Biotherapy of Arteriosclerosis

Clinical Significance
Non-pharmacological &
Pharmacological intervention
Pathogenesis related with Ang II-mediated oxidative stress

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부산

Central arterial stiffness

- Central arterial stiffness, is an independent predictor of CV outcome in patients with hypertension, DM, and ESRD, and predicted CV outcome above and beyond mean arterial pressure in general population

- PWV: index of arterial elasticity and stiffness

  Moens-Korteweg equation: $PWV^2=E \cdot h/2r \cdot \rho$ (E: Young modulus, h: wall thickness,·r: internal radius at end-diastole, ρ: blood density)

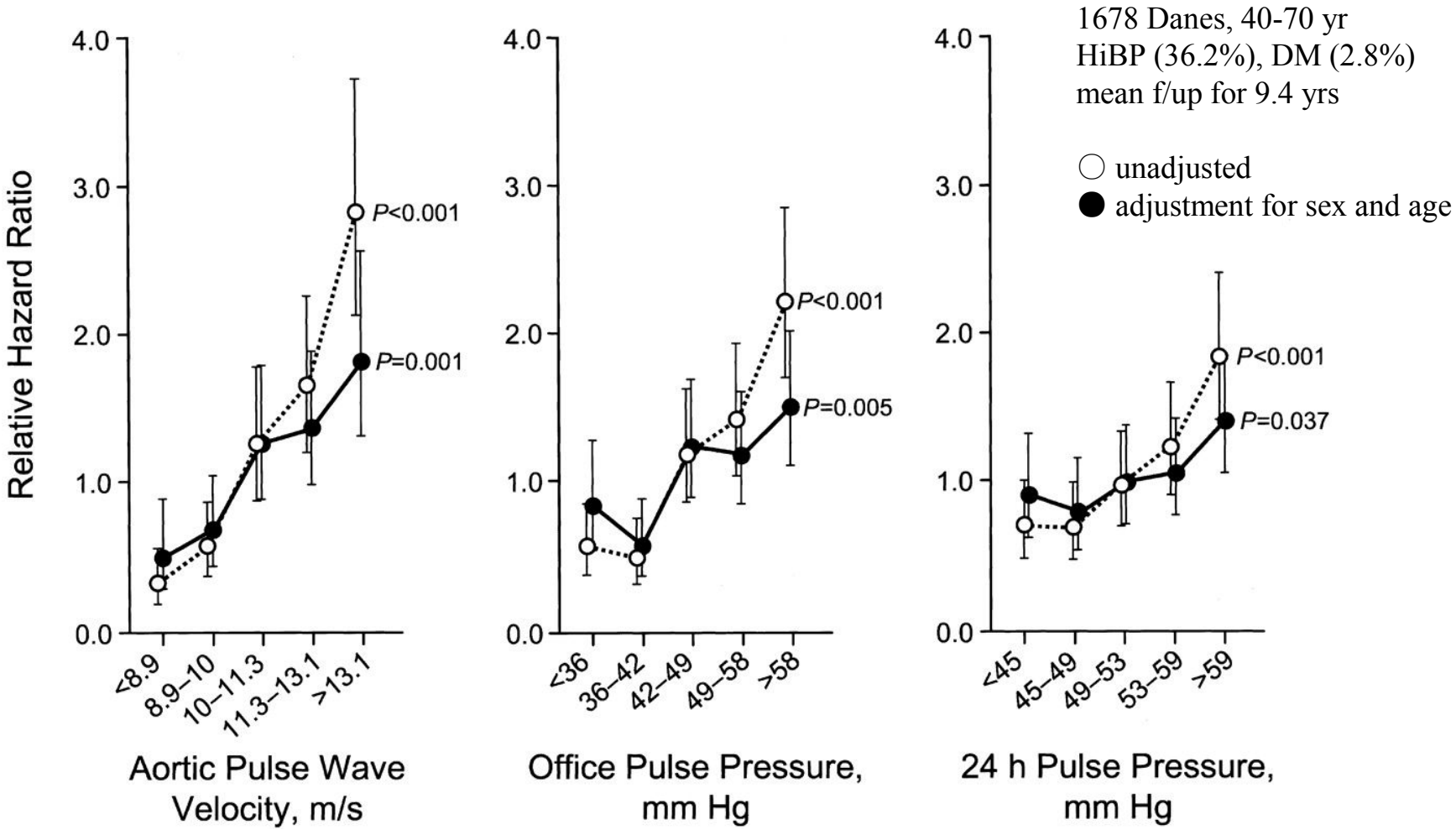
- Contributing factors

  age, blood pressure

  arterial wall structure (ex, increased collagen, degeneration of elastic fiber, Ca^{2+} deposition, AGE, atherosclerosis, DIT)

  Functional factors (Ang II. NO, endothelin, natriuretic factor, catecholamine, prostaglandin, autonomic NS)

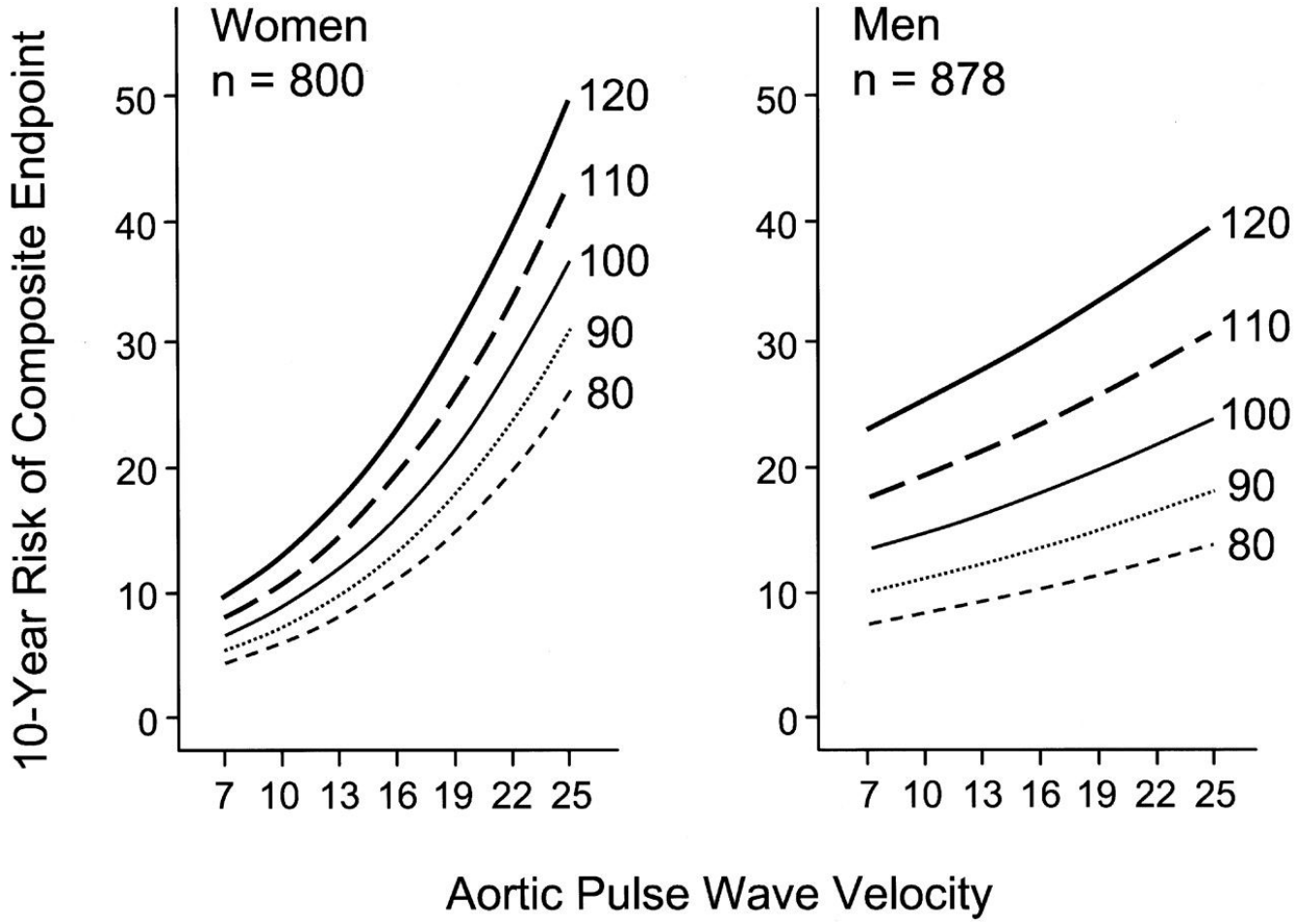
Relative hazard ratios for the composite CV end point
by distribution of APWV and office and 24-hour pulse pressures



Willum Hansen, T. et al. Circulation 2006;113:664-670

Absolute risk associated with APWV at different levels of office mean arterial pressure

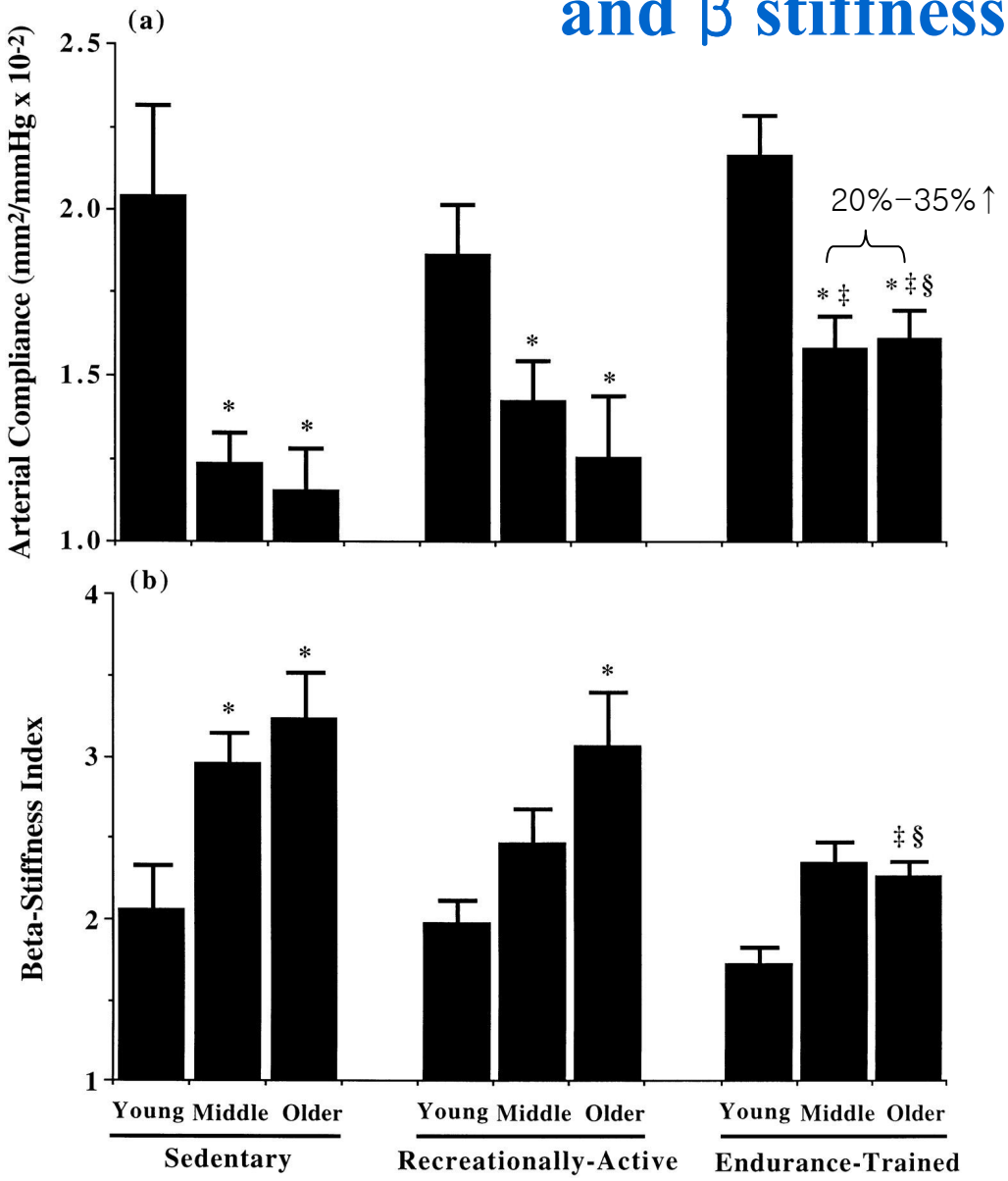
controlling for age, BMI, smoking, and alcohol intake



Willum Hansen, T. et al. Circulation 2006;113:664-670

Effect of Exercise on Arterial Stiffness

Effect of aerobic exercise on arterial compliance and β stiffness index

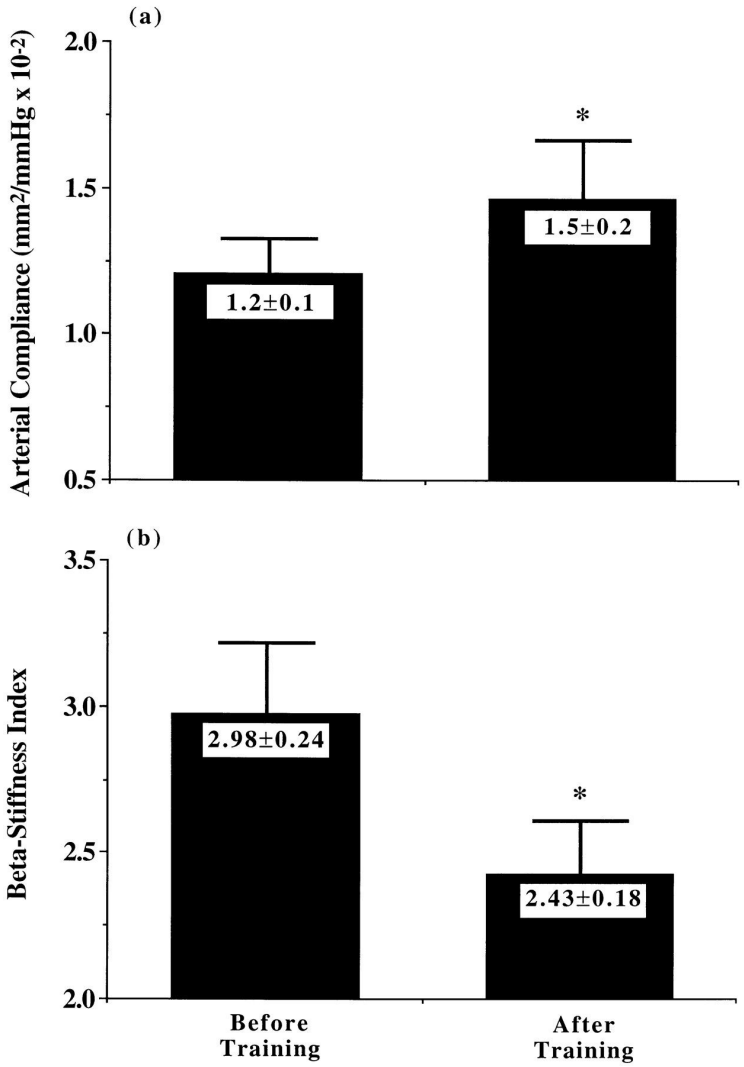


- Cross sectional study
- subjects: 151 healthy men
- sedentary
- recreational active
  light-moderate exercise ≥3/wk
- vigorous aerobic-endurance
  exercise ≥5/wk
- Central arterial compliance of common carotid artery

*p<0.05 vs young
within same activity group
‡p<0.05 vs sedentary of same age group
§p<0.05 vs recreational active of same age group

Hirofumi Tanaka, Circulation. 2000;102:1270

Aerobic exercise produced an increase in central arterial compliance and reduction in β stiffness index



- intervention study
- 20 healthy middle or older aged sedentary subjects
- walking exercise for 3 mo: 4-6/wk, 40-45min/d, 70-75% of Max heart rate

