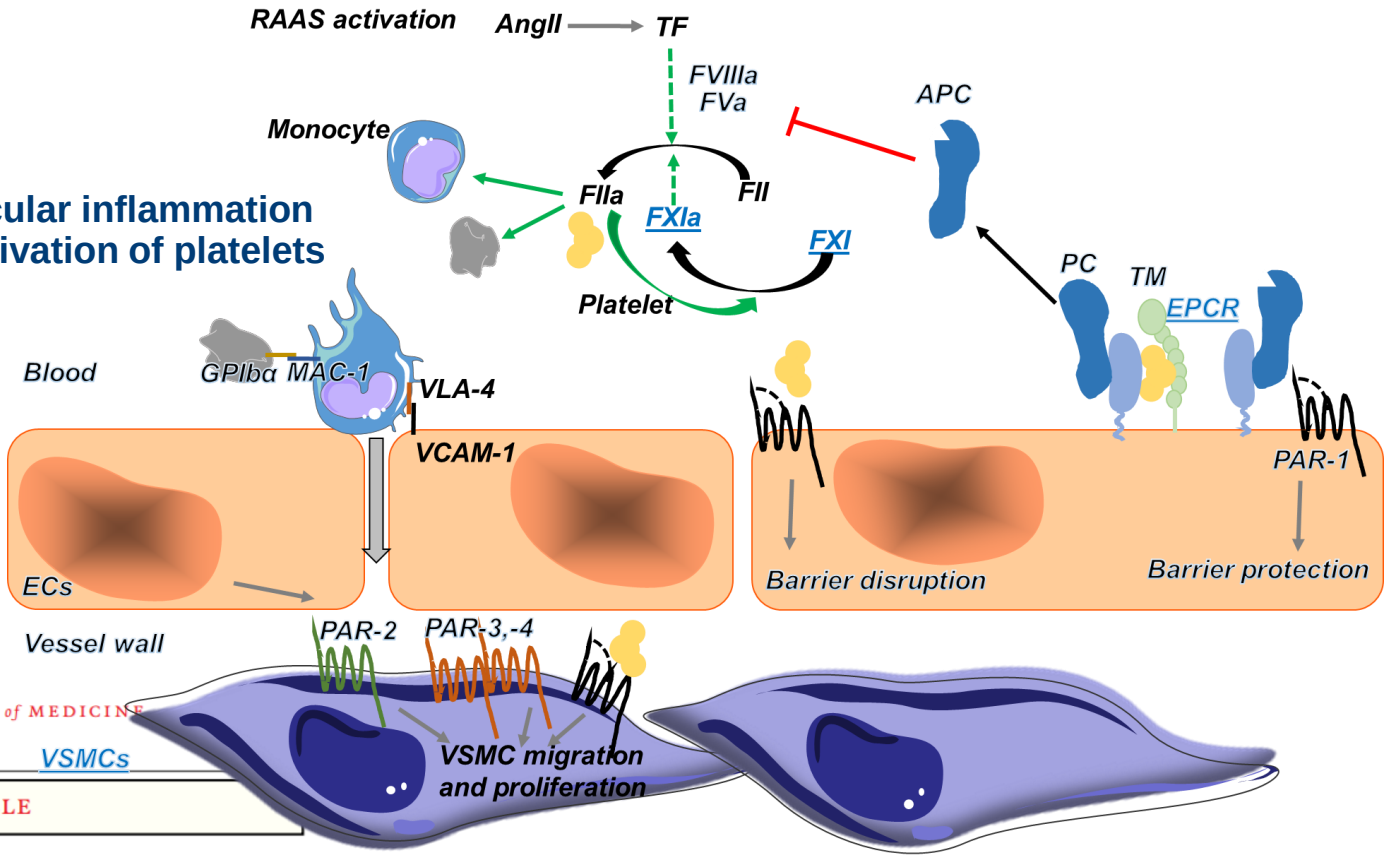
Summary

- **VSMCs are responsible for the increase of thrombin generation within the vascular wall**
- **MR increases the EPCR**
- **FXI inhibition limits vascular inflammation induced by thrombin activation of platelets and immune cells**



