Oxidative Stress in Experimental and Clinical Study

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2008년 **4**월 **19**일 춘계순환기학회, 부산

Definition

“Oxidative stress”

: conditions involving increased Reactive oxygen species (ROS) levels.

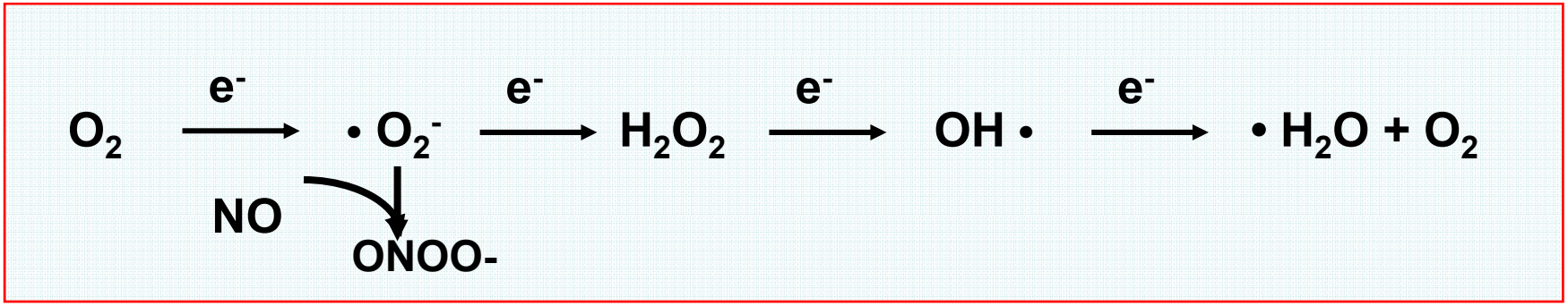
“Reactive oxygen species”

= “oxygen-derived species” or “oxidants,”

- **intermediates in reduction-oxidation (redox) reactions leading from O_2 to H_2O.**
- **two major groups:**

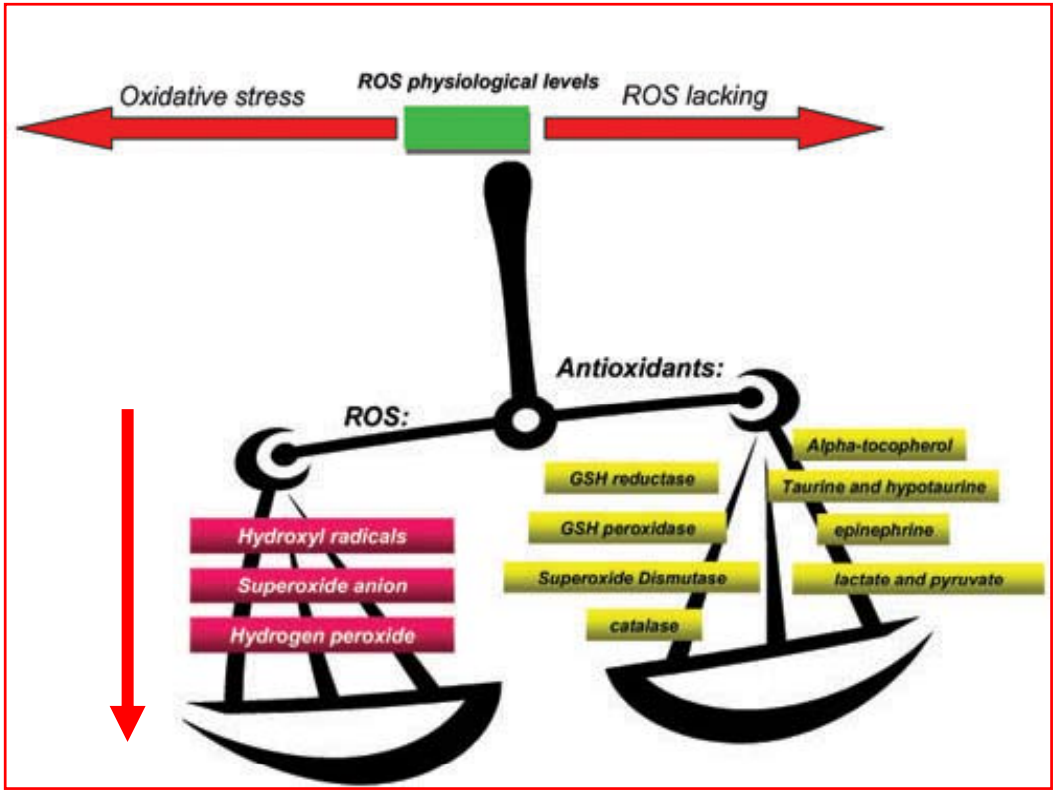
  free radicals : superoxide [O_2], hydroxyl[OH], nitric oxide [NO]