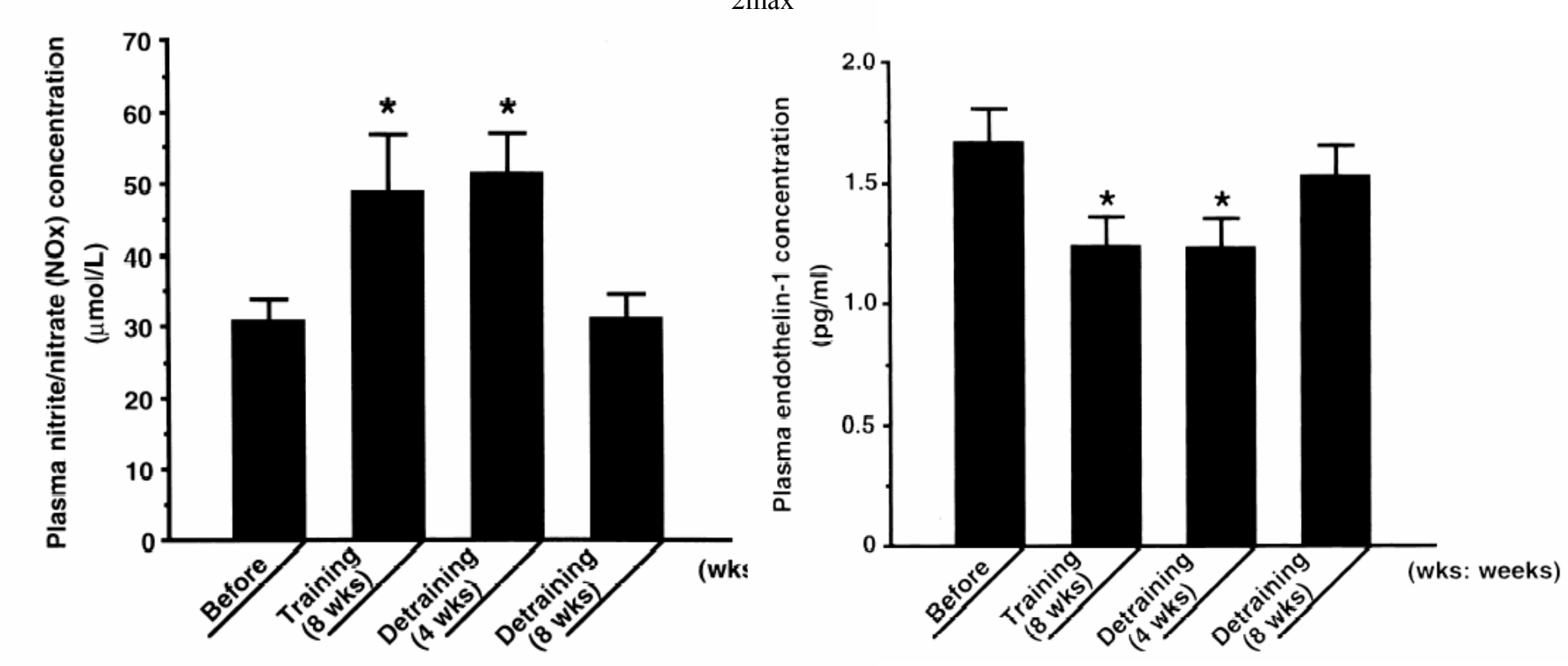
Hirofumi Tanaka, Circulation. 2000;102:1270

Exercise and arterial Elasticity

- Both moderate and vigorous physical activity led to comparable reduction in arterial stiffness in postmenopausal woman. (Sugawara J, Am J Hypertens 2006;19:1032-6)
- Aerobic exercise improve most of the classical risk factors (Body fatness, insulin resistance, BP), endothelial function, inflammation, and sympathetic activity
- Exercise failed to exert any beneficial impact in patients with isolated systolic hypertension. (Tanaka H, Safar ME. Am J Hypertens 2005;18:137-44) Thus aerobic exercise may be more effective when initiated early, as a preventive rather than treatment (Ferreira I,2006)

Plasma nitrite/nitrate (NOx) and endothelin-1 (ET-1) Changes before and after 8 wk exercise

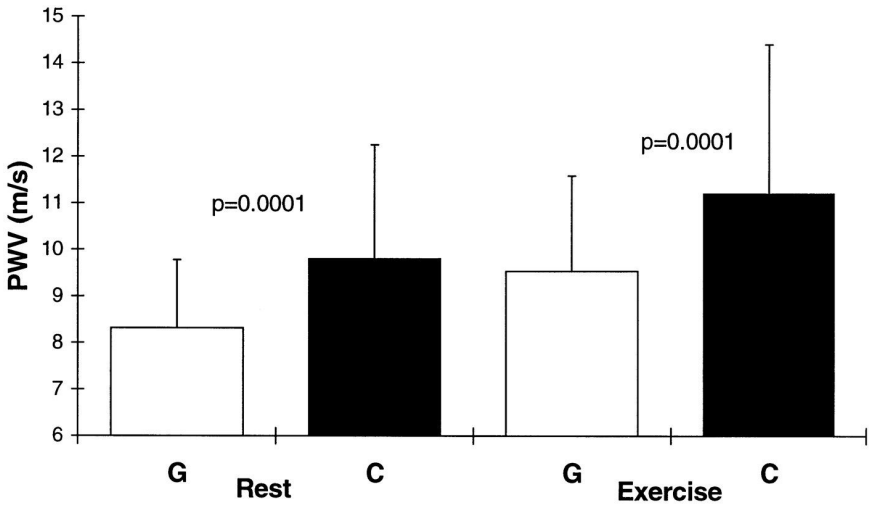
- 8 healthy male 20 yr
- Cycle ergonometer 3-4d/wk, 8wk
- 70% of VO_{2max}

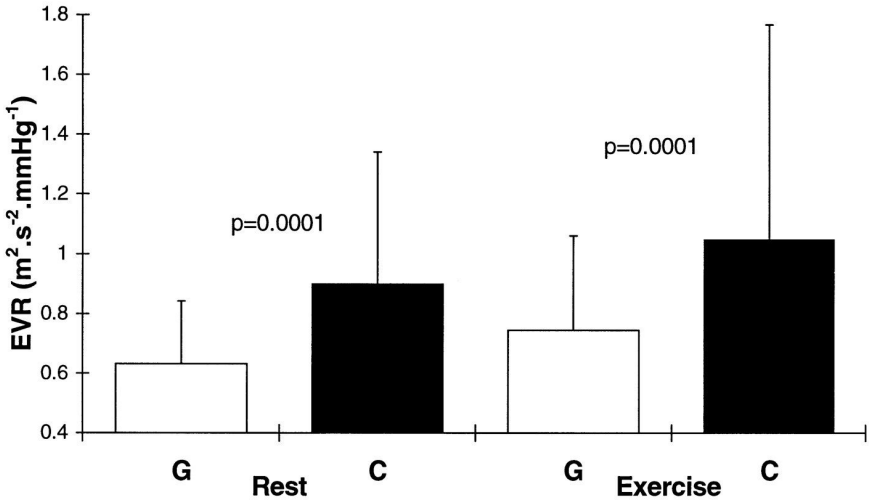


Maeda S, Life Sci 2001;1005-16

Effect of Diet & Nutrition on arterial Stiffness

Effect of chronic garlic intake on elastic properties of Aorta

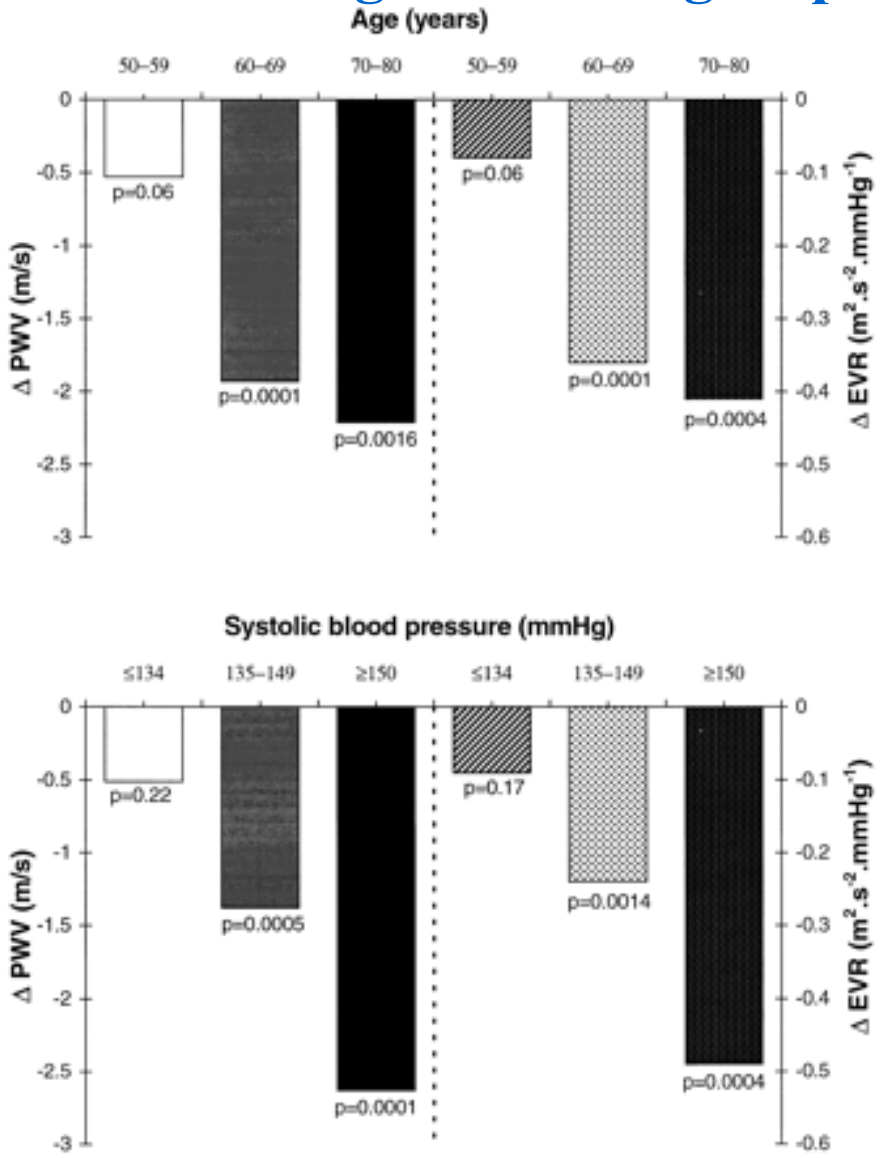




- Cross-sectional observational study
- Subjects: healthy (50-80yrs)
  Garlic group (G: n=101): ≥300 mg/d for 7.1 yrs / Control group (C: n=101)
- CF-PWV & EVR at rest and during isometric exercise

Kerstin Breithaupt-Grögler, Circulation. **1997;96:2649-55**

Differences in group mean PWV (left) and EVR (right) for different age and SBP groups



Kerstin Breithaupt-Grögler, Circulation. 1997;96:2649-2655

Effects of fish oil vs olive oil on Pulse-Contour and Impedance parameters

- Double-blind, placebo-controlled, cross-over study
- 20 Pts with NIDDM, three 6 wk phase
- Fish oil: eicosapentanoic acid (1.8g) & docosapentanoic acid (1.8g)
- Olive oil for placebo

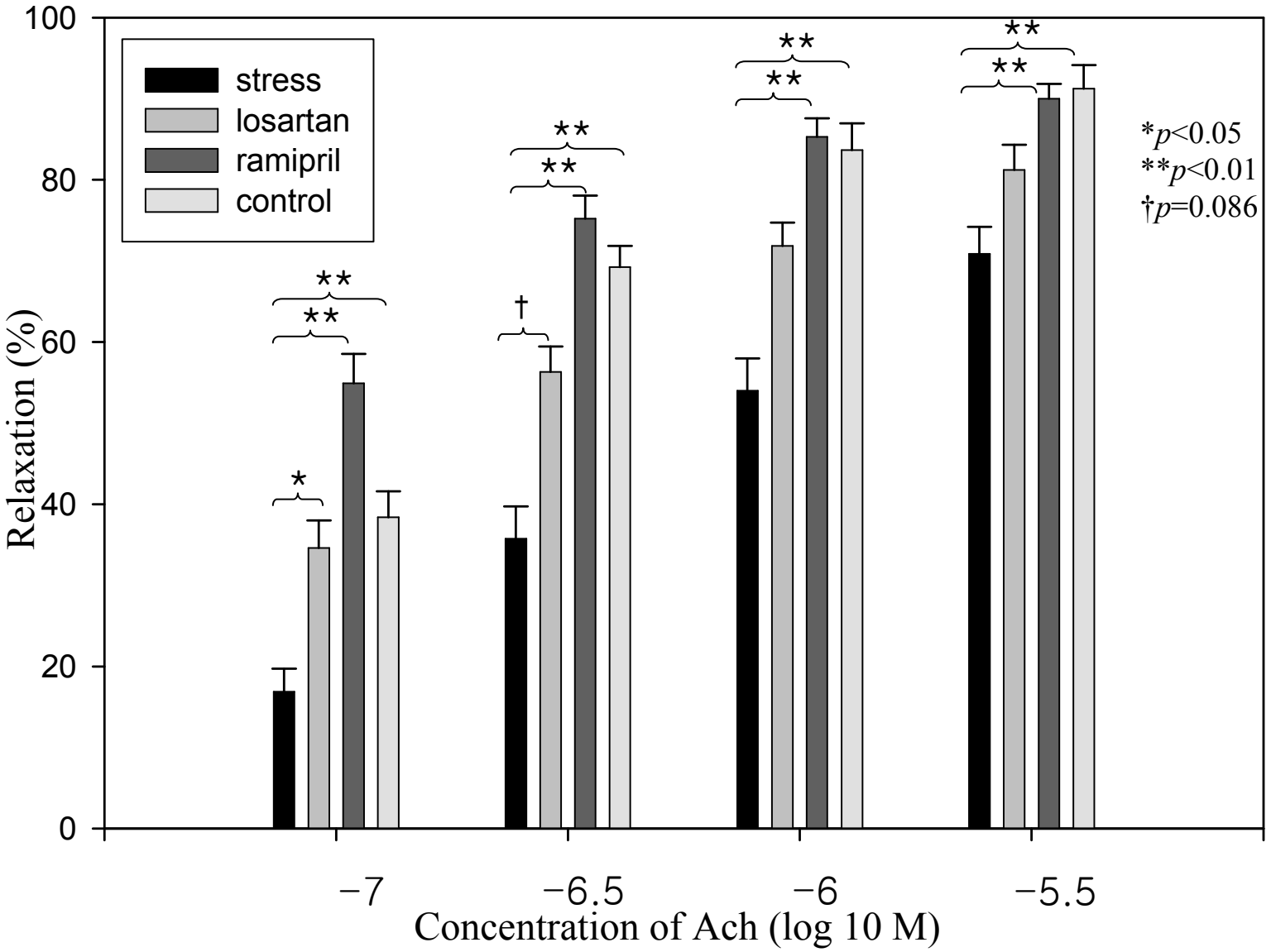
Parameters	Baseline		Olive Oil		Fish Oil	
Pulse contour						
A_2, s^{-1}	0.76	(0.70-0.82)	0.75	(0.70-0.80)	0.68	(0.63-0.73)*
A_4, s^{-1}	86	(56-116)	80	(45-115)	45	(32-58)†
A_5, s^{-1}	23	(16-30)	21	(15-27)	27	(18-36)
Impedance						
C_1, mL/mm Hg	1.50	(1.31-1.69)	1.52	(1.35-1.69)	1.68	(1.52-1.84)*
C_2, mL/mm Hg	0.015	(0.011 0.019)	0.017	(0.013 0.021)‡	0.022	(0.016-0.028)†
R, dyne · s · cm^{-5}	1268	(1144-1392)	1254	(1131-1377)	1210	(1128-1292)
L, mL · mm Hg^{-1} · s^{-2}	0.02	(0.01-0.03)	0.02	(0.01-0.03)	0.02	(0.01-0.03)

A2, exponentially decaying pressure; A4 damping of diastolic oscillation; A5 frequency of diastolic oscillation
C1, larger artery compliance estimate; C2, oscillatory compliance estimate; R, systemic resistance; L, inertance
*p<.01 fish oil vs baseline and olive oil; †p<.05 fish oil vs baseline; ‡p=.08 fish oil vs olive oil