Summary

- **VSMCs are responsible for the increase of thrombin generation within the vascular wall**
- **MR increases the EPCR**
- **FXI inhibition limits vascular inflammation induced by thrombin activation of platelets and immune cells**



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Factor XI Antisense Oligonucleotide for Prevention of Venous Thrombosis

Harry R. Büller, M.D., Claudette Bethune, Ph.D., Sanjay Bhanot, M.D., Ph.D., David Gailani, M.D., Brett P. Monia, Ph.D., Gary E. Raskob, Ph.D., Annelise Segers, M.D., Peter Verhamme, M.D., and Jeffrey I. Weitz, M.D., for the FXI-ASO TKA Investigators*

- **FXI inhibition to limit vascular diseases progression?**

Acknowledgements

UNIVERSITÉ DE LORRAINE

Instituts thématiques Inserm

UMR_S 1116

Véronique Regnault
Patrick Lacolley

Mélusine Didelot
Amel Mohamadi
Karima Ait Aissa
Simon Thornton
Denis Wahl
Huguette Louis
Cécile Lakomy
Jean-Pierre Max

Sabine Kossmann
Susanne H. Karbach
Venkata Garlapati
Katharina Perius
Stefanie Finger
Rebecca Schüler
Tanja Knopp

Philip Wenzel

Group vascular inflammation

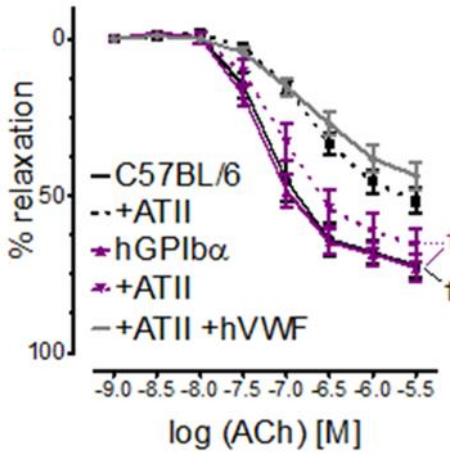
JG|U UNIVERSITÄTSmedizin. MAINZ

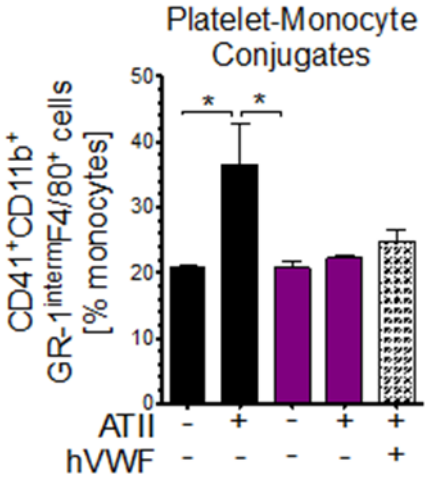
CTH Center for Thrombosis and Hemostasis Mainz