  nonradical derivatives of O_2 : H_2O_2, $ONOO^-$



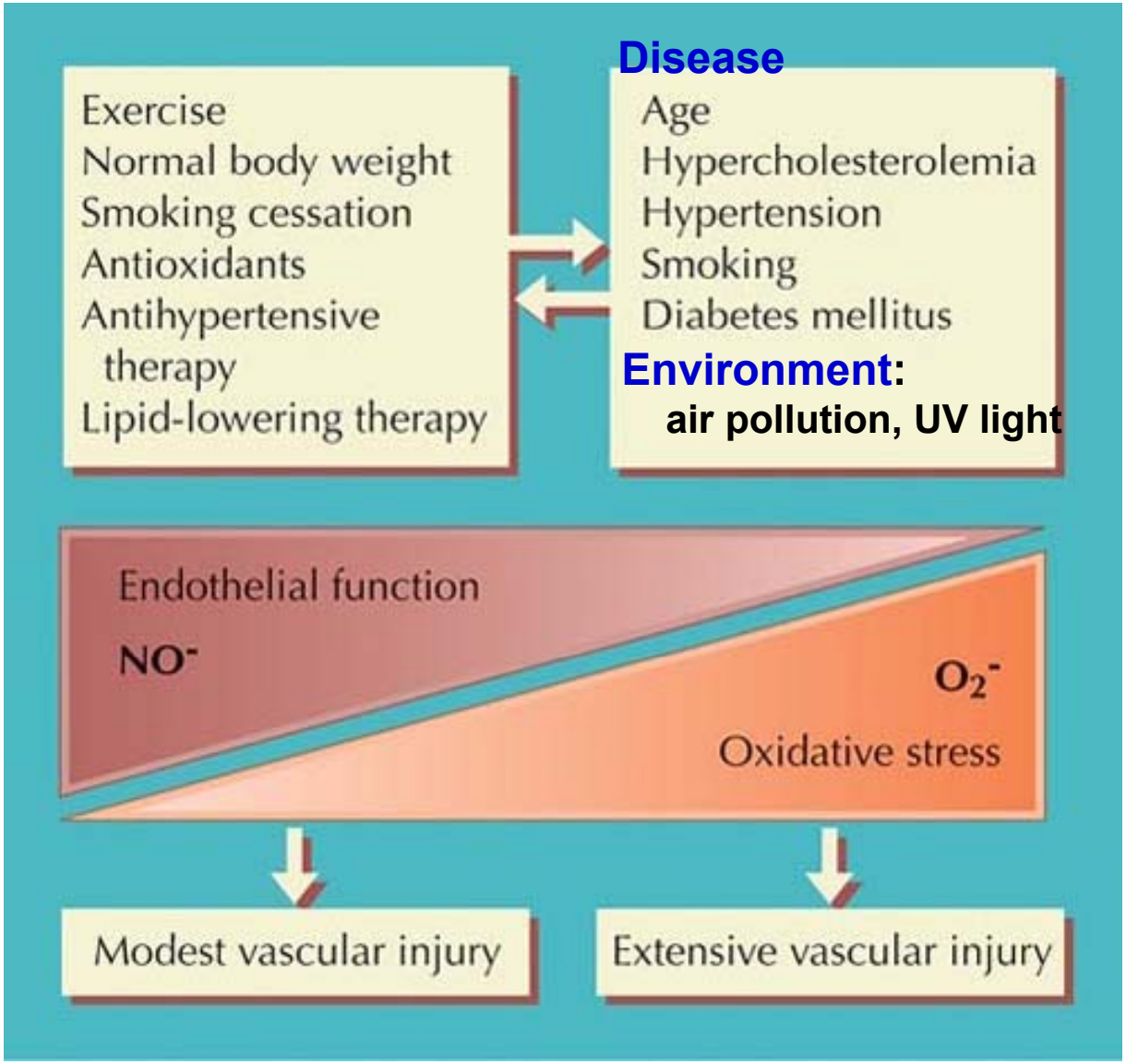
Background

원인: **Reactive oxygen** 생산과 **reactive intermediates** 를 해독하거나 손상을 수리할 수 있는 **biological system's ability** 와의 불균형

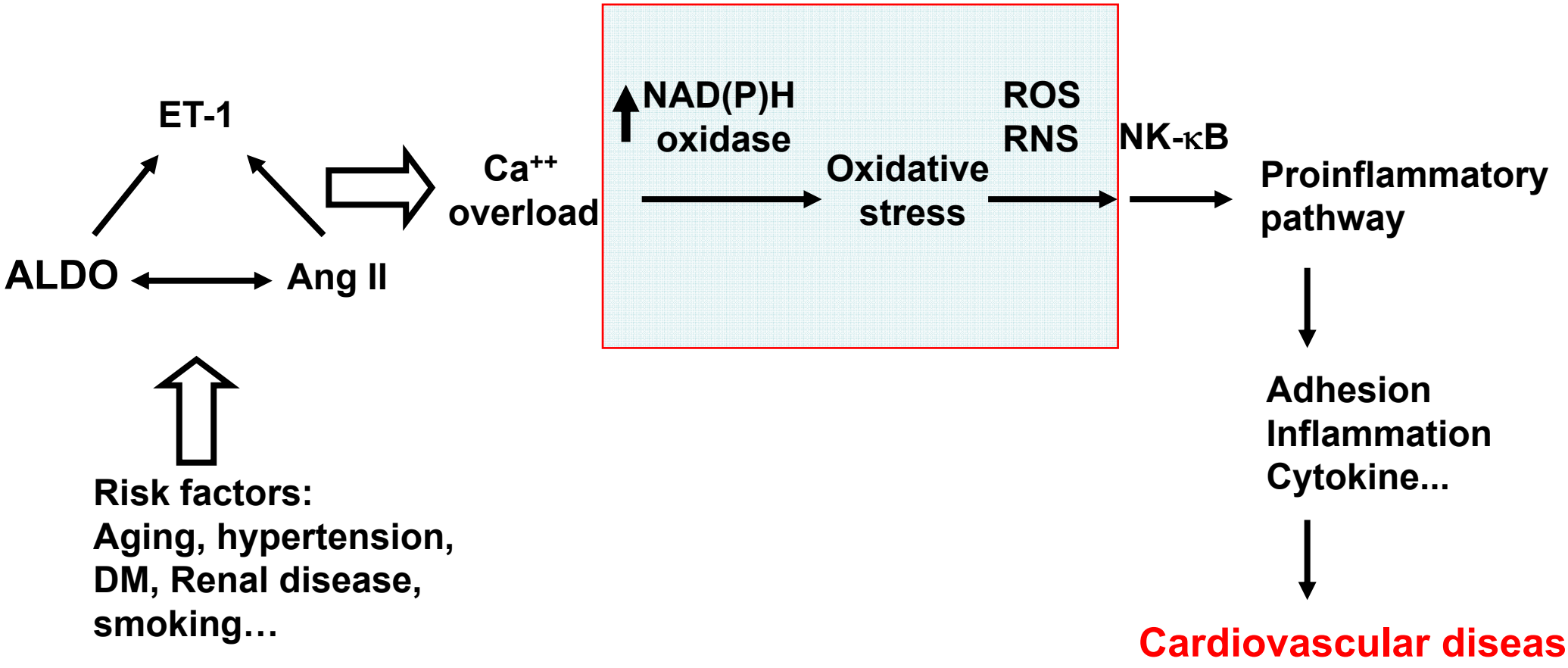


Cardiovascular disease (hypertension, atherosclerosis, diabetes, cardiac hypertrophy, heart failure, ischemia-reperfusion injury, and stroke)