McVeigh GE Arterioscler Thromb Vasc Biol 1994;14:1425-9

Effect of psychological stress on endothelial function

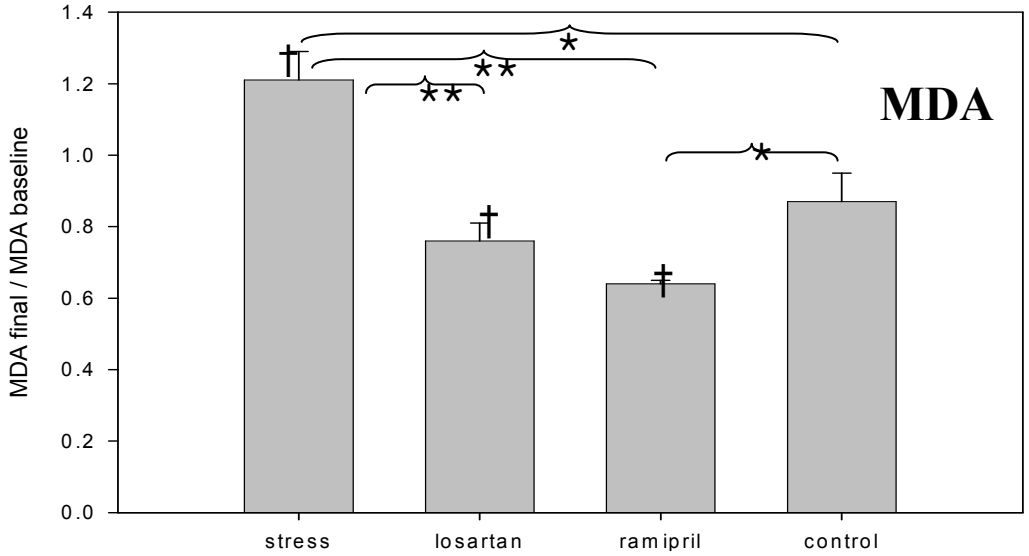
Changes in Ach-induced arterial relaxation by immobilization stress

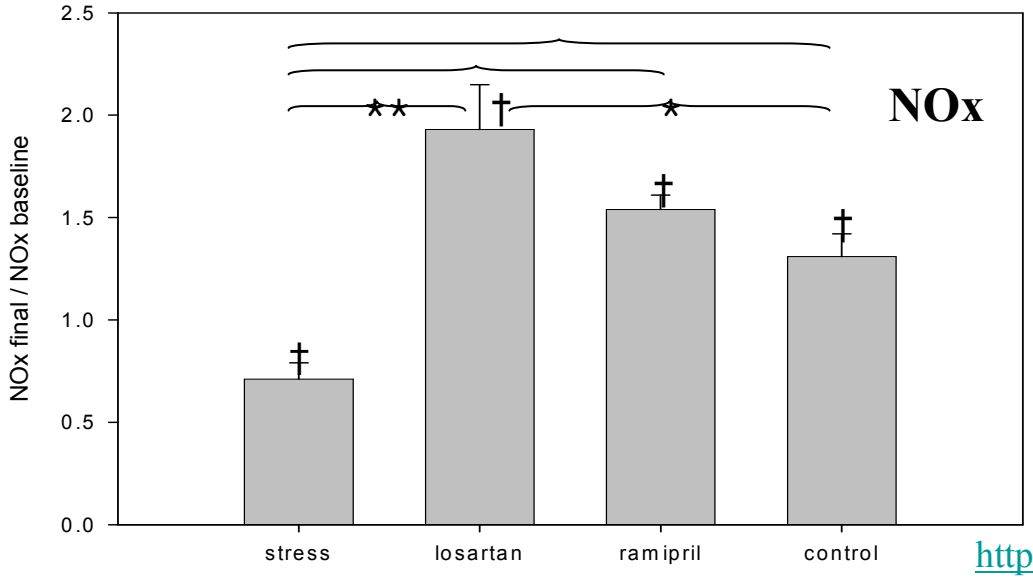


Chung I-M. Circ Res 2004;93:1523

http://circres.ahajournals.org/cgi/data/94/12/1523/DC1/1

Changes in NOx and MDA by immobilization stress

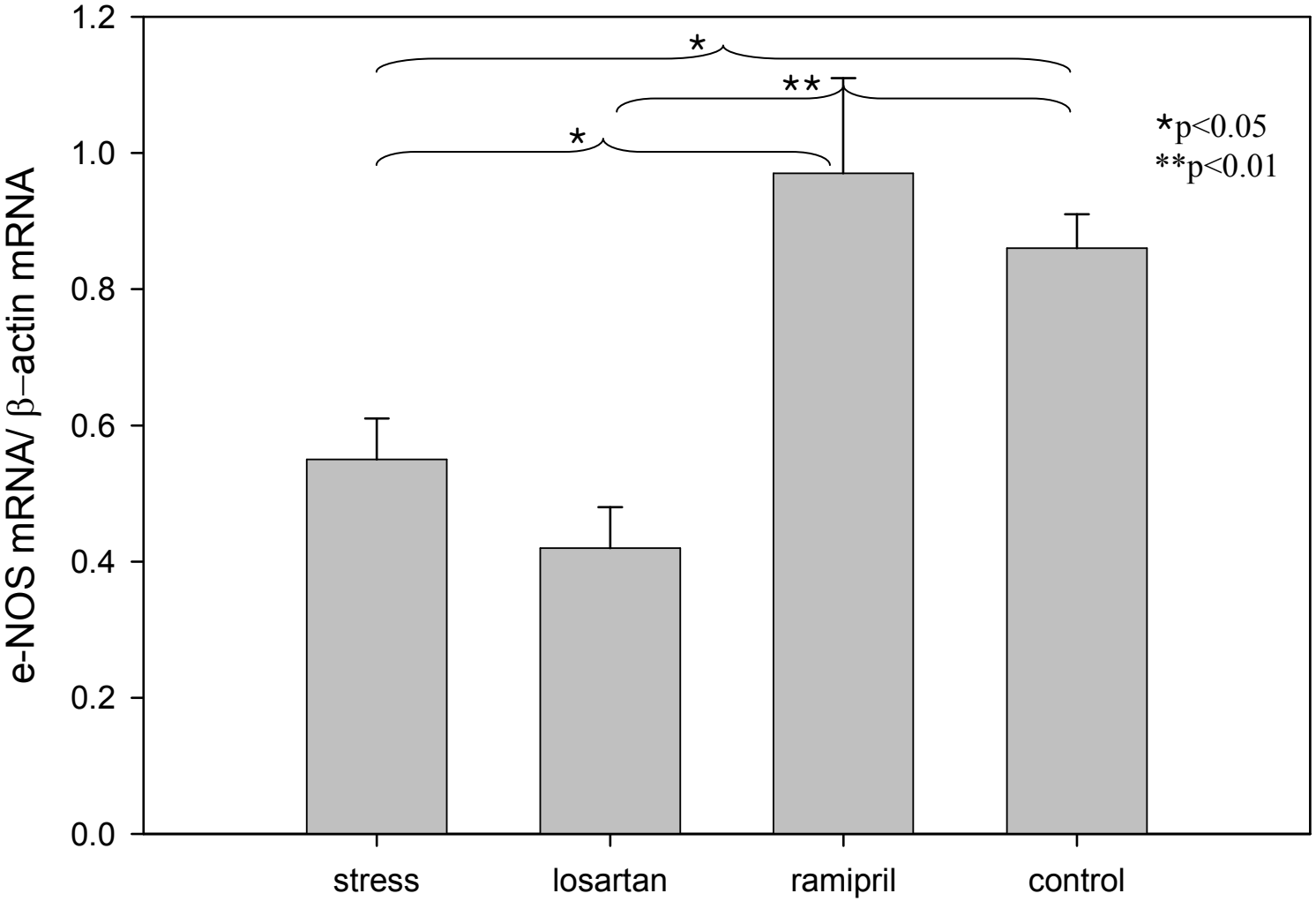




*p<0.05
**p<0.01
†p<0.05 for final vs baseline
M±SEM

Chung I-M. Circ Res 2004;93:1523
http://circres.ahajournals.org/cgi/data/94/12/1523/DC1/1

Changes in eNOS mRNA by immobilization stress



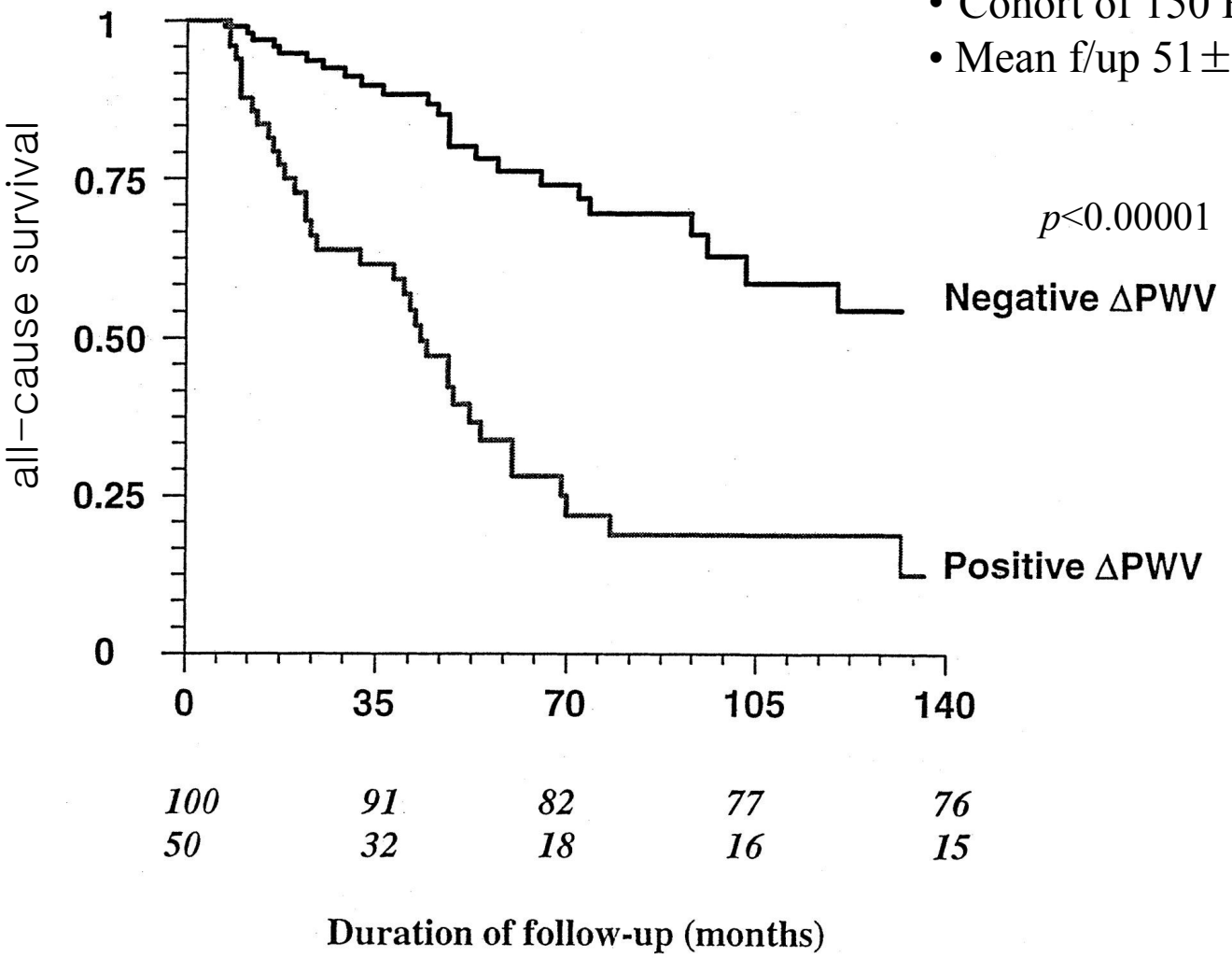
Chung I-M. Circ Res 2004;93:1523

http://circres.ahajournals.org/cgi/data/94/12/1523/DC1/1

Pharmacological Intervention of Arteriosclerosis

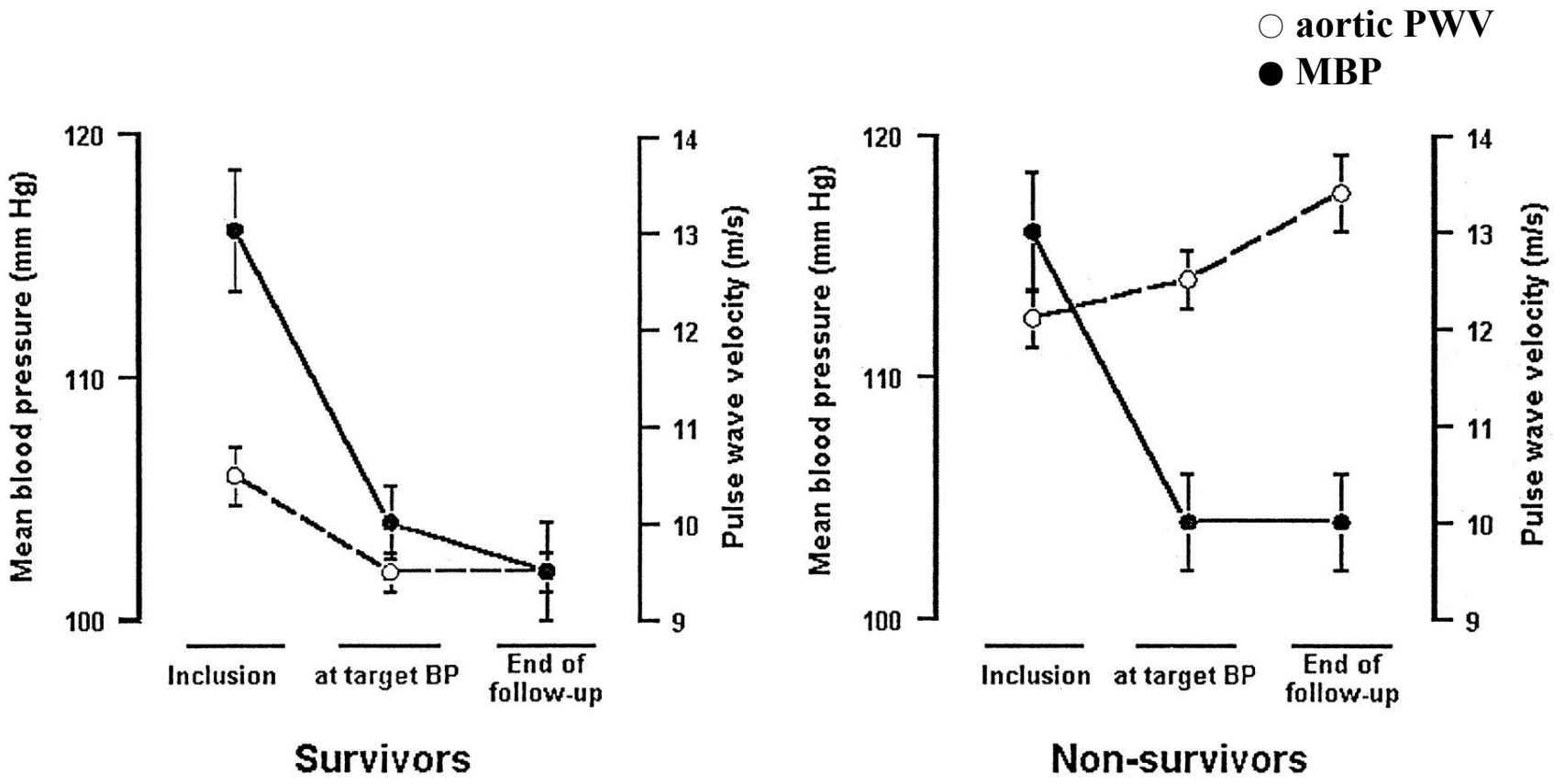
Probability of all-cause survival according to ΔPWV under antihypertensive therapy