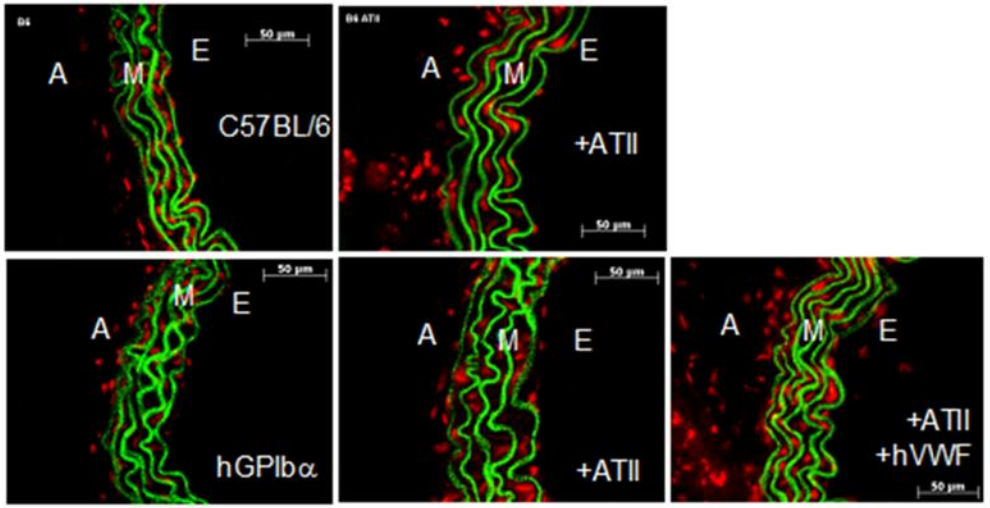
CTH Centrum für Thrombose und Hämostase Mainz