Oxidative stress/Antioxidant balance and vascular damage

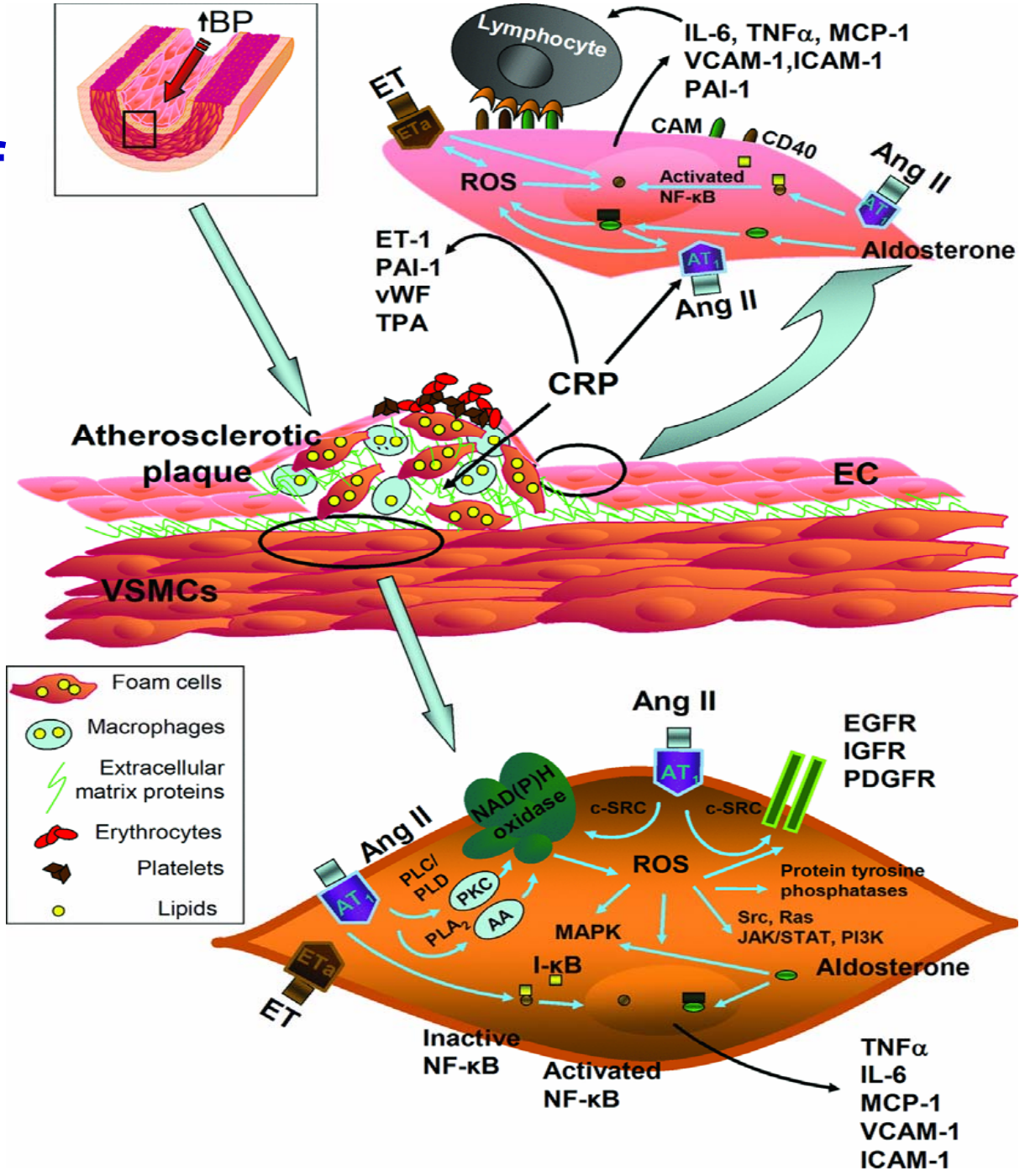


RAAS, NAD(P)H oxidase and Vascular damage



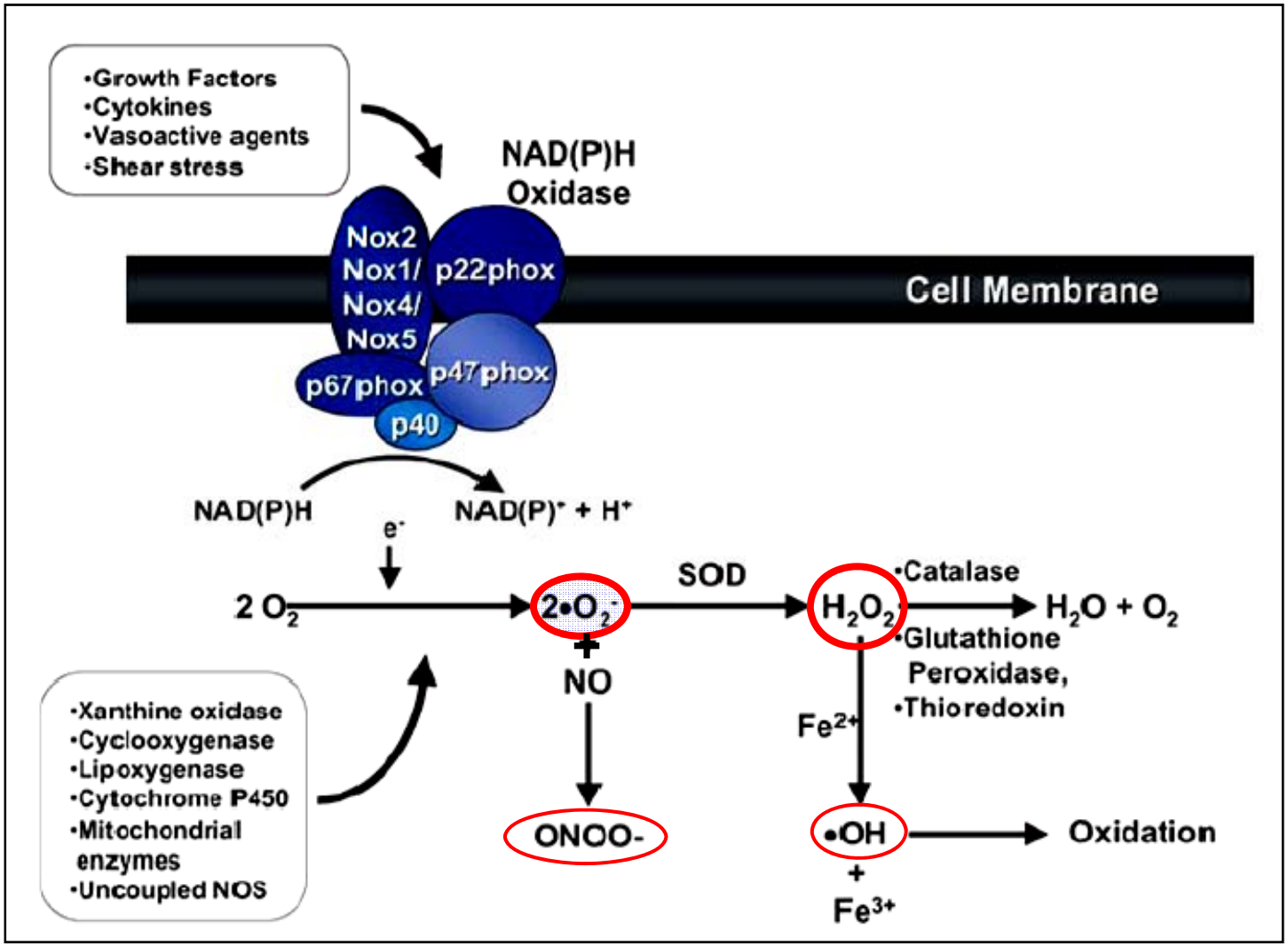
2005 Park JB

Molecular mechanism of ROS and inflammation



Clin. Sci. (2007) 112, 375-384

Generation of O_2^- and H_2O_2 from O_2 in vascular cell



Oxidant (ROS):

- •O2-, H_2O_2,
- •OH, ONOO-, …

Antioxidant:

- •<u>Enzymes</u>

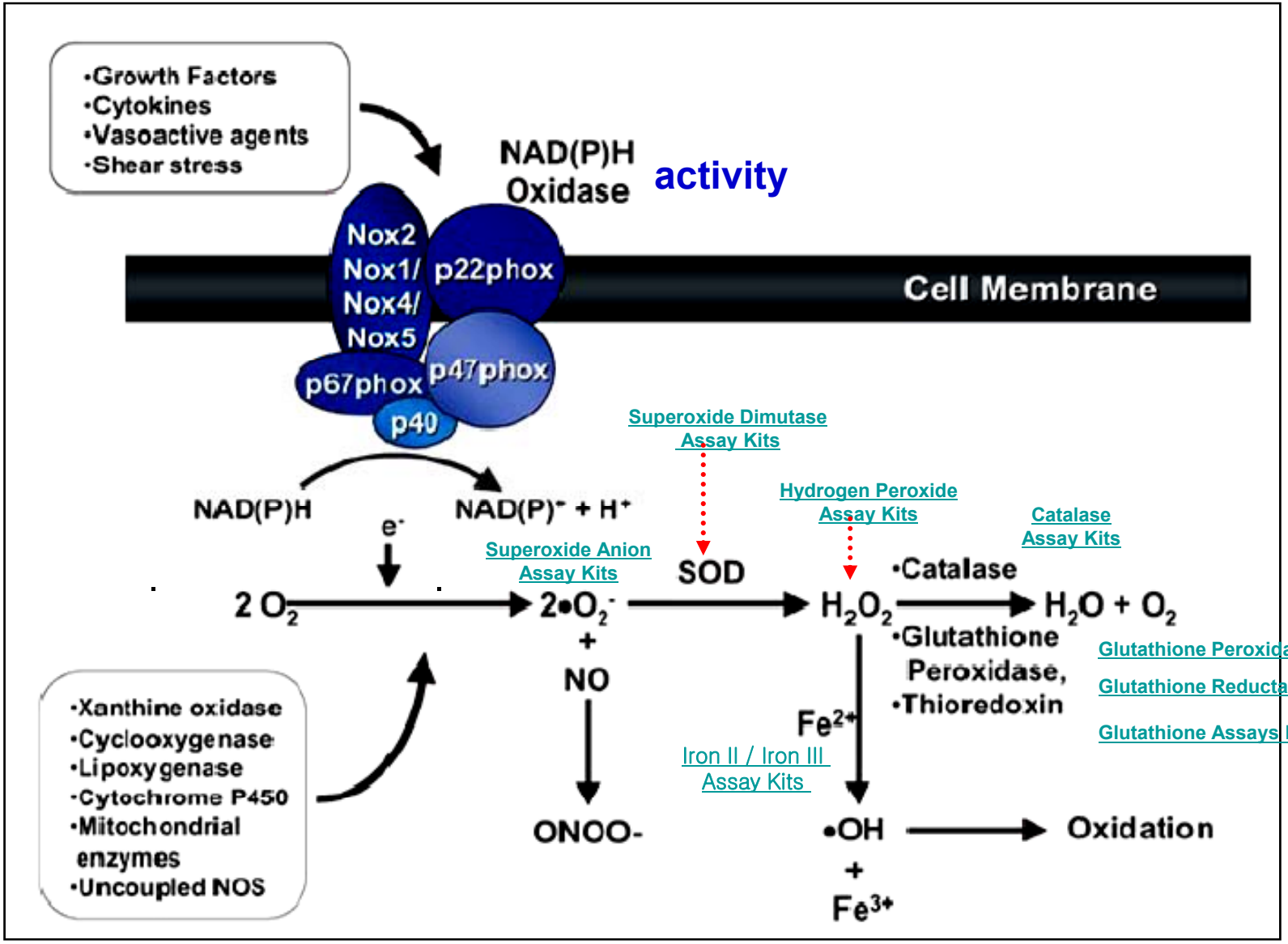
SOD,
Glutathione peroxidase,
Catalase,
Thioredoxin Reductase

- •<u>Non-enzymes</u>

Vitamins,
Thiols,
Small molecules

*SOD: superoxide dismutase

Oxidative Stress/Free Radical Assays

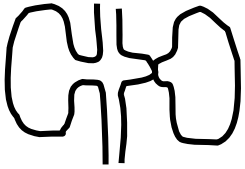


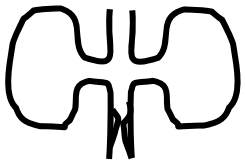
Monoamine Oxidase Assay Kits | hROS Detection Kits | Protein Oxidation Assay Kits | Lipid Hyperoxide Assay Kits | Aconitase Assay Kits

SSAO (Semicarbazide-Sensitive Amine Oxidase) Assay Kits | Malondialdehyde (MDA) Assay Kits | Tissue Hypoxia Assay Kits

Oxidative Stress
in Experimental Study

Myocardial fibrosis experimental model (Aldosterone-salt Hypertension)