- Cohort of 150 ESRD
- Mean f/up 51±38 mo



Guerin, A. P. et al. Circulation 2001;103:987-992

Changes of MBP and aortic PWV for survivors and nonsurvivors



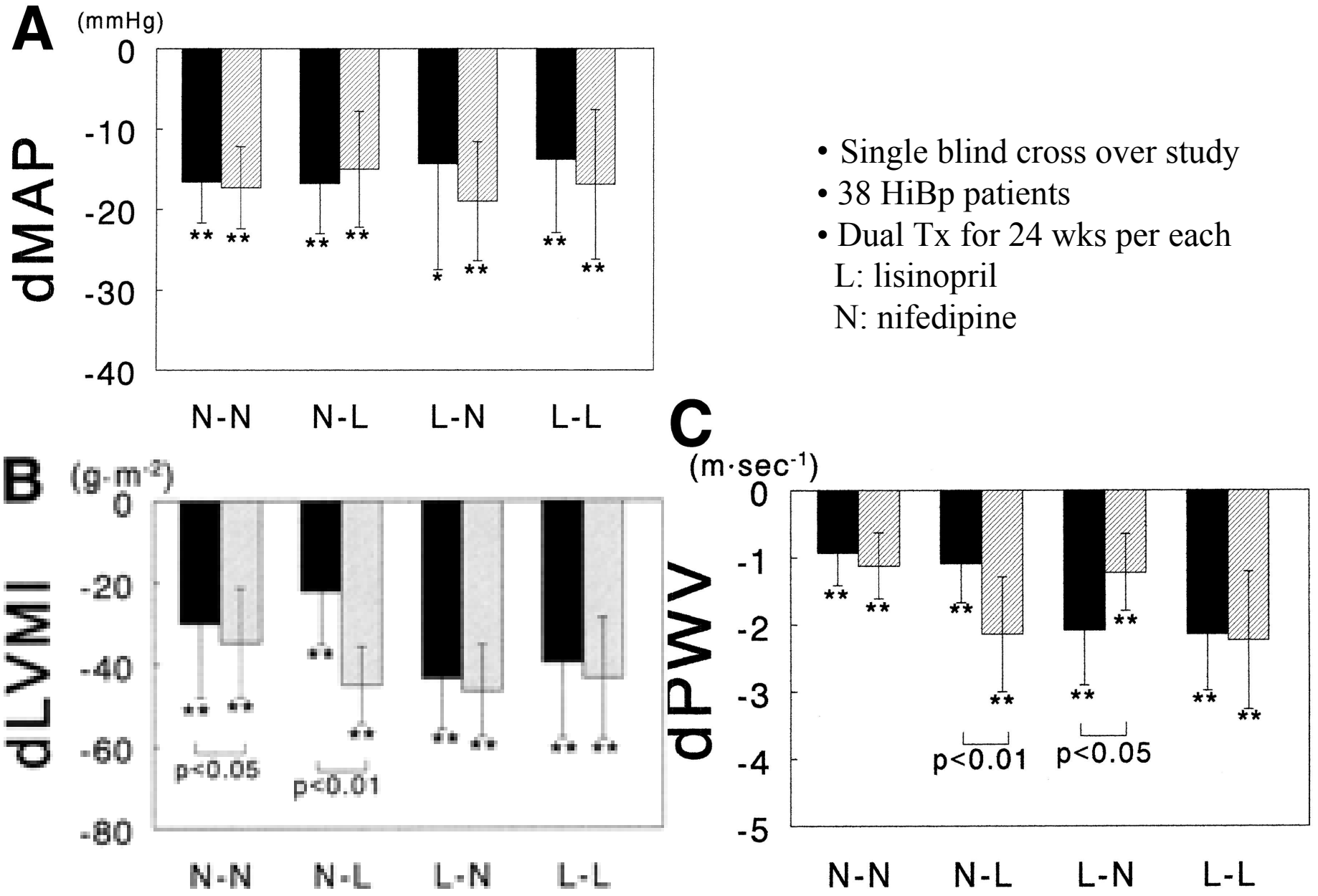
Guerin, A. P. et al. Circulation 2001;103:987-992

Proportional Hazard Regression Analyses of All-Cause and Cardiovascular Mortality

Variable	RR (95% CI)	*z* Statistic	*P*	Pseudo-r^2
All-cause mortality				
Age (10 y)	1.69 (1.32–2.17)	4.15	0.00003	0.15346
LV mass index (10-g increase)	1.08 (1.04–1.15)	2.27	0.02322	0.05144
PWV (1=positive/0=negative)	2.59 (1.51–4.43)	3.46	0.00053	0.11215
ACE inhibitor (1=yes/0=no)	0.19 (0.14–0.43)	−3.93	0.00027	0.13956
Cardiovascular mortality				
CVD (yes/no)	4.72 (1.91–11.61)	3.36	0.00077	0.13097
LV mass index (10-g increase)	1.11 (1.03–1.19)	2.63	0.00844	0.00847
PWV (1=positive/0=negative)	2.35 (1.23–4.51)	2.57	0.01004	0.08110
ACE inhibitor (1=yes/0=no)	0.18 (0.06–0.55)	−3.00	0.00274	0.10689

Guerin, A. P. et al. Circulation 2001;103:987-992

Lisinopril reverses LVH through improved aortic compliance

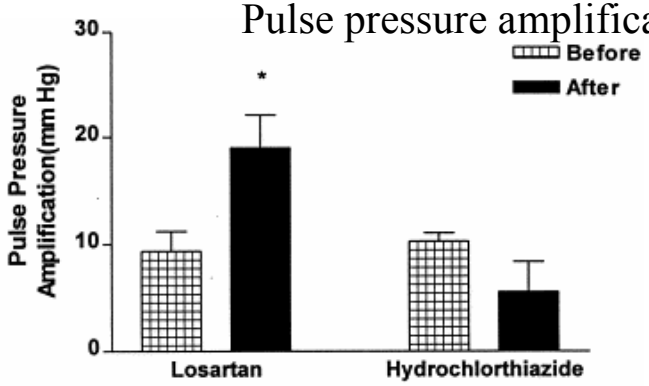


- Single blind cross over study
- 38 HiBp patients
- Dual Tx for 24 wks per each
  L: lisinopril
  N: nifedipine

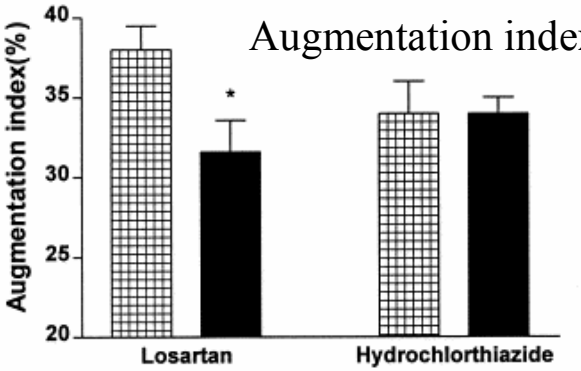
Shimamoto H, Hypertension. 1996;28:457-63

Effect of Losartan vs Hyderochlorthiazide on arterial stiffness

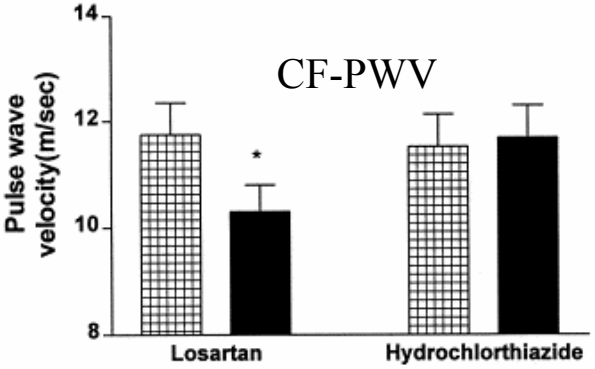
Pulse pressure amplification



Augmentation index %



CF-PWV

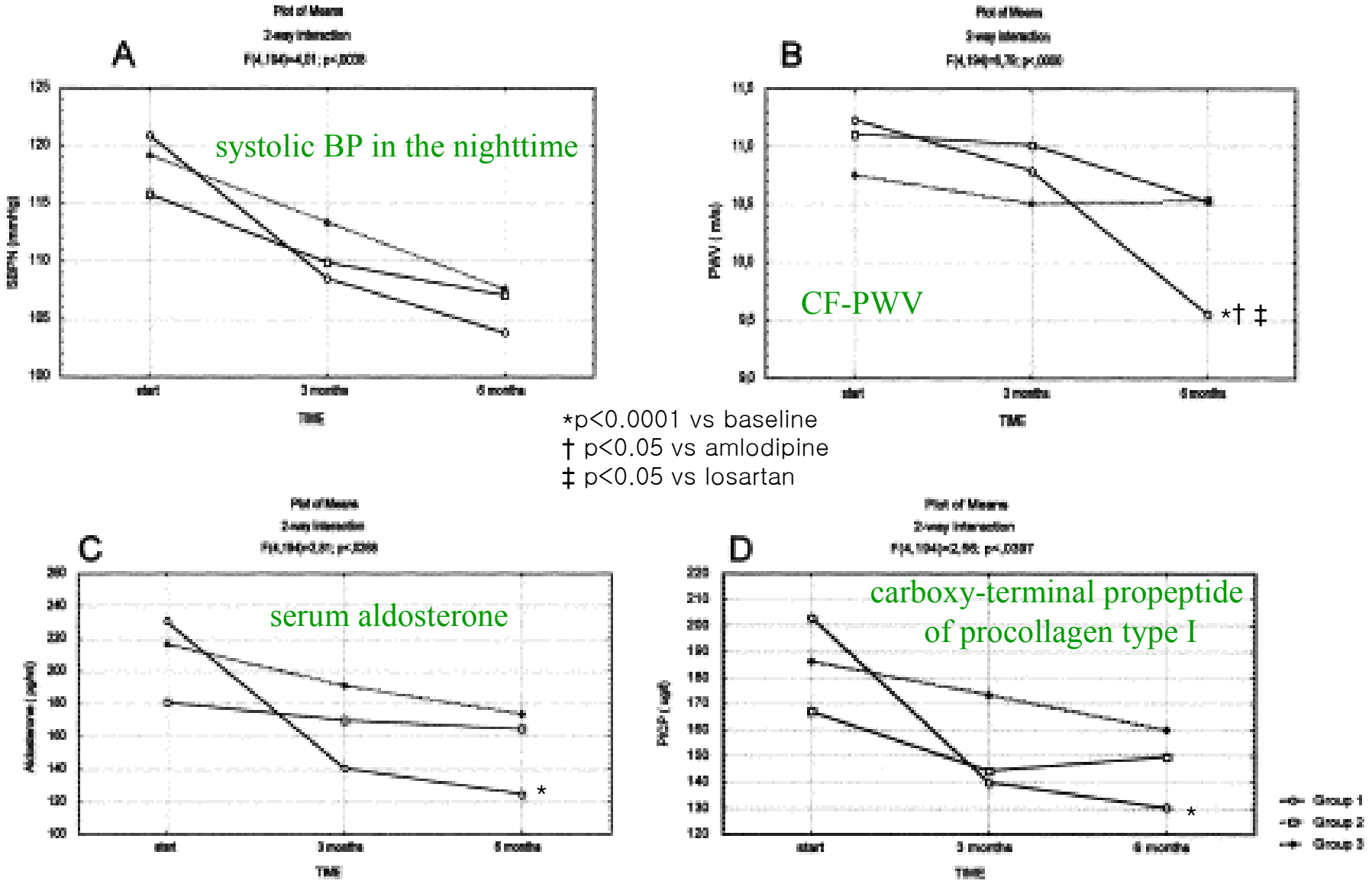


- HiBp pts (n=11)
- Single blind randomized crossover study
- 4wk Tx each, 4 wk washout

Mahmud A, Am Journal of Hypertens 2002:15:1092

Effects of antihypertensive drugs in patients with hypertension

○: quinapril (20mg) □: amlodipine (10mg) ●: losartan 2x50mg



*p<0.0001 vs baseline
† p<0.05 vs amlodipine
‡ p<0.05 vs losartan

Rajzer M, Am J Hypertens 2003;16:439-44

PWV as endpoint in large-scale intervention trial. The Complior® Study

- assess the feasibility of using PWV as endpoints in a large scale intervention
- essential HiBp (n=2187, 18-79 yrs)
- intervention with perindopril 4 to 8 mg / indapamide (2.5 mg) Tx for 6 mo
- carotid-femoral PWV using the Complior®

Table 1 **Treatment effects on blood pressure and pulse wave velocity; mean values and changes from baseline (M0) during (M2) and at the end of the study (M6)**

Variables	M0	M2	M6	Δ(M2−M0)	*P*	Δ(M6−M0)	*P*
SBP (mmHg)	158 ± 15	139 ± 16	134 ± 13	−20 ± 17	< 0.001	−24 ± 17	< 0.001
DBP (mmHg)	98 ± 7	86 ± 9	84 ± 8	−12 ± 10.1	< 0.001	−14 ± 10	< 0.001
MAP (mmHg)	118 ± 8	103 ± 10	100 ± 9	−15 ± 11	< 0.001	−18 ± 11	< 0.001
PP (mmHg)	59 ± 15	52 ± 12	50 ± 10	−7 ± 14	< 0.001	−9 ± 15	< 0.001
HR (bpm)	75 ± 10	75 ± 9	75 ± 10	−0.4 ± 10	NS	−0.3 ± 10	NS
PWV (m/s)	11.6 ± 2.6	10.7 ± 2.2	10.5 ± 2.1	−0.9 ± 1.4	< 0.001	−1.1 ± 1.4	< 0.001

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP: mean arterial pressure; PP, pulse pressure; HR, heart rate; PWV, pulse wave velocity.

Asmar R. J Hypertens 2001;19:813-8

Arterial stiffness in HiBp can be reversed by an antihypertensive Tx based on ACE inhibition through mechanisms partly independent of BP reduction

Table 2 **Changes in blood pressure and pulse wave velocity according to the initial hypertension grade (a) and pre-study treatment (b). Results are adjusted for age**

(a) Hypertension grade (SBP/DBP mmHg)	ΔSBP (mmHg)		ΔDBP (mmHg)		ΔPWV (m/s)	
	Men	Women	Men	Women	Men	Women
Grade I (140–159/90–99)	−19 ± 15	−21 ± 16	−12 ± 10	−12 ± 10	−1.00 ± 1.39	−1.12 ± 1.39
Grade II (160–179/100–109)	−28 ± 16	−33 ± 14	−18 ± 8	−18 ± 8	−1.03 ± 1.29	−1.22 ± 1.44
Grade III (180/110)	−44 ± 12	−50 ± 14	−22 ± 8	−27 ± 18	−2.03 ± 1.33	−1.48 ± 1.35

± SD; SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity; Δ, change from baseline.

(b) Pre-study treatment status	Δ SBP (mmHg)		ΔDBP (mmHg)		Δ PWV (m/s)	
	Men	Women	Men	Women	Men	Women
Previously treated	−20 ± 18	−23 ± 17	−12 ± 9	−11 ± 10	−1.00 ± 1.55	−0.98 ± 1.42
Previously untreated	−22 ± 16	−26 ± 17	−14 ± 9	−16 ± 9	−1.03 ± 1.33	−1.19 ± 1.40

± SD; SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, pulse wave velocity; Δ, change from baseline.

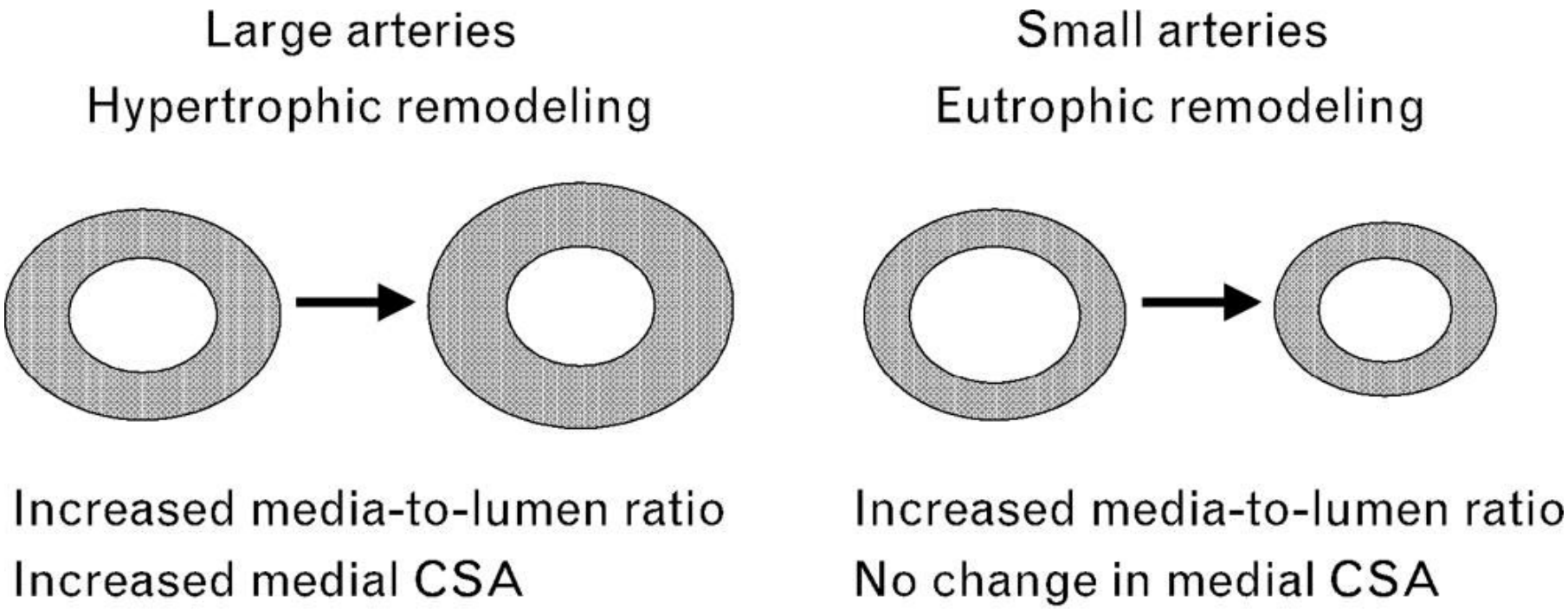
Asmar R. J Hypertens 2001;19:813-8

PWV changes observed in double-blind studies with antihypertensive Tx

Study		Short-term Tx <28 d		Long-term Tx ≥ 28d	
Class/Author	**Drug**	**Aorta**	**Arm/Leg**	**Aorta**	**Arm/Leg**
Vasodilators					
Lacolley	Cadralazine	≡			
B blockers	Dilevalol			↘	↘
Kelly	Atenolol			↘	↘ (≡)
Asmar	Bisprolol			↘	↘/≡
Barenbrock	Metoprolol			≡	
Simon	Metoprolol				≡
Ca2+ antagonists					
Pancera	Lacidipine				↘
	Nifedipine				↘
Pannier	Lacidpine	≡			
Asmar	Nitrendipine			↘	≡
	Felodipine			↘	↘
ACE inhibitors					
Lacolley	Captopril	↘	↘		
Asmar	Lisinopril	↘		distensibility↗	
Barenbrock	Lisinopril				
Kool	Perindopril			distensibility↗	
Topouchian	Quinapril	↘			
Topouchian	Trandolapril			↘	
Diuretics					
Asmar	HCTZ			≡	≡
Kool	HCTZ+amiloride			≡	
Benetos	HCTZ+amiloride			↘	