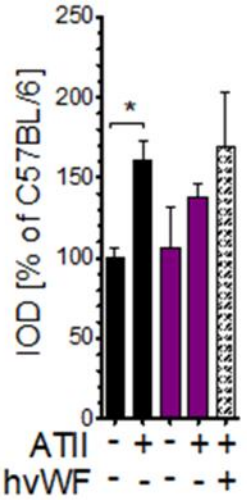
Nexts steps

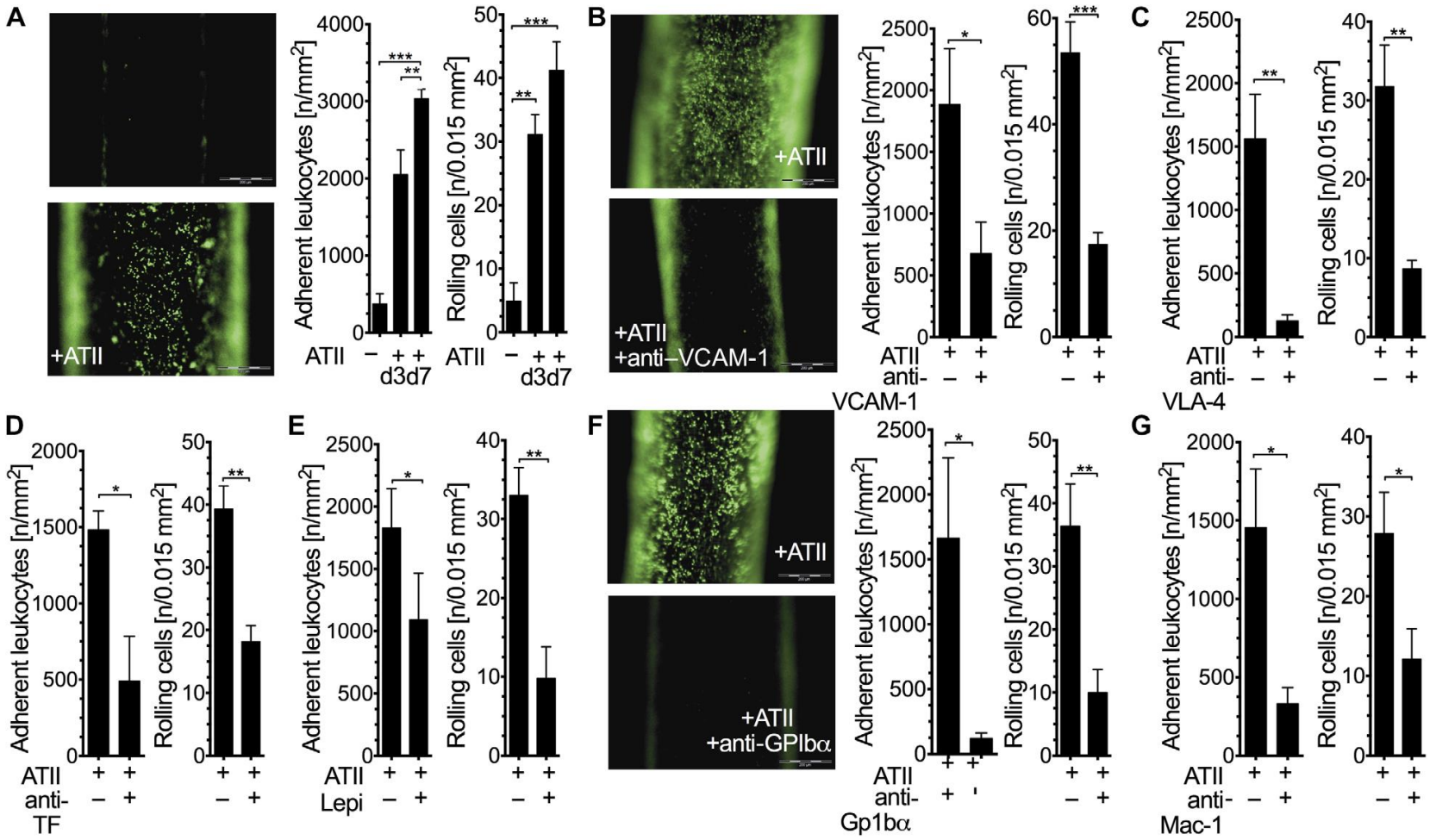
Implication of VWF and GPIbalpha










A
+ATII
Adherent leukocytes [n/mm²]
Rolling cells [n/0.015 mm²]
ATII
d3d7
B
+ATII
+anti–VCAM-1
anti-VCAM-1
C
anti-VLA-4
D
anti-TF
E
Lepi
F
+anti-GPIbα
anti-Gp1bα
G
anti-Mac-1

Results

Patient characteristics of all participants of the FACTO-RR study (1)

	Controlled Hypertension	Uncontrolled Hypertension	
		Hypertension grade I	Hypertension grade II or >II
n	19	16	36
Male (%)	7 (36.8%)	10 (62.5%)	16 (44.4%)
Age [years]	65.4±3.5	62.8±3.1	65.3±1.9
Blood pressure [mmHg]			
Systolic	**122.6±2.5**	**138.8±1.2**	**179.0±1.9 *,#**
Diastolic	**70.8±1.7**	**85.6±1.6 ***	**88.8±1.7 ***
Mean	**88.1±1.5**	**103.3±1.2 ***	**118.8±1.5 *,#**
Heart rate (bpm)	72.5±2.1	75.7±3.6 *	83.3±2.0
BMI [kg per m^2]	26.5±1.0	28.3±1.3	29.7±1.0
Medication (%)			
ACE inhibitors	6 (31.6%)	2 (12.5%)	11 (30.6%)
AT_1 receptor blockers	5 (26.3%)	10 (62.5%) *	12 (33.3%)
Ca^{2+} channel blockers	2 (10.5%)	4 (25.0%)	8 (22.2%)
ß blockers	13 (68.4%)	9 (56.3%)	13 (36.1%) *
thiazide diuretics	2 (10.5%)	8 (50.0%) *	8 (22.2%)
loop diuretics	3 (15.8%)	4 (25%)	5 (13.9%)
aldosterone antagonists	5 (26.3%)	1 (6.3%)	0 *
renin inhibitors	1 (5.3%)	0	1 (2.8%)
alpha blockers	1 (5.3%)	1 (6.3%)	3 (8.3%)
statins	6 (31.6%)	7 (43.8%)	5 (13.9%) #
acetylsalicylic acid	8 (42.1%)	10 (62.5%)	14 (38.9%)
anti diabetic drugs	4 (21.1%)	4 (25%)	5 (13.9%)
NSAIDs	1 (5.3%)	2 (12.5%)	1 (2.8%)
thyroid hormone	2 (10.5%)	4 (25%)	11 (30.6%)

ACE, angiotensin converting enzyme; NSAIDs, non steroidal anti-inflammatory drugs; BMI, body mass index; MI, myocardial infarction; Kruskal-Wallis test with Dunn´s multiple comparison test or Fisher´s exact test were used. *, $p<0.05$ vs. Control. #, $p<0.05$ vs. Hypertension grade I.