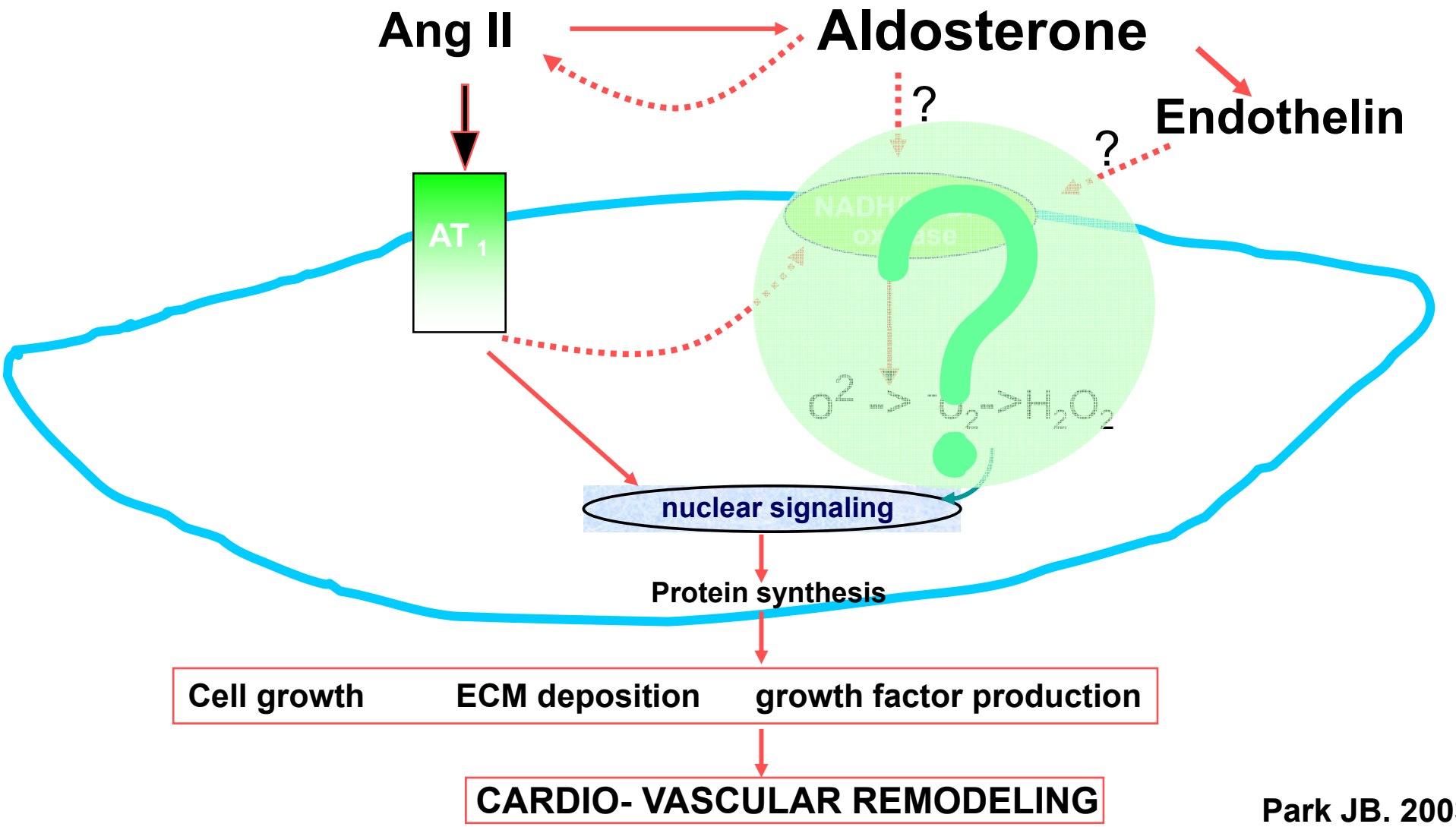




	HBP	LVH	Fibrosis	Artery
Ang II ↑ Aldo ↑	+	+	+	hypertrophic
Ang II → Aldo ↑	+	+	+	hypertrophic
Ang II → Aldo →	+	+	−	eutrophic

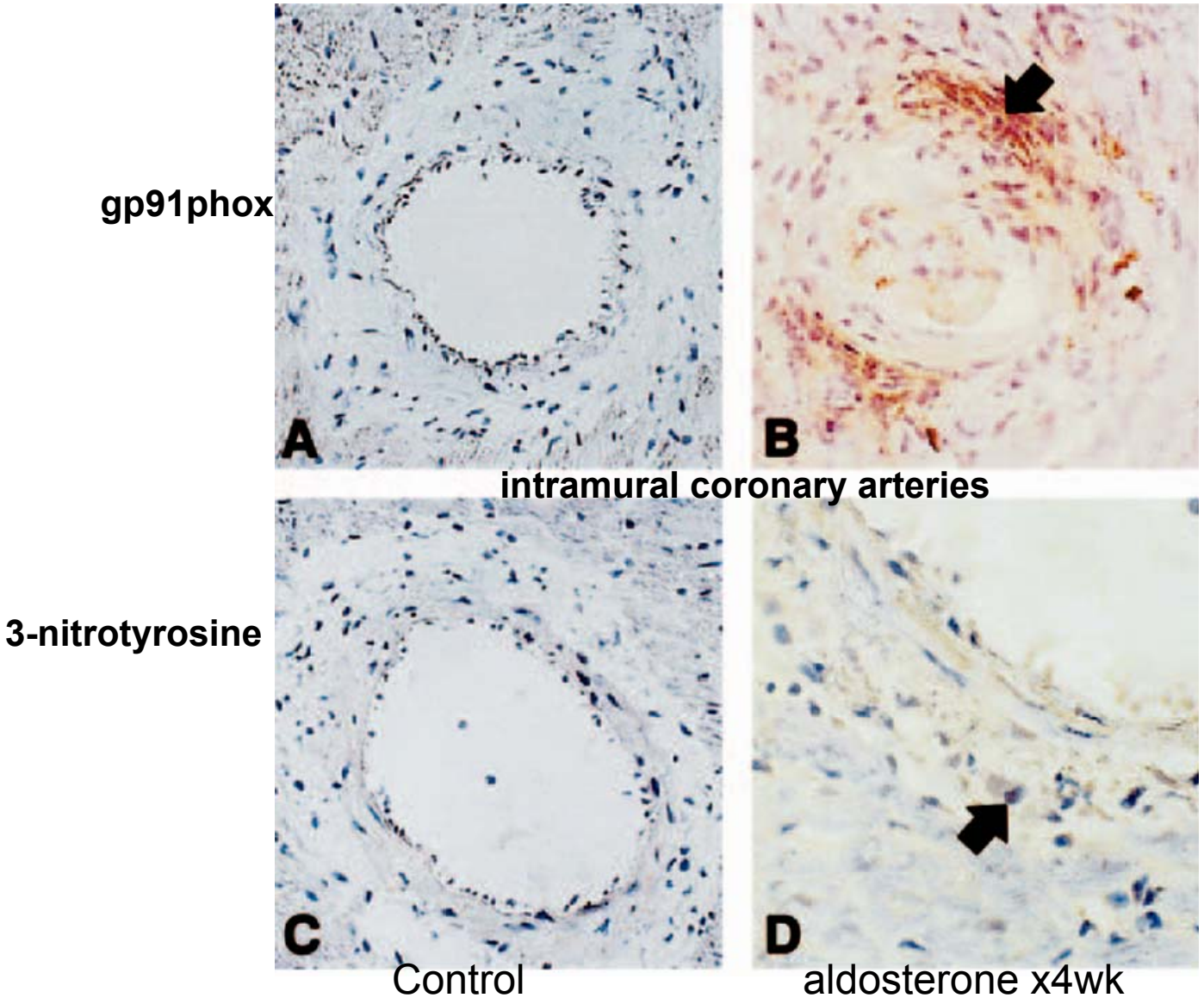
Modified from Weber K group.