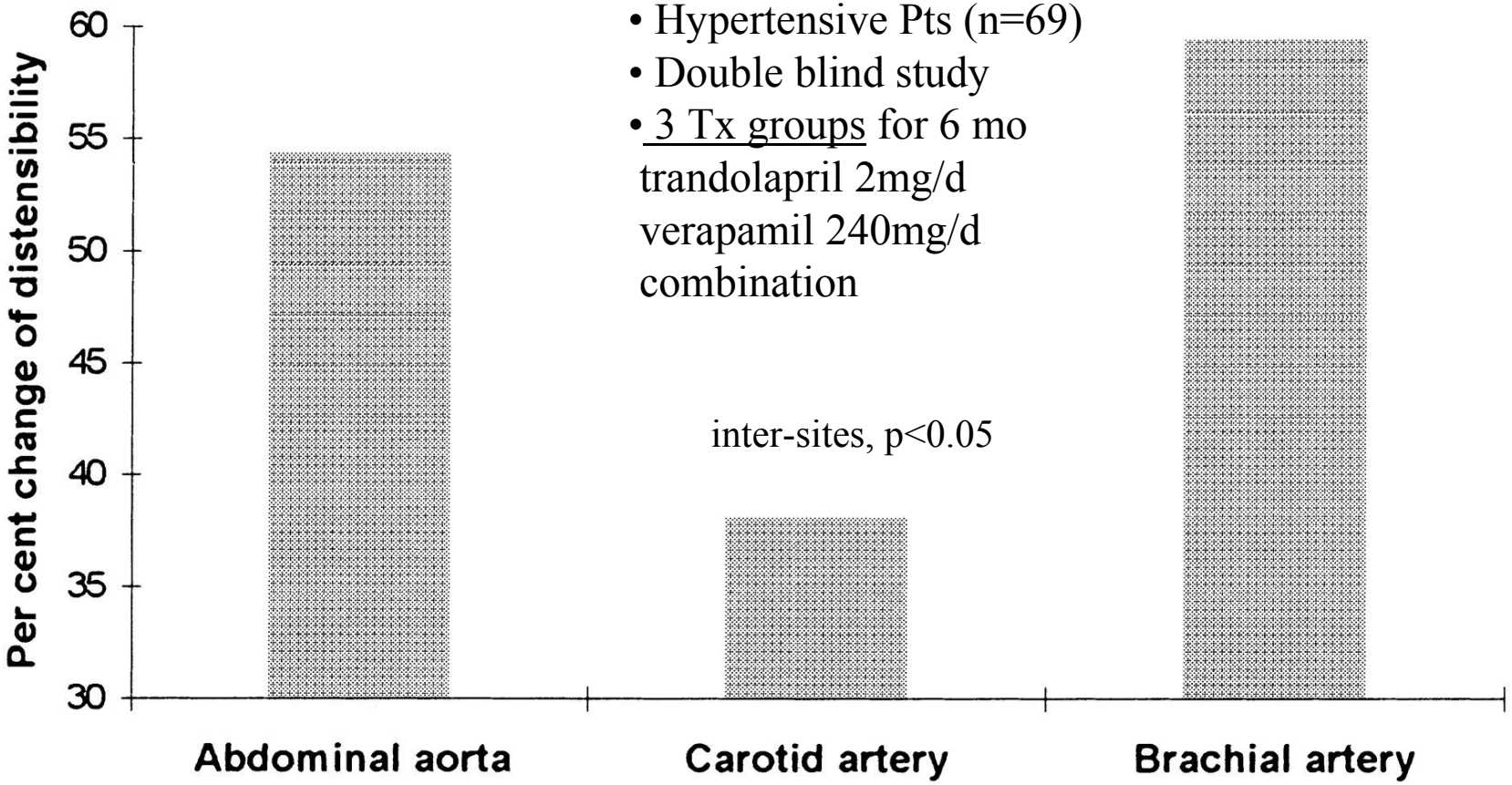
Roland Asmar. 1999

Vascular Remodeling



Vascular remodeling of large and small arteries. CSA, cross-sectional area.

Percent change in distensibility in 3 studied arterial territories

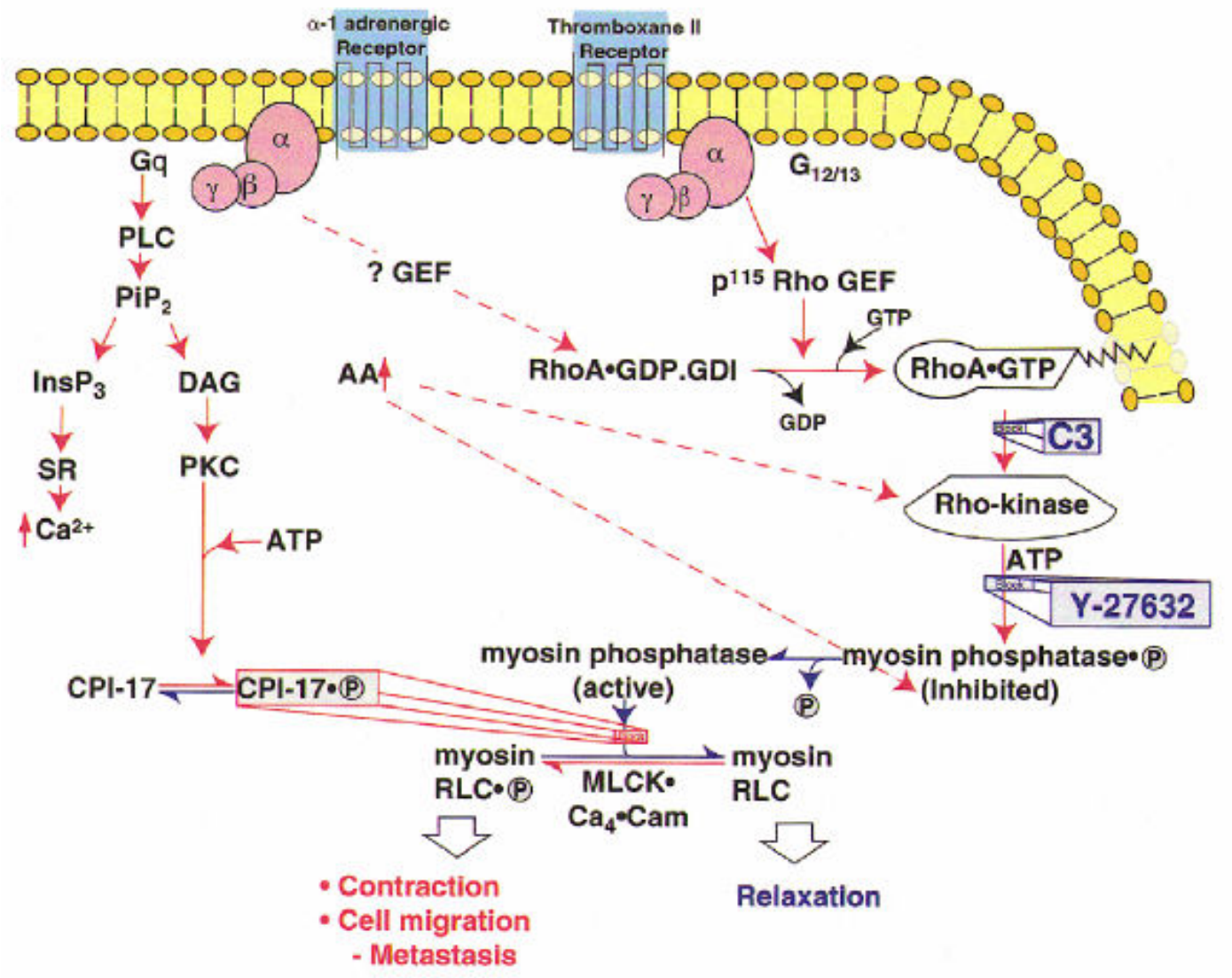


- Hypertensive Pts (n=69)
- Double blind study
- 3 Tx groups for 6 mo
  trandolapril 2mg/d
  verapamil 240mg/d
  combination

inter-sites, $p<0.05$

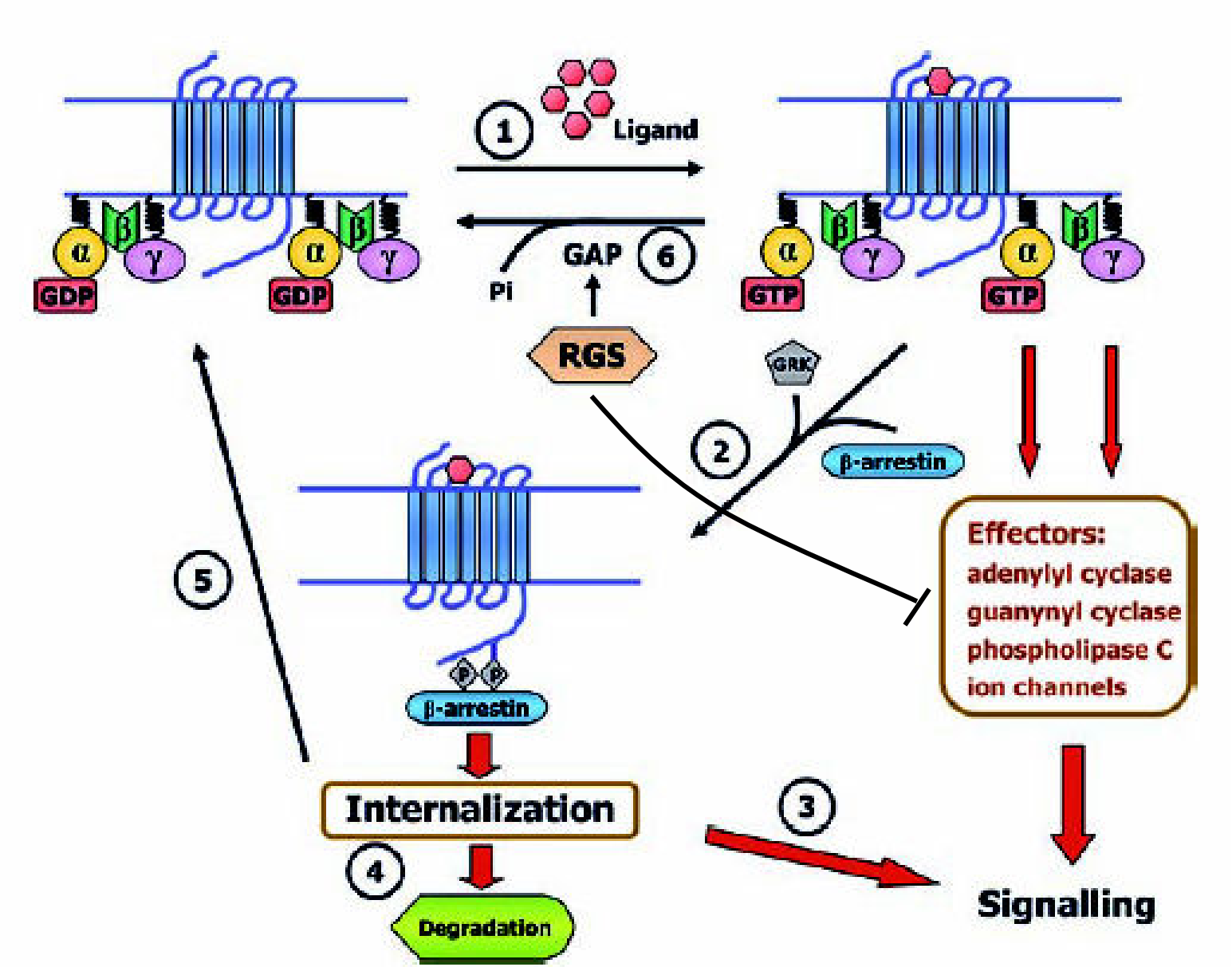
Topouchian, J. et al. Stroke 1999;30:1056-1064

Regulation of myosin II in SMC and non-muscle cells



Somlyo AP. J Phys 2000;522:1777

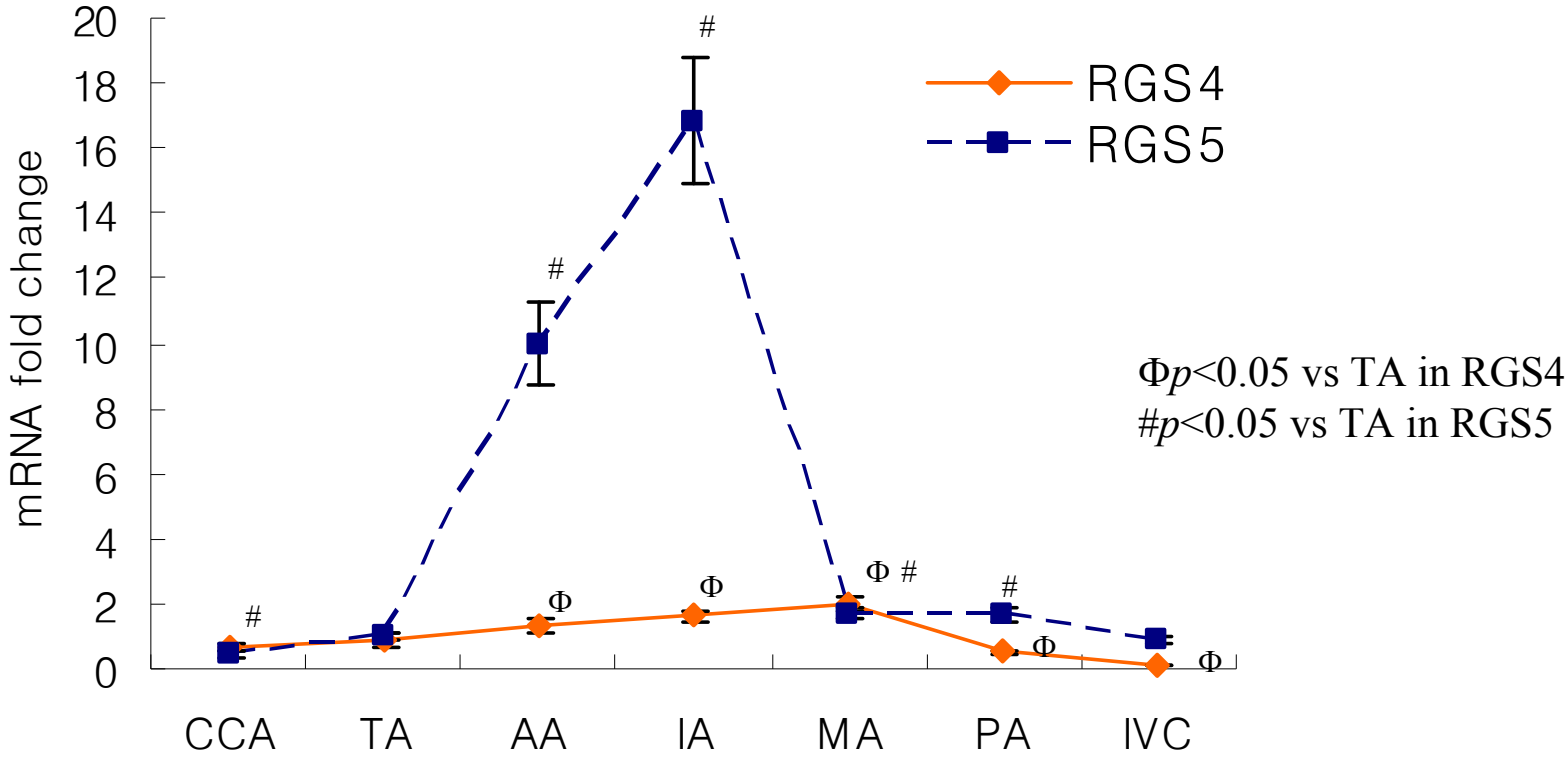
G-Protein Coupled Receptor (GPCR) Signaling