Results

Patient characteristics of all participants of the FACTO-RR study (2)

	Controlled Hypertension	**Uncontrolled Hypertension**	
		Hypertension grade I	**Hypertension grade II or >II**
n	19	16	36
Male (%)	7 (36.8%)	10 (62.5%)	16 (44.4%)
Age [years]	65.4±3.5	62.8±3.1	65.3±1.9
Platelet count [10^3 per µl]	**183.7±19.1**	**228.4±14.8**	**191.2±12.0**
Comorbidites			
Diabetes	8 (42.1)	3 (18.8%)	9 (25%)
Cigarette Smoking	1 (5.3%)	2 (12.5%)	2 (5.6%)
History of Smoking	5 (26.3%)	5 (31.3%)	9 (25%)
Dyslipidemia	8 (42.1)	7 (43.8%)	11 (30.6%)
Obesity, BMI>30kg/m^2	5 (26.3%)	5 (31.3%)	11 (30.6%)
History of MI or CAD	12 (63.2)	6 (37.5%)	5 (13.9%) *
History of Stroke	1 (5.3%)	1 (6.3%)	2 (5.6%)
PAD	2 (10.5%)	3 (18.8%)	1 (2.8%)
COPD	1 (5.3%)	0	0
Family history of CVD	6 (31.6%)	8 (50%)	17 (47.2%)
Alcohol abuse	8 (42.1%)	8 (50%)	13 (36.1%)
Cancer	3 (15.8%)	2 (12.5%)	1 (2.8%)
Chronic kidney disease	5 (26.3%)	0 *	4 (11.1%)

CAD, coronary artery disease; PAD, peripheral artery disease; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease. Kruskal-Wallis test with Dunn´s multiple comparison test or Fisher´s exact test were used. *, $p<0.05$ vs. Control. #, $p<0.05$ vs. Hypertension grade I.

Vascular disease and hemostasis: example of hypertension

In humans :

- Increases thrombotic risk due to atherosclerosis
- Increased platelet reactivity
- Increased FVII and D-dimers…
- …but also antithrombin and protein C

Arikan *et al., Thromb Hemost*. 2005
Nadar *et al., Am j Cardiol*. 1994
Junker *et al., J. Hypertens*. 1998
Lip *et al., J. Hypertens*. 1998
Regnault *et al., Arterioscler Thromb Vac Biol*. 2011