Possible Pathway of Aldosterone on CV remodeling



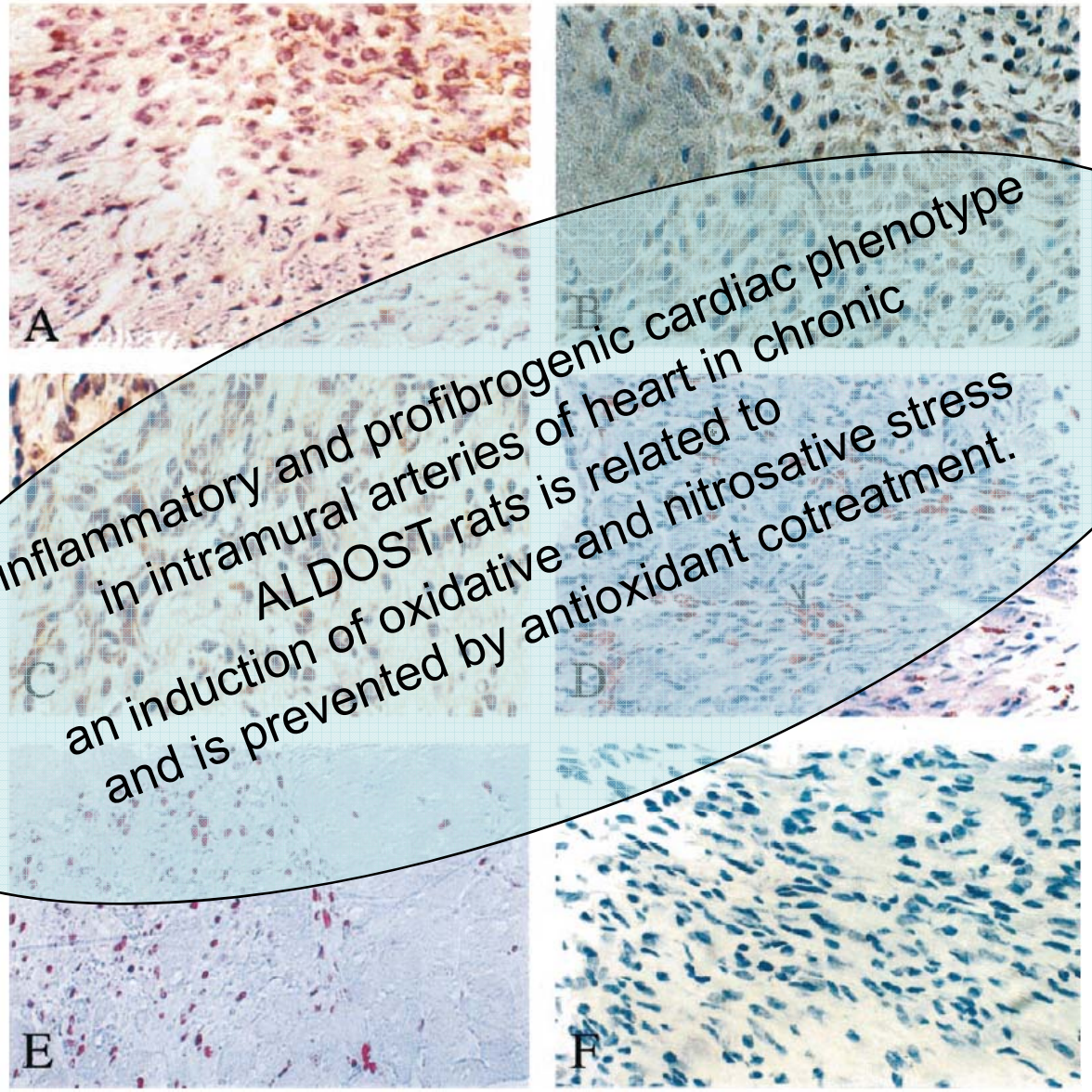
Park JB. 2003

Immunohistochemical evidence of gp91^{phox} expression & presence of 3-nitrotyrosine at *week 4* of ALDOSTERONE



Gerling IC et al.
AJP Heart Circ Physiol
2003

Aldosterone-Induced Inflammation in the Rat Heart
Role of Oxidative Stress



NADPH Oxidase Expression
gp91phox

3-Nitrotyrosine Staining

Activated NF-κB

ED-1-positive macrophages

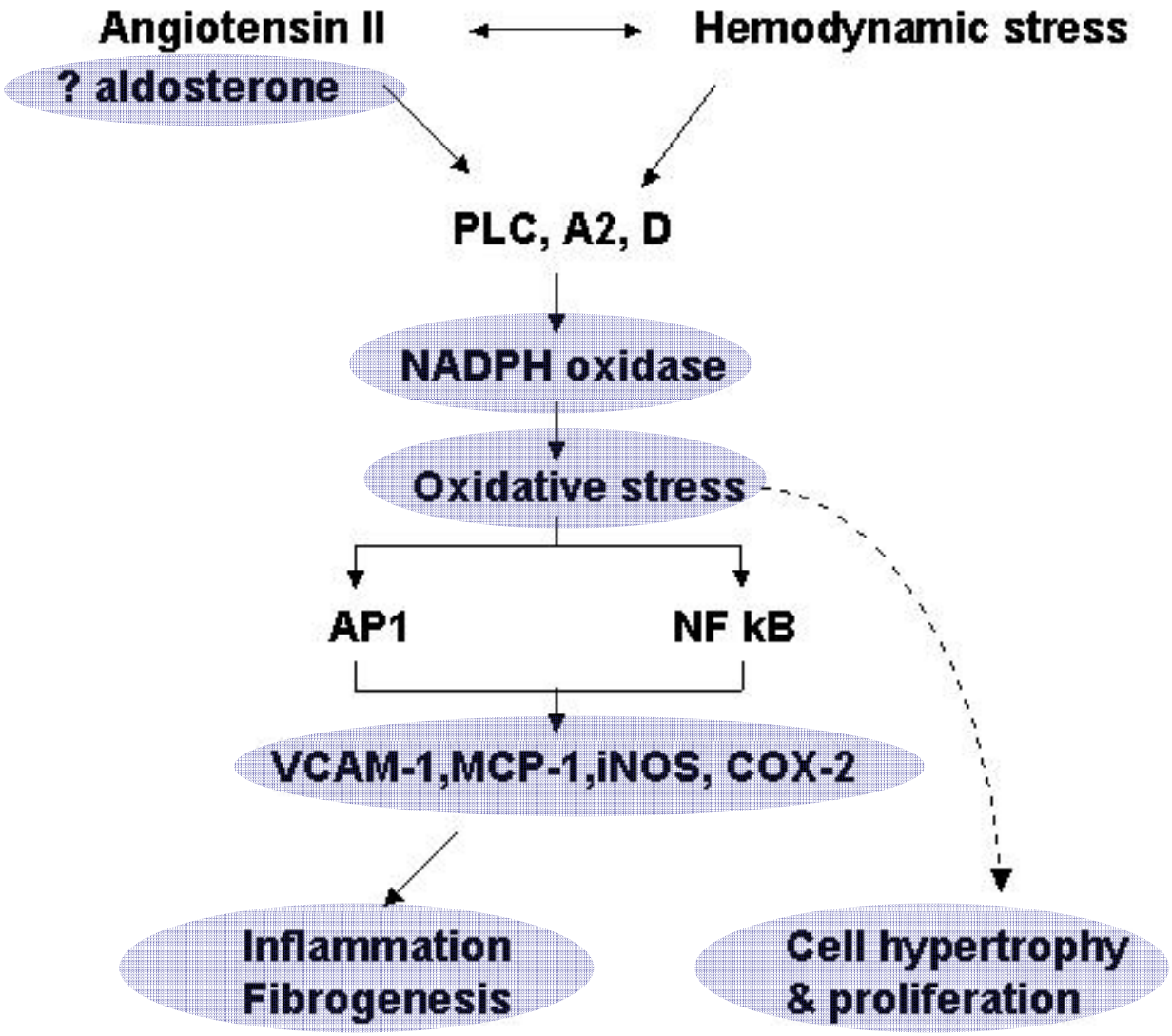
BrdU-positive cells

Negative control

Proinflammatory and profibrogenic cardiac phenotype in intramural arteries of heart in chronic ALDOST rats is related to an induction of oxidative and nitrosative stress and is prevented by antioxidant cotreatment.