Jean-Baptise. Cell Mol Life Sci 2006;63:11969

Comparisons of R4 RGS mRNA between vessels

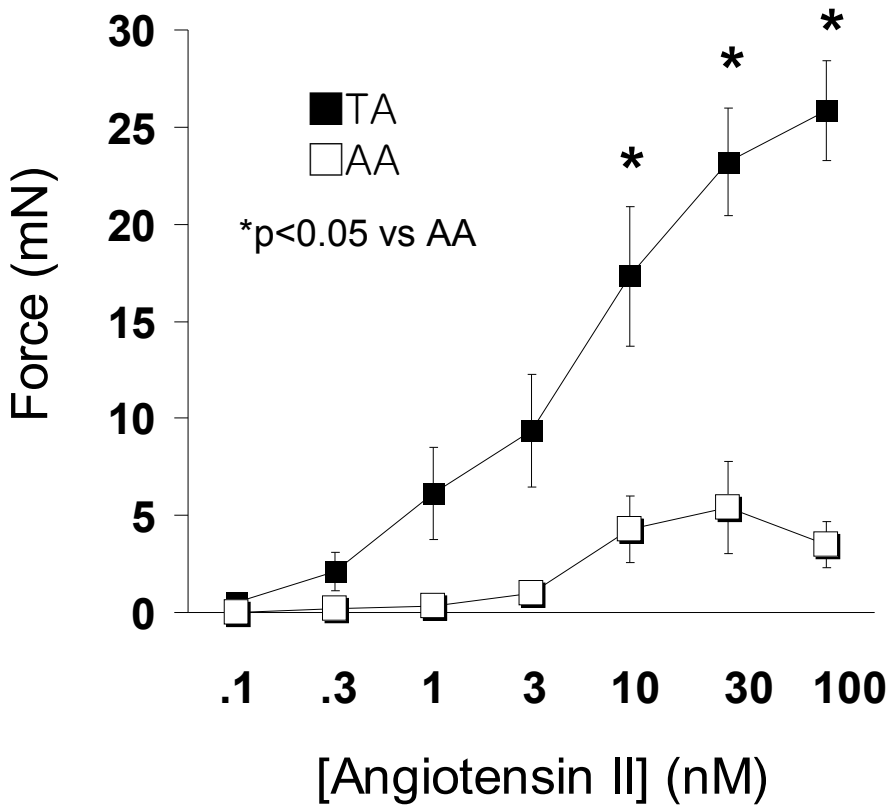
in SD rats, real-time Q RT-PCR

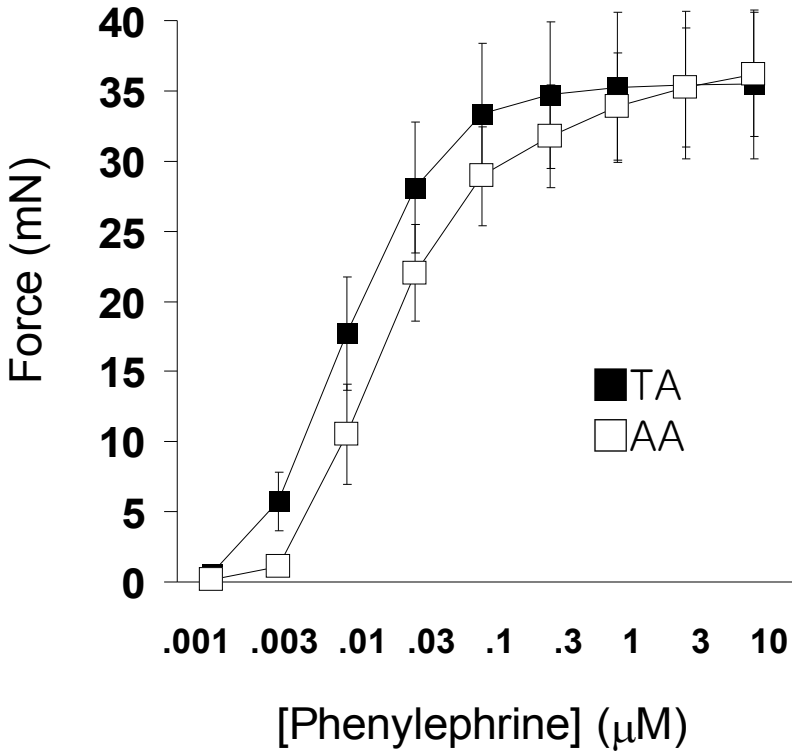


Chung I-M et al. Circulation. suppl 2006;114: II-248

Arterial contraction assay

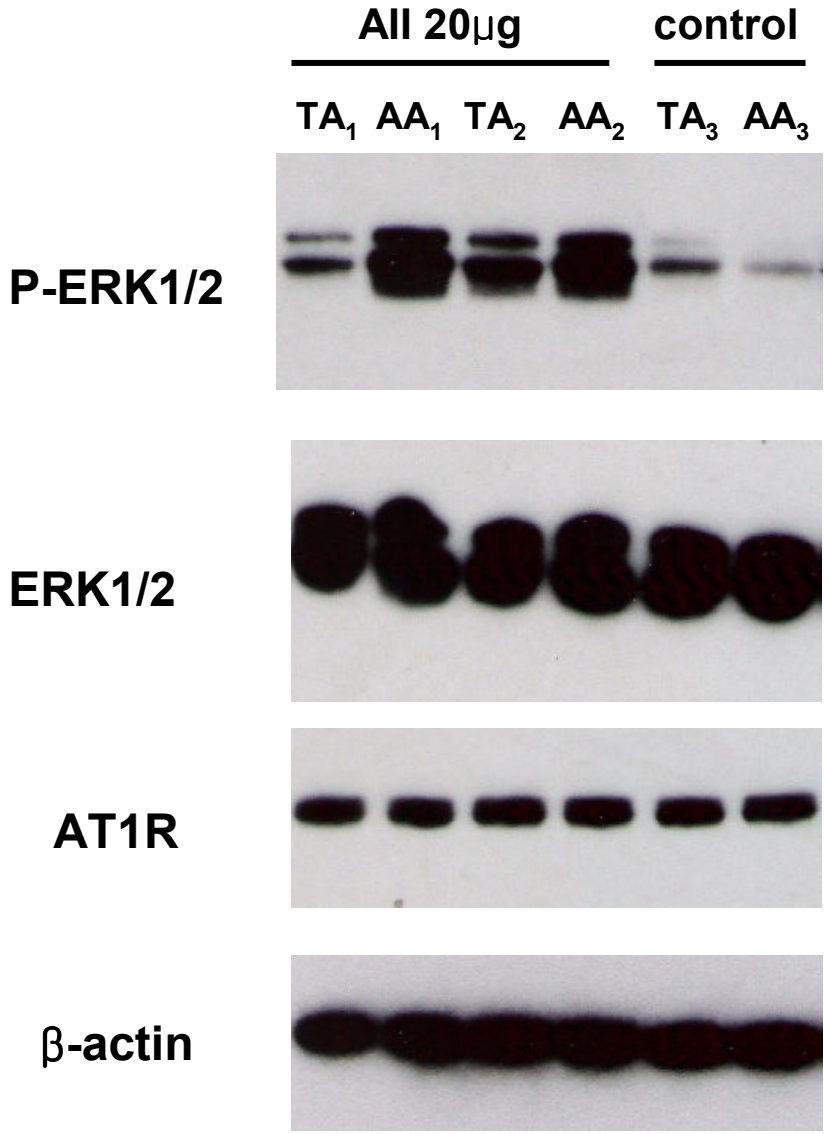
Ang II vs Phenylephrine



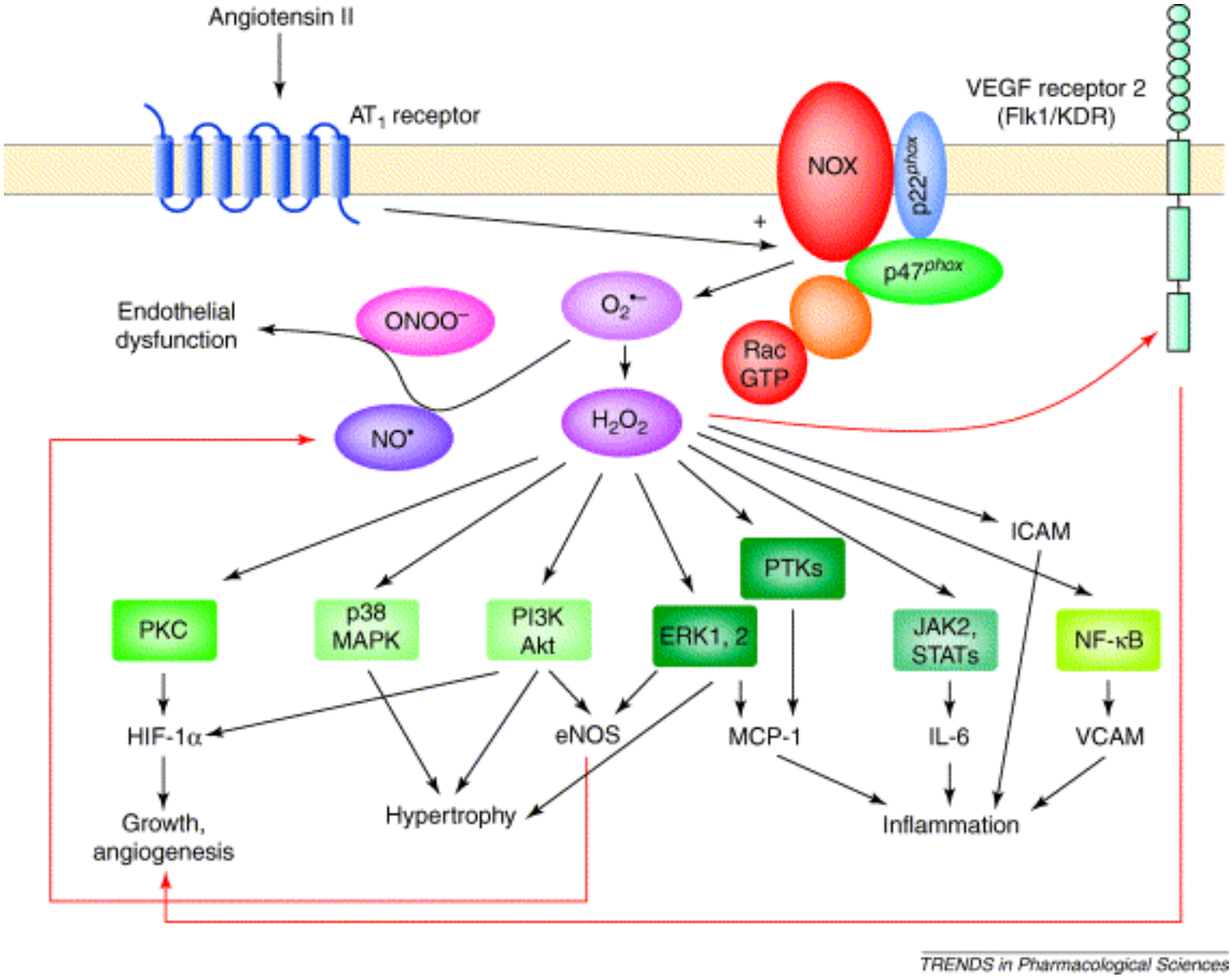


Chung I-M et al. Circulation. suppl 2006;114: II-248

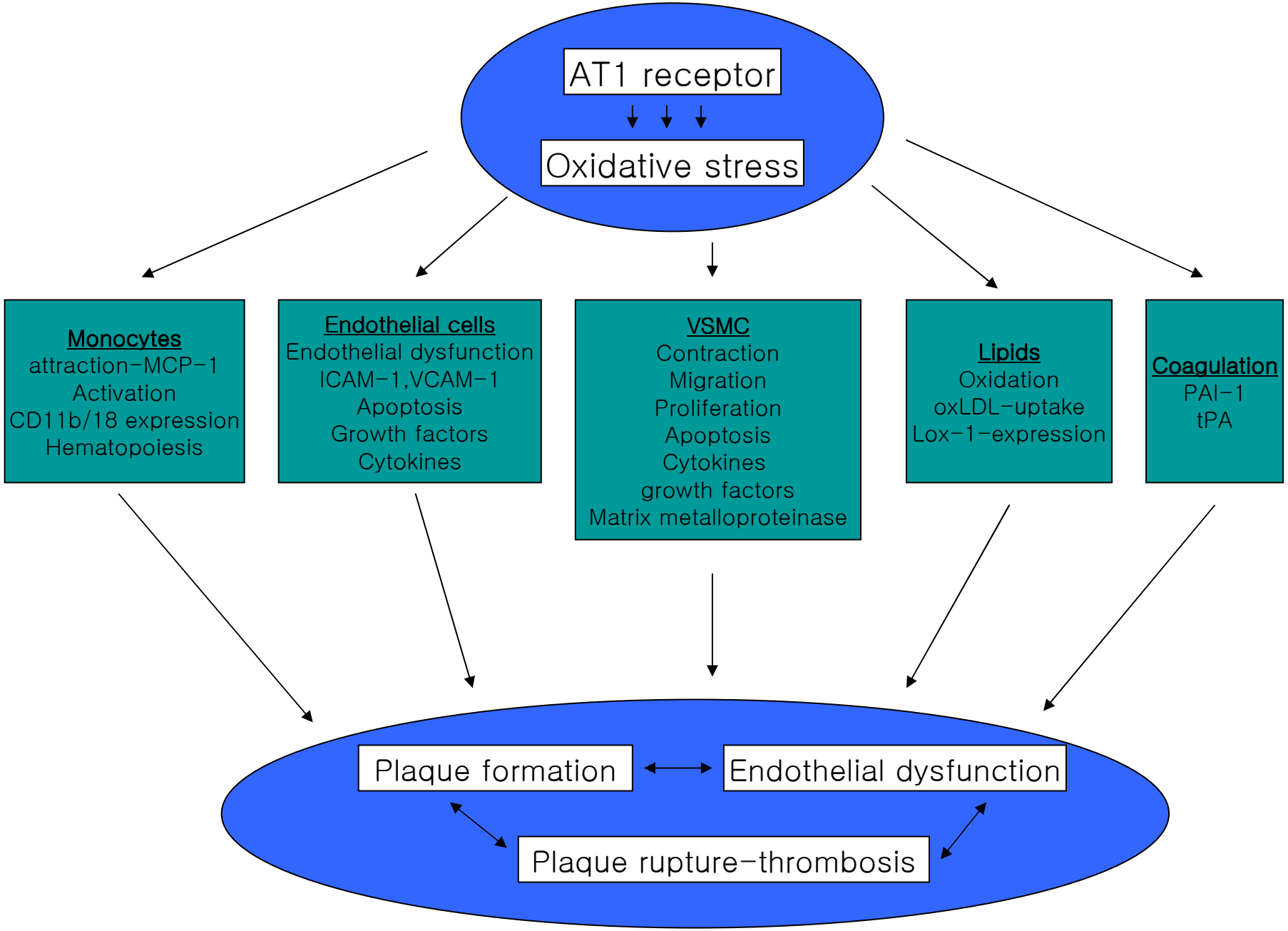
Angiotensin II-mediated ERK1/2 activation
Comparison between thoracic and abdominal aorta