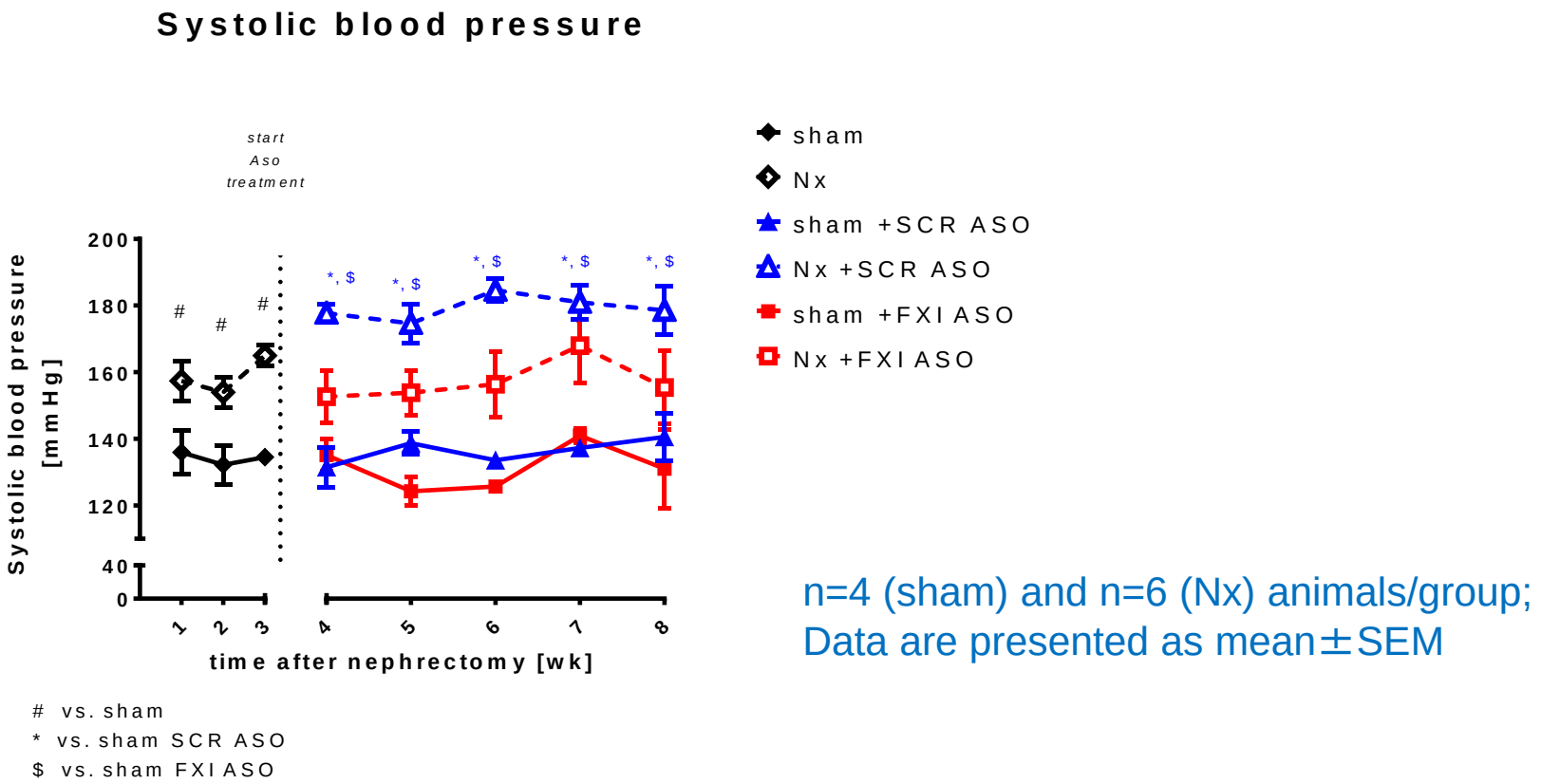
In animal models :

- Increased TF, prothrombin, TAT, fibrinogen
- Decreased TM

Sawada *et al., Clin. Exp. Hypertens*. 2003
Corseaux *et al., Mol. Med*. 2002
Ait Aissa*, Lagrange* *et al*. *Arterioscler Thromb Vac Biol*. 2015