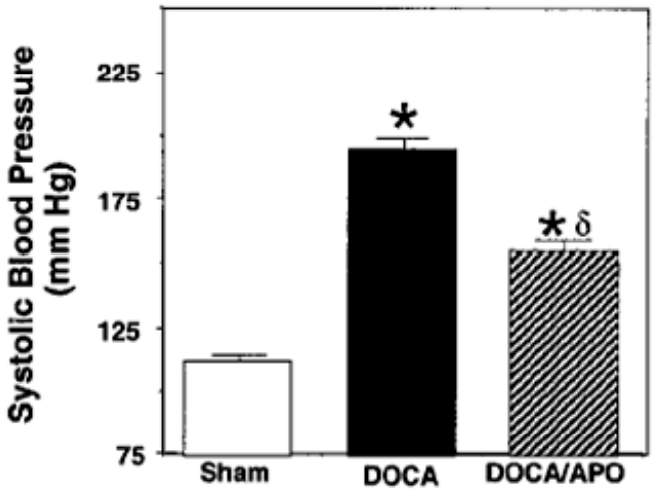
Sun Y et al.
Am J Pathol 2002

Relationship between aldosterone, and oxidative stress, inflammation, fibrosis

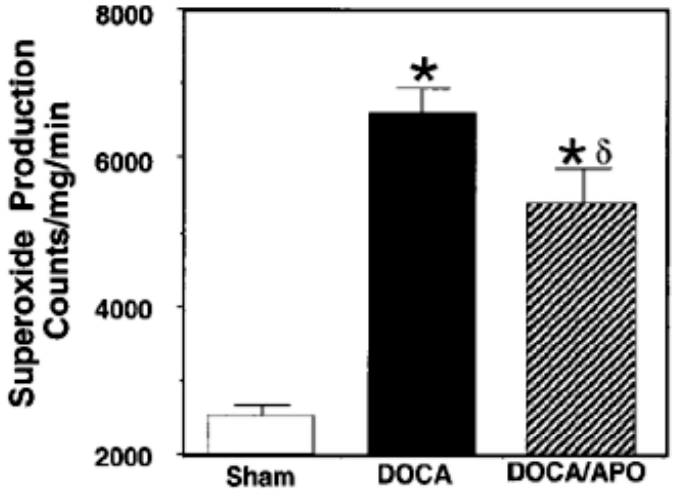


NAD(P)H Oxidase and p22^{phox} In DOCA Hypertension

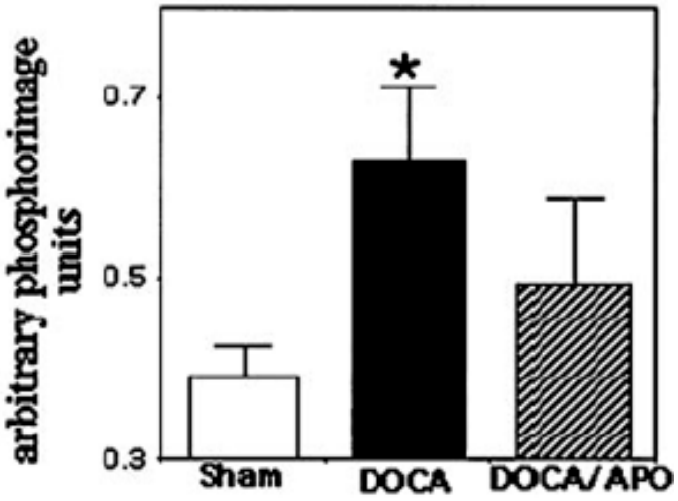
Blood pressure



Superoxide production

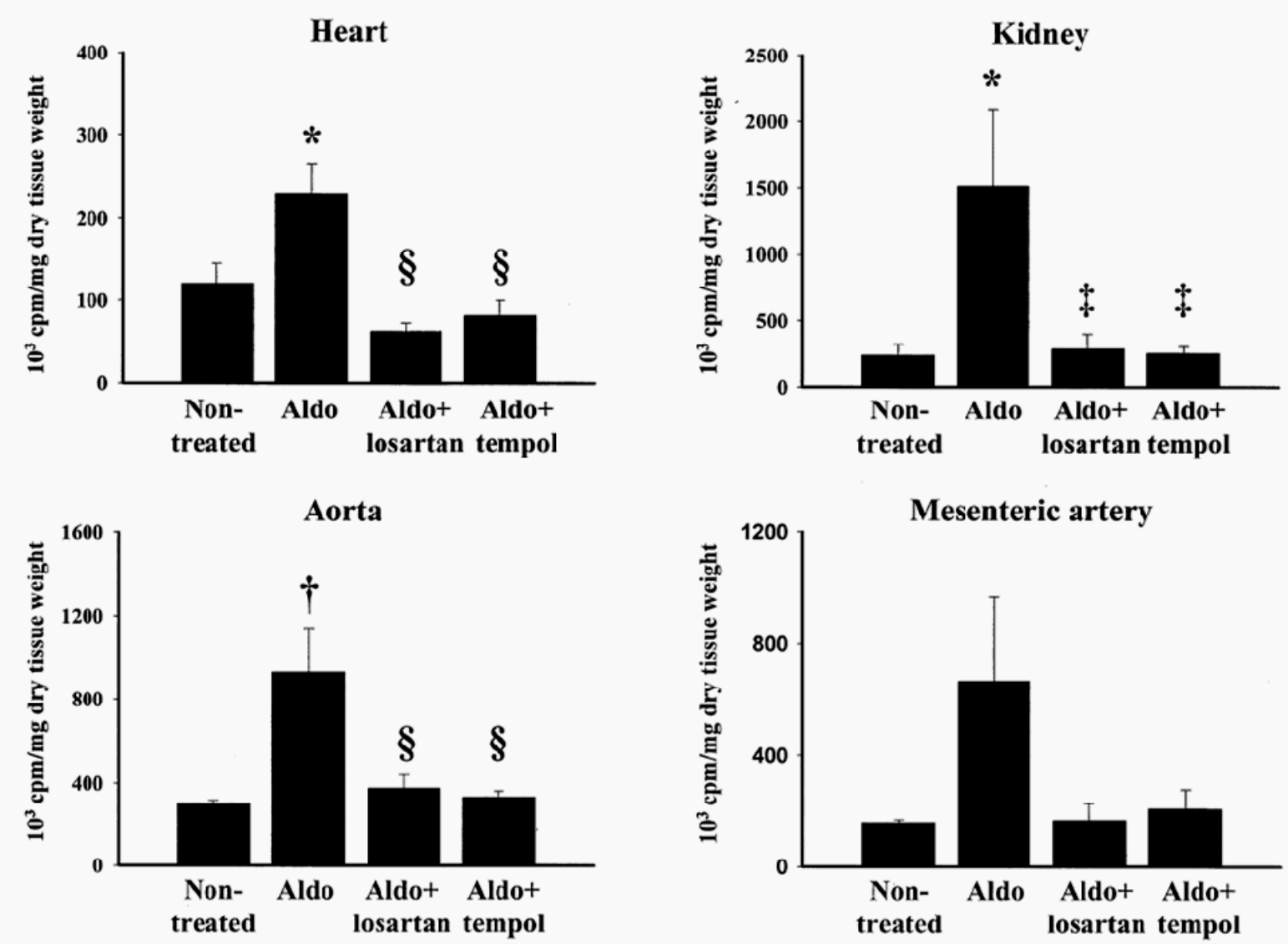


p22^{phox} mRNA



Beswick RA et al. Hypertension 2001

Effect of aldosterone on NA(D)PH oxidase activity in aldosterone-induced fibrosis



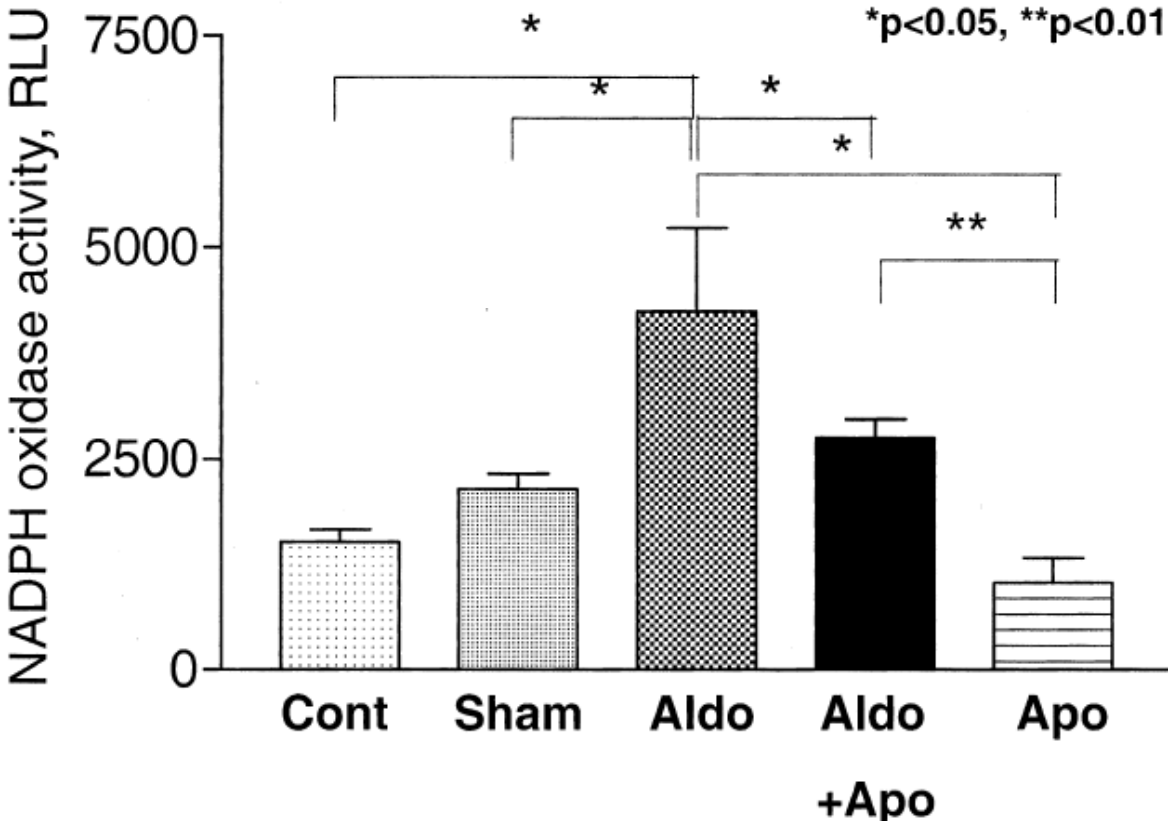
Iglarz M et al. 2004 AJH

NAD(P)H oxidase inhibitor prevents blood pressure elevation and cardiovascular hypertrophy in aldosterone-infused rats☆

Young Mee Park,[a] Mi Young Park,[a] Yeon-Lim Suh,[b] and Jeong Bae Park[a,*]

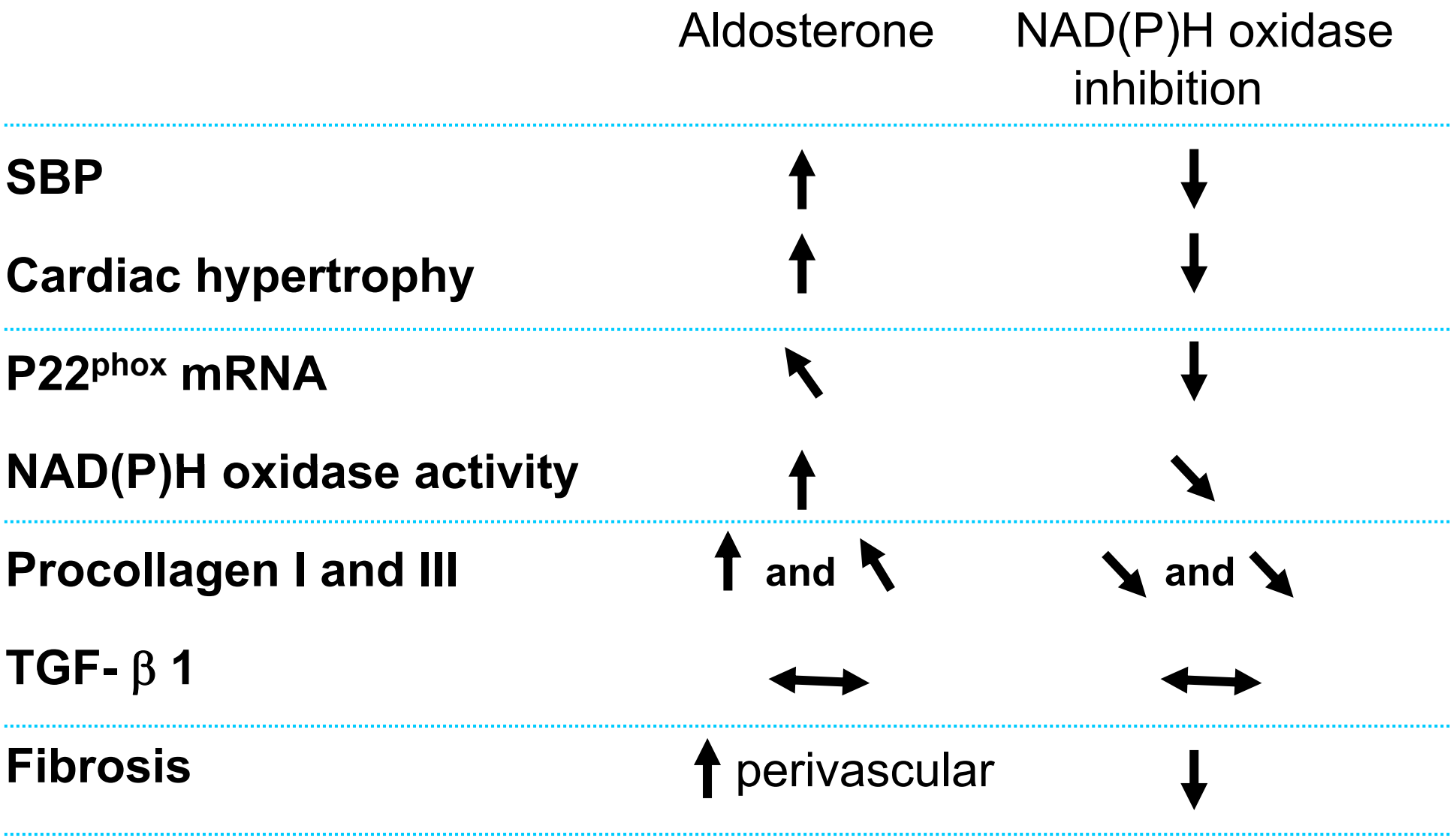
[a] *Department of Medicine/Cardiology, Samsung Cheil Hospital, Sungkyunkwan University School of Medicine and Samsung Biomedical Research Institute, Seoul, Republic of Korea*

[b] *Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea*