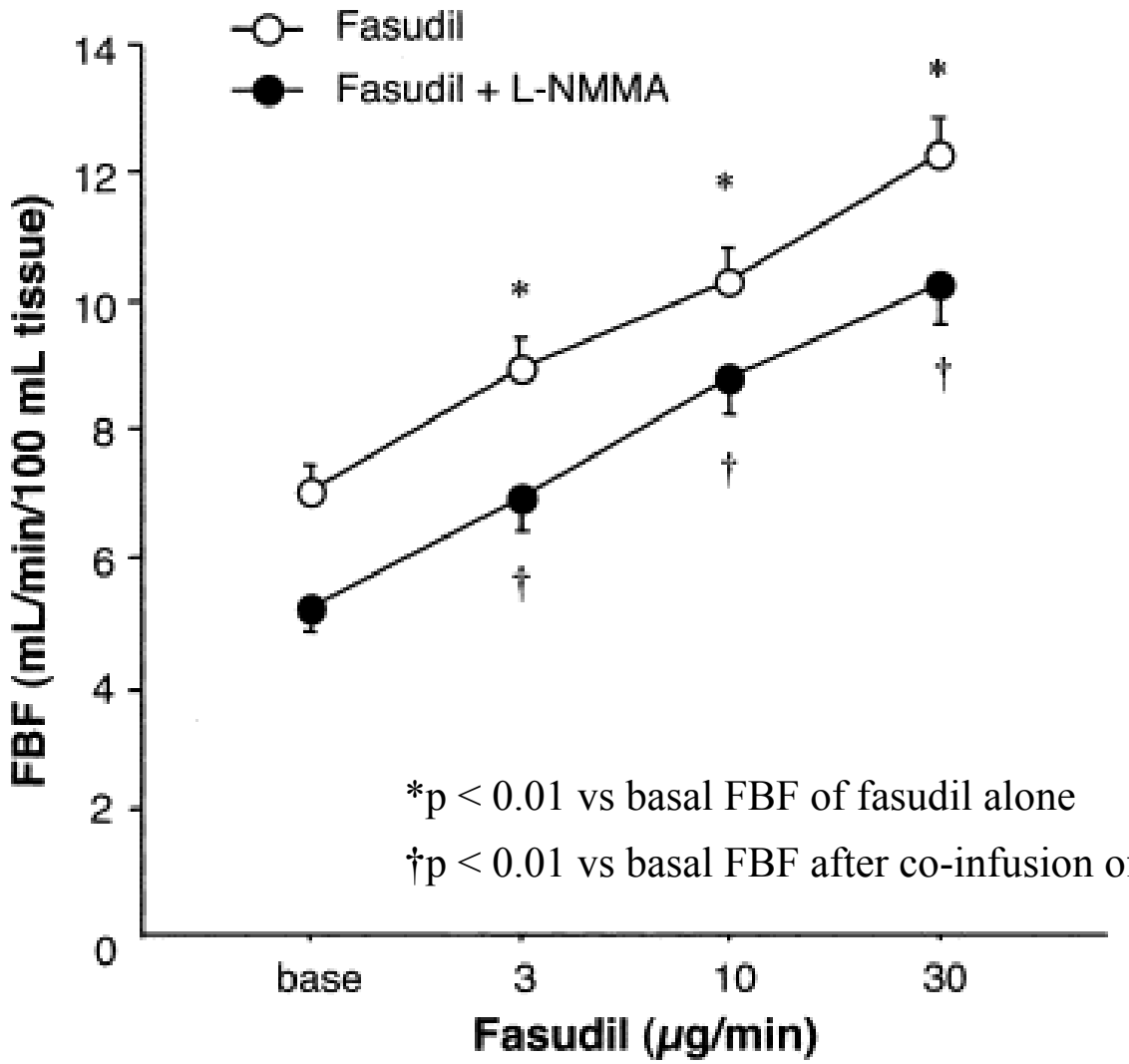
Chung I-M et al. Circulation. suppl 2006;114: II-248



Cai H et al. Trends in Pharmacological Sciences 2003;24:471–478


AT1 receptor
Oxidative stress
Monocytes
attraction-MCP-1
Activation
CD11b/18 expression
Hematopoiesis
Endothelial cells
Endothelial dysfunction
ICAM-1,VCAM-1
Apoptosis
Growth factors
Cytokines
VSMC
Contraction
Migration
Proliferation
Apoptosis
Cytokines
growth factors
Matrix metalloproteinase
Lipids
Oxidation
oxLDL-uptake
Lox-1-expression
Coagulation
PAI-1
tPA
Plaque formation
Endothelial dysfunction
Plaque rupture-thrombosis

Forearm blood flow response to Fasudil (ROCK inhibitor)



*p < 0.01 vs basal FBF of fasudil alone

†p < 0.01 vs basal FBF after co-infusion of L-NMMA

Noma K, J Am Coll Cardiol 2007;49:698-705

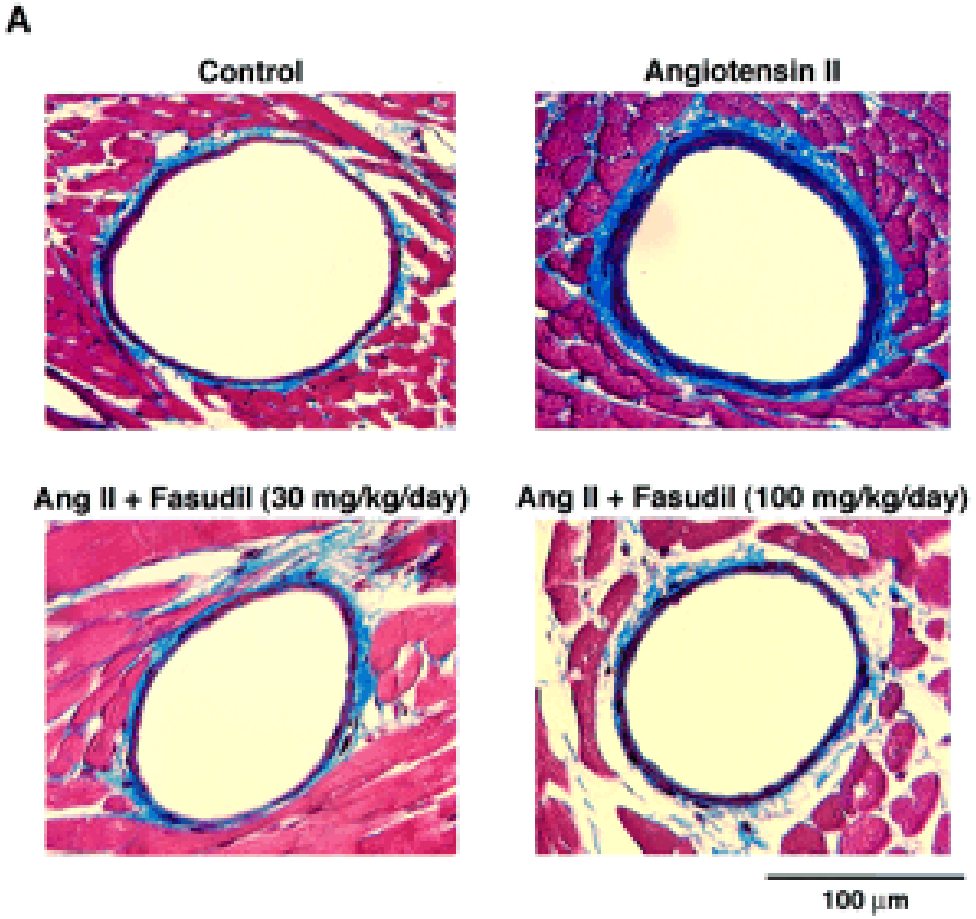
Long-Term Inhibition of Rho-Kinase Suppresses Angiotensin II–Induced Cardiovascular Hypertrophy in Rats In Vivo

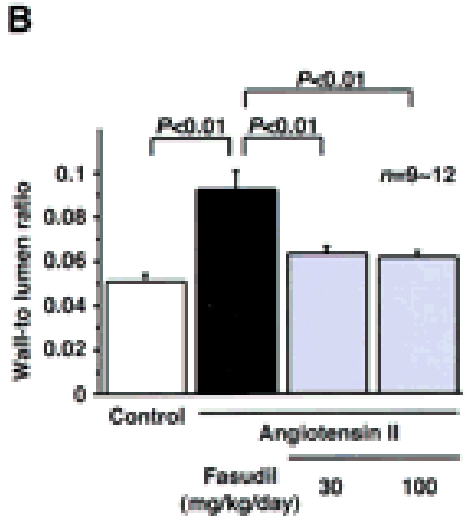
Effect on Endothelial NAD(P)H Oxidase System

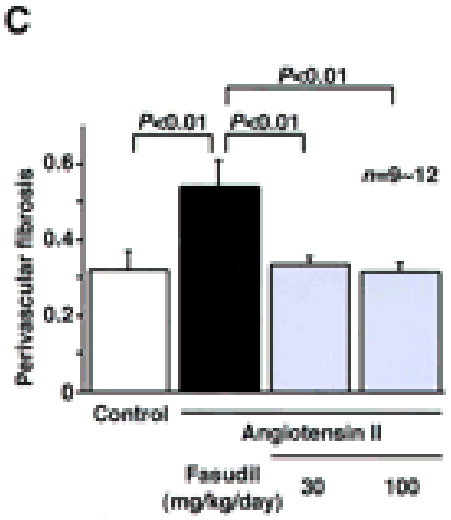
Midoriko Higashi, Hiroaki Shimokawa, Tsuyoshi Hattori, Junko Hiroki,
Yasushi Mukai, Keiko Morikawa, Toshihiro Ichiki, Shosuke Takahashi, Akira Takeshita

Abstract—Intracellular signaling pathway mediated by small GTPase Rho and its effector Rho-kinase plays an important role in regulation of vascular smooth muscle contraction and other cellular functions. We have recently demonstrated that Rho-kinase is substantially involved in angiotensin II–induced gene expressions and various cellular responses in vitro. However, it remains to be examined whether Rho-kinase is involved in the angiotensin II–induced cardiovascular hypertrophy in vivo and, if so, what mechanisms are involved. Long-term infusion of angiotensin II for 4 weeks caused hypertrophic changes of vascular smooth muscle and cardiomyocytes in rats. Both changes were significantly suppressed by concomitant oral treatment with fasudil, which is metabolized to a specific Rho-kinase inhibitor, hydroxyfasudil, after oral administration. Angiotensin II caused a perivascular accumulation of macrophages and Rho-kinase activation, both of which were also significantly suppressed by fasudil. Vascular NAD(P)H oxidase expression (nox1, nox4, gp91phox, and p22phox) and endothelial production of superoxide anions were markedly increased by angiotensin II, both of which were also significantly suppressed by fasudil. Thus, fasudil ameliorated the impaired endothelium-dependent relaxations caused by angiotensin II without affecting vasodilator function of vascular smooth muscle. These results provide evidence that Rho-kinase is substantially involved in the angiotensin II–induced cardiovascular hypertrophy in rats in vivo. The suppression of endothelial NAD(P)H oxidase upregulation and resultant superoxide production and the amelioration of endothelial vasodilator function may be involved in this process. (*Circ Res*. 2003;93:767-775.)