3: FXI implication in hypertension development

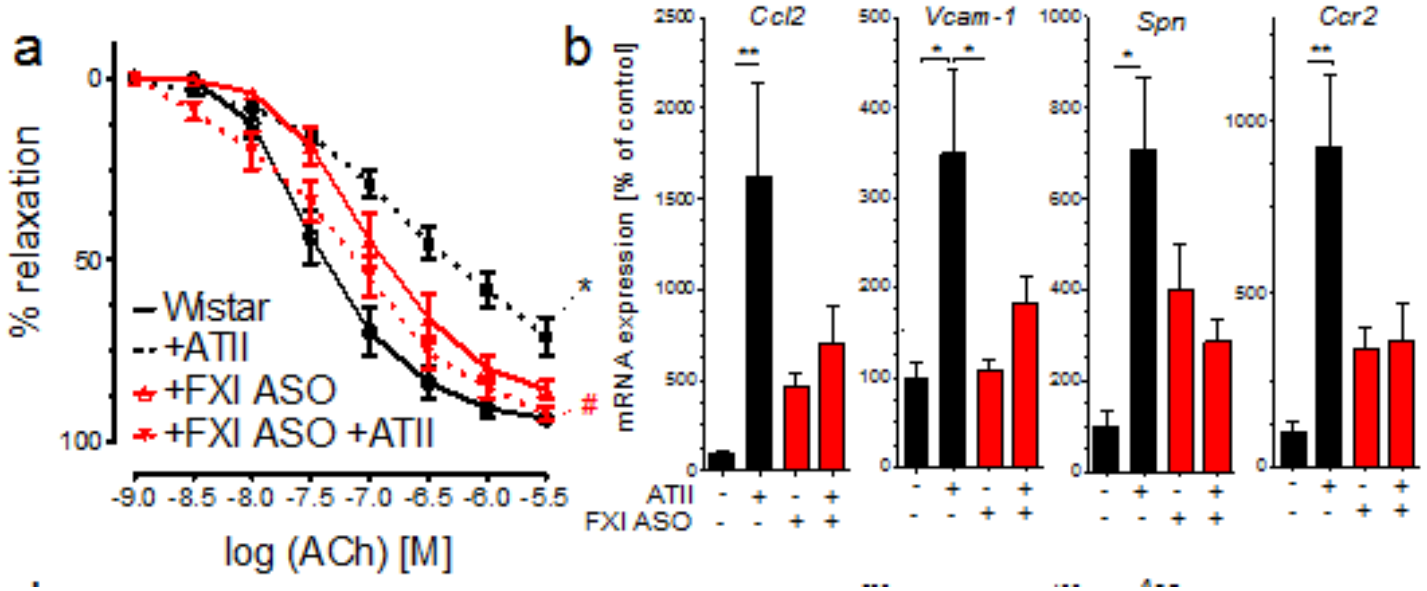
FXI inhibition decreased blood pressure in 5/6 nephrectomized rats even after increased blood pressure



n=4 (sham) and n=6 (Nx) animals/group;
Data are presented as mean±SEM

Results

Improved vascular reactivity and decreased inflammation in FXI ASO treated ATII infused rats

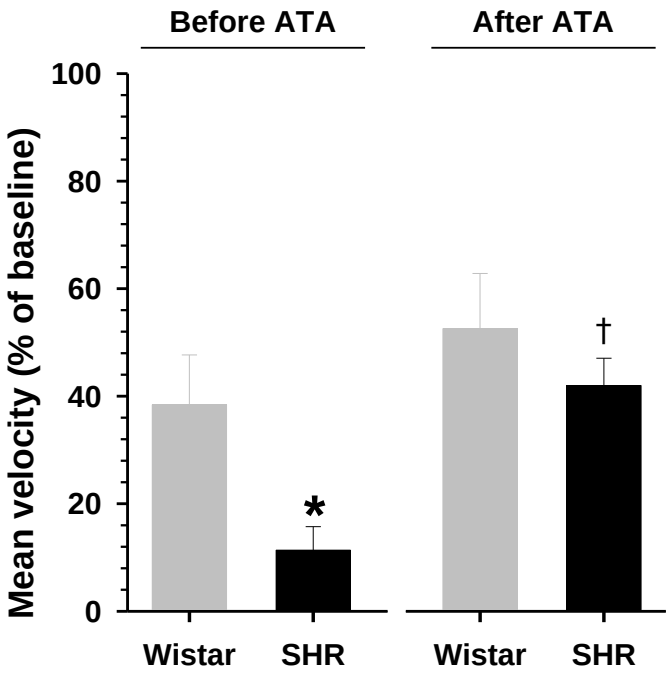


1-way ANOVA and Bonferroni`s Multiple Comparison test of maximal relaxation, n=5-9 animals/group; *, p<0.05 vs. Wistar, #, p<0.05 vs. Wistar +ATII
Data are presented as mean±SEM

PCR: Kruskal-Wallis and Dunn´s Multiple Comparison test, n=3-6 animals/group; *, p<0.05; **, p<0.01
Data are presented as mean±SEM

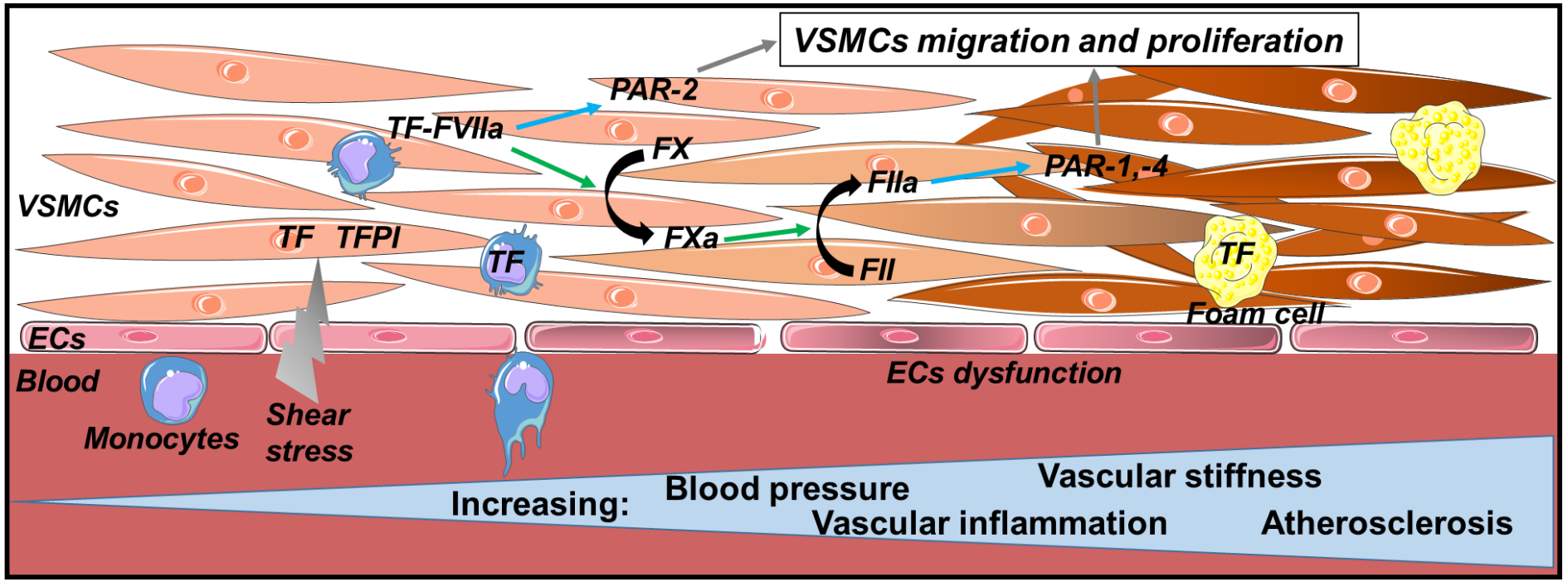
Nexts steps

GPIba binding domain of vWF is involved in SHR parietal hypercoagulability



Aurintricarboxylic acid (ATA)
Results are means ± SEM of 4 experiments
* P<0.05 versus Wistar rats; † P<0.05 vs SHR before ATA

Summary


VSMCs migration and proliferation
PAR-2
TF-FVIIa
FX
FXa
FIIa
FII
PAR-1,-4
VSMCs
TF
TFPI
TF
TF
Foam cell
ECs
Blood
Monocytes
Shear stress
ECs dysfunction
Increasing:
Blood pressure
Vascular stiffness
Vascular inflammation
Atherosclerosis