Ang II-mediated coronary vascular hypertrophy Role of Rho-kinase

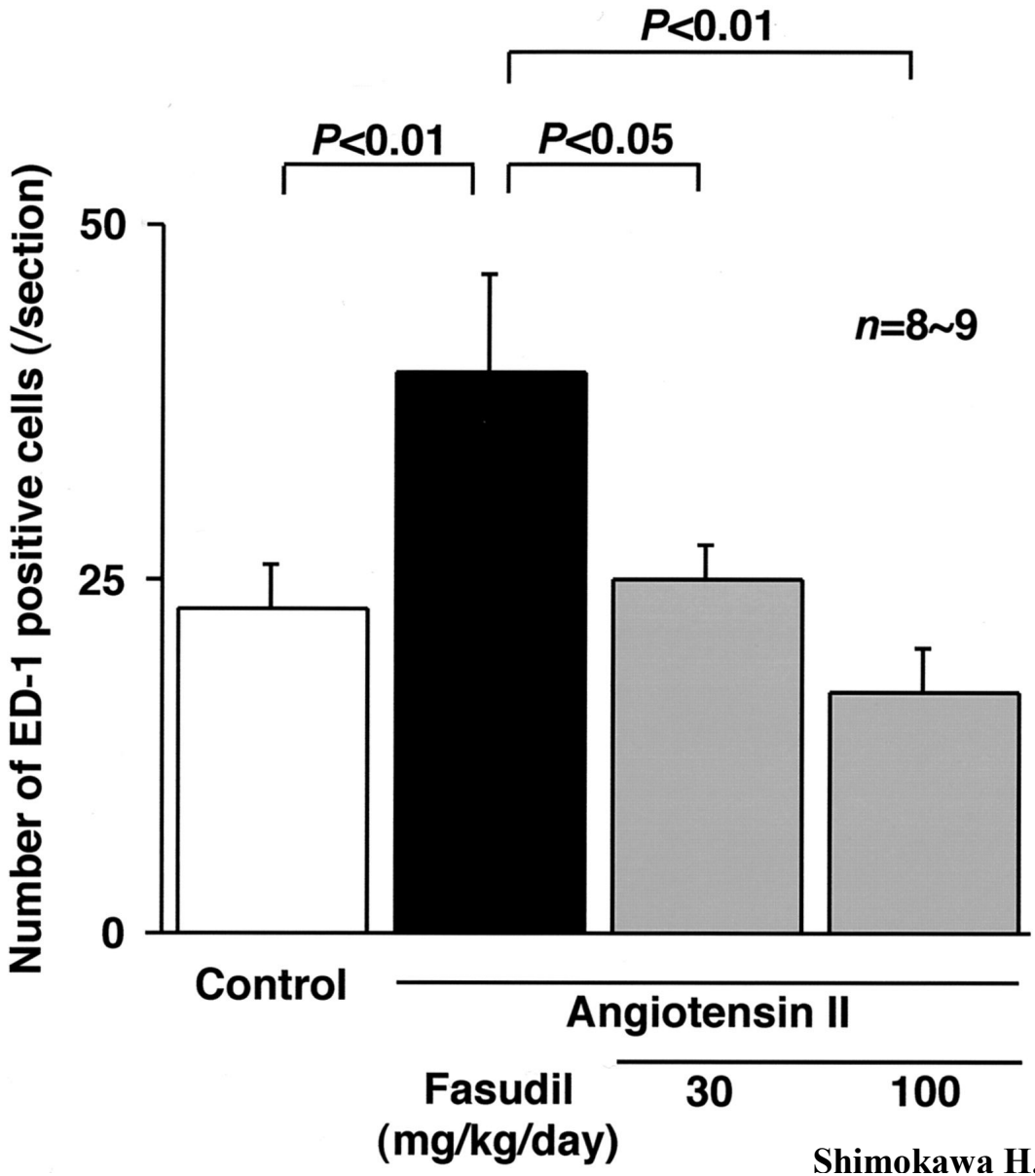






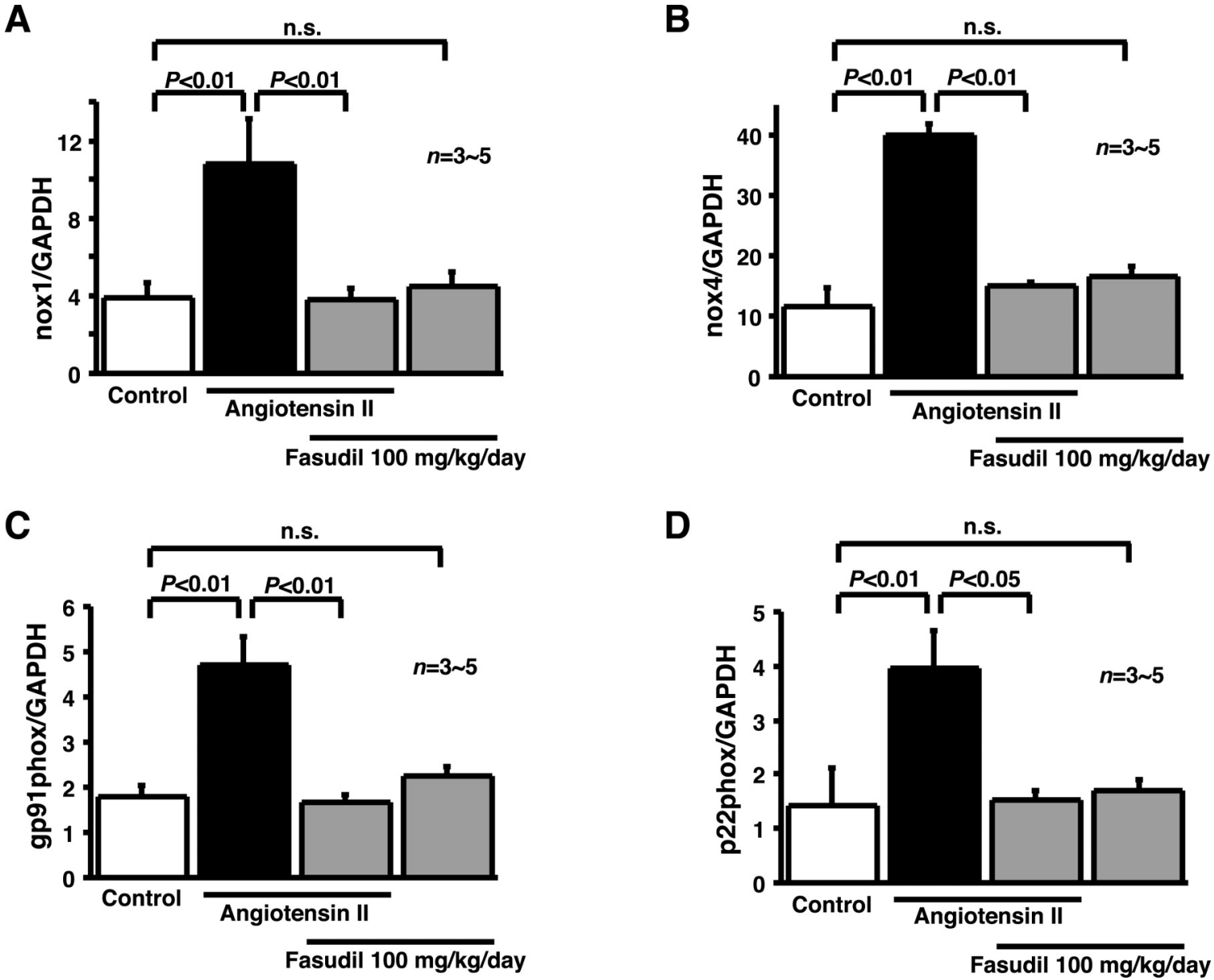
Shimokawa H. Circ Res 2003;93:767-75

Ang II-mediated vascular macrophage accumulation
Role of Rho-kinase



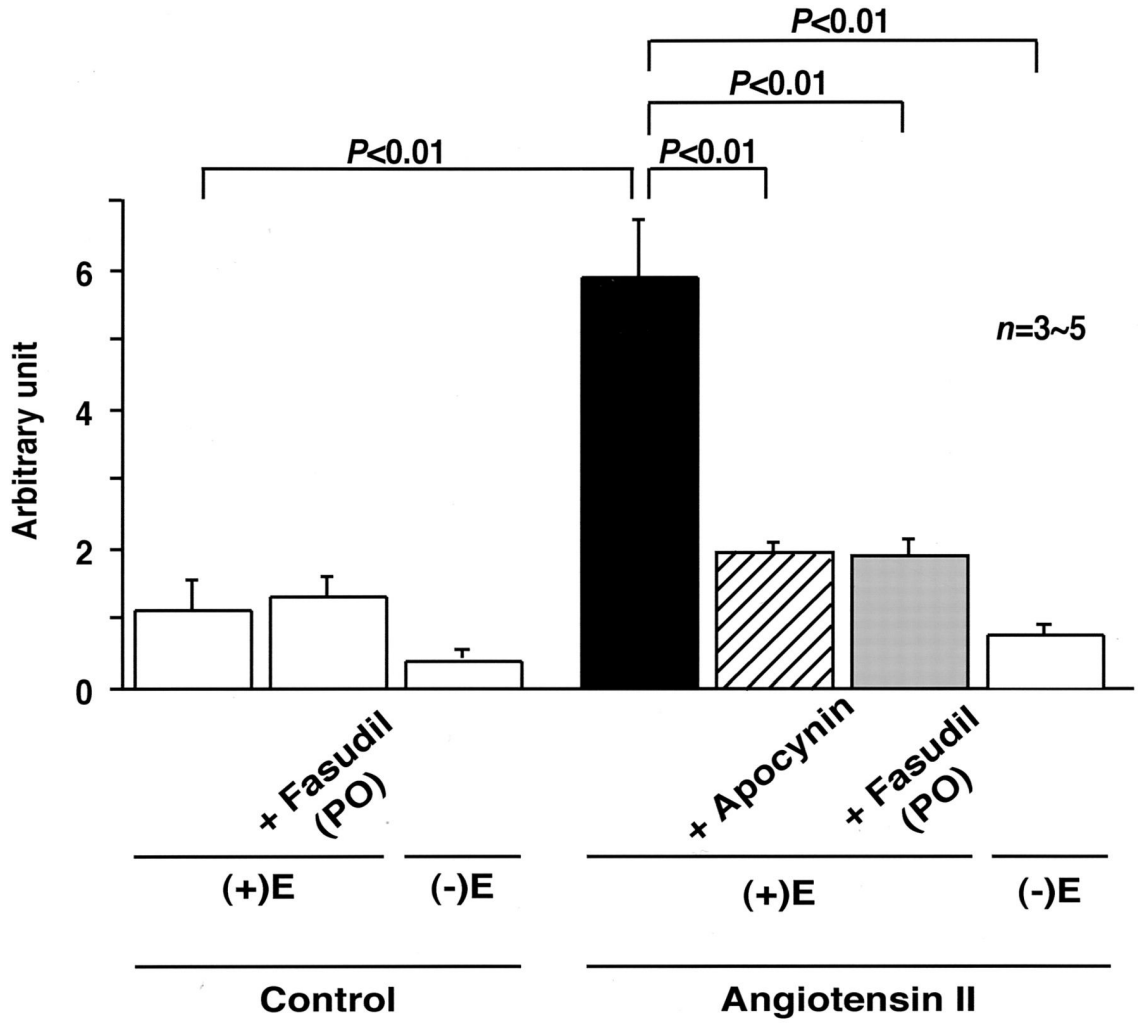
Shimokawa H. Circ Res 2003:93:767-75

Ang II-mediated NADPH oxidase: Role of Rho-kinase



Shimokawa H. Circ Res 2003;93:767-75

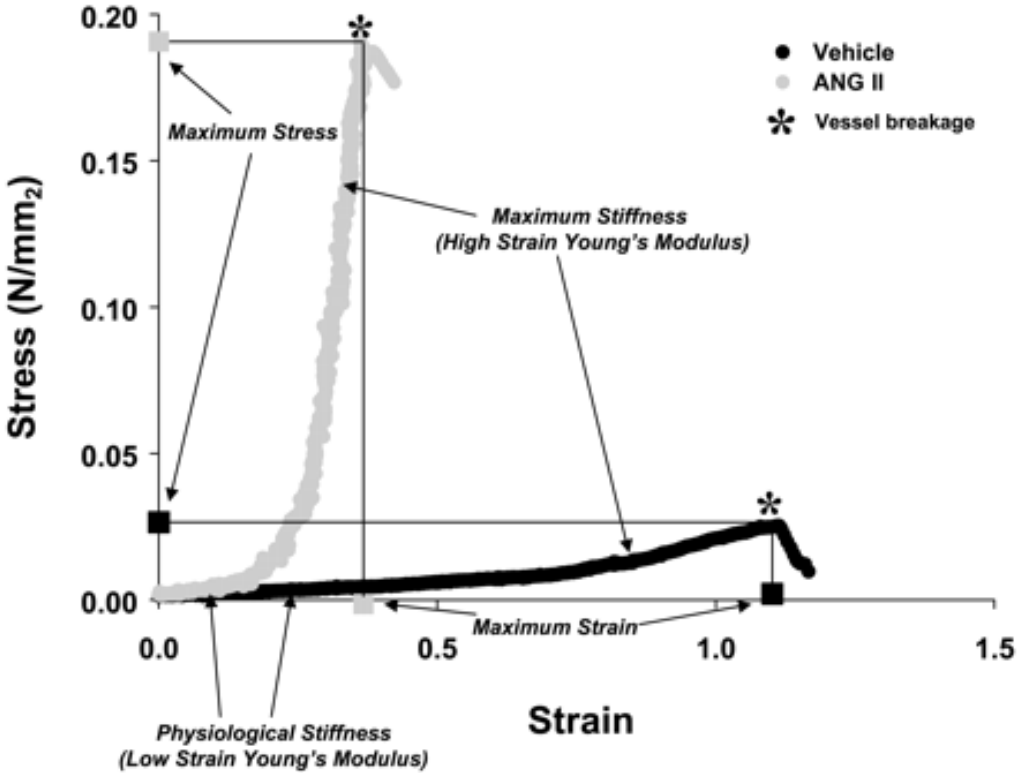
Long-term treatment with fasudil suppresses angiotensin II-induced endothelial production of superoxide anions

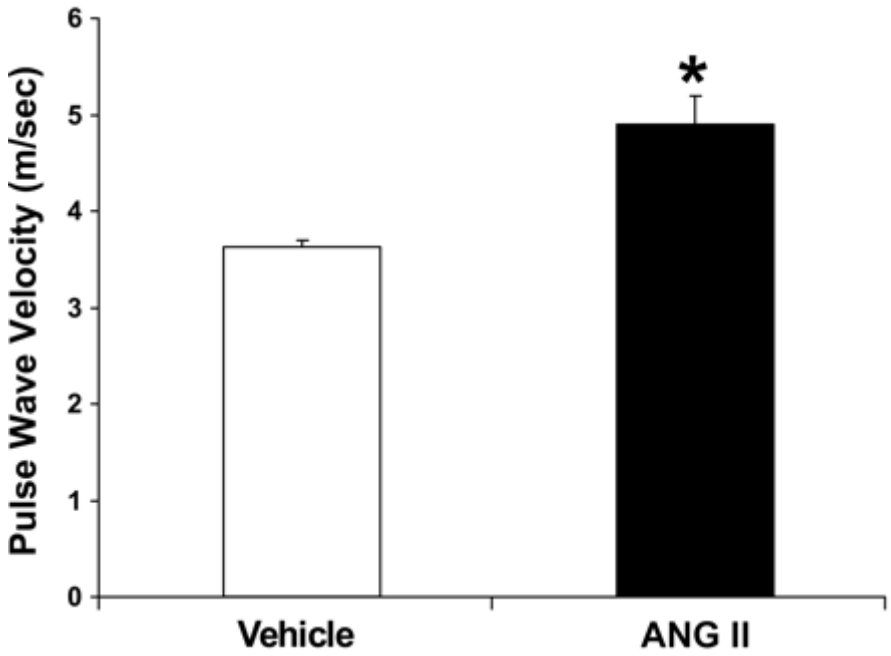


Higashi, M. et al. Circ Res 2003;93:767-775

Stress vs strain & PWV
thoracic aorta from apo-E KO mice treated with Ang II

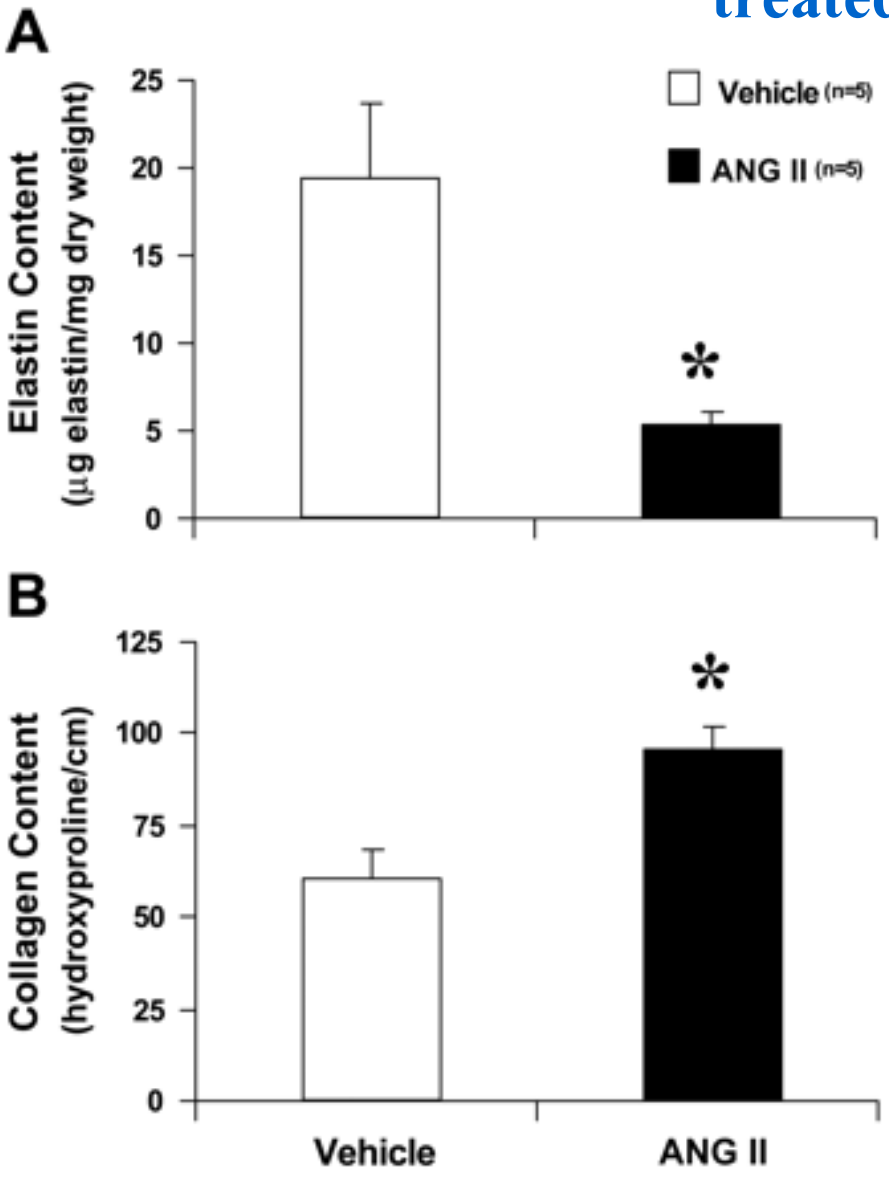
- Stress: vessel tension developed per vessel area
- Strain: fractional change in vessel width ($w-w_0/w_0$)

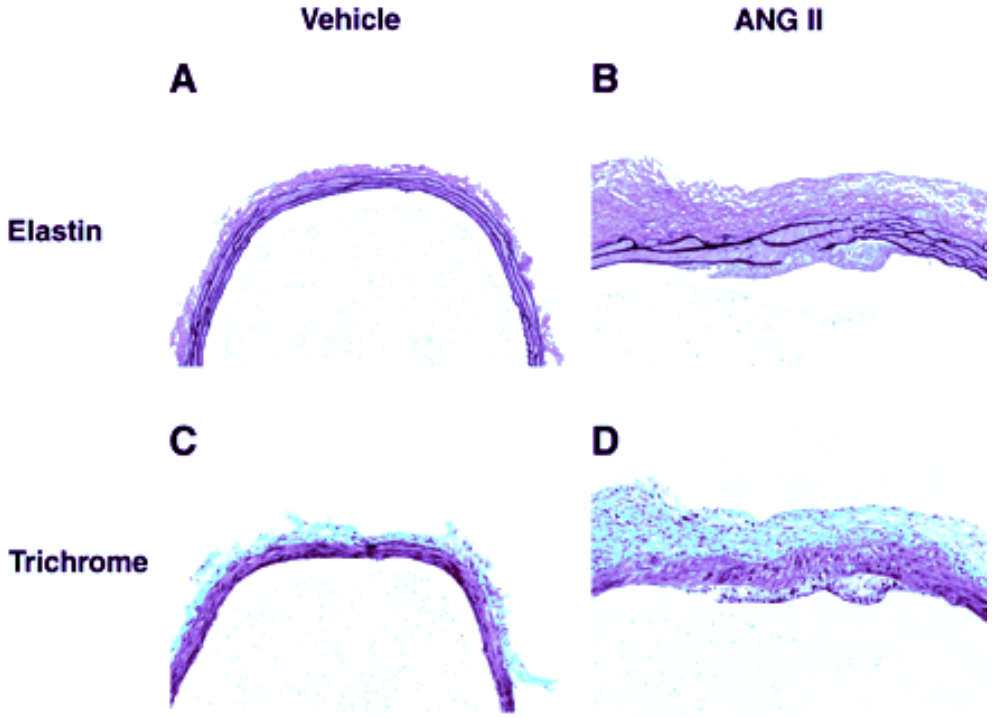




Tham DM, *Am J Physiol Regul Integr Comp Physiol* 283: R1442-R1449, 2002.

Histological changes in aorta from apo-E KO mice treated with Ang II





Elastin van Gieson & Trichrome stain of suprarenal artery from apoE-KO mice

Conclusion

- Arterial stiffness, an independent risk factor for future cardiovascular disease, can be measured non-invasively and simply by PWV
- Complex mechanisms including Ang II-mediated oxidative stress and subsequent vascular remodeling and inflammatory change may play a role in arterial stiffness
- Improvement of arterial stiffness can be induced by 1) non-pharmacological approach, such as nutrients (low salt, garlic, fish oil etc), aerobic exercise, or 2) pharmacological approach (ACEI, AT1R blocker, CCB, ROCK inhibitors etc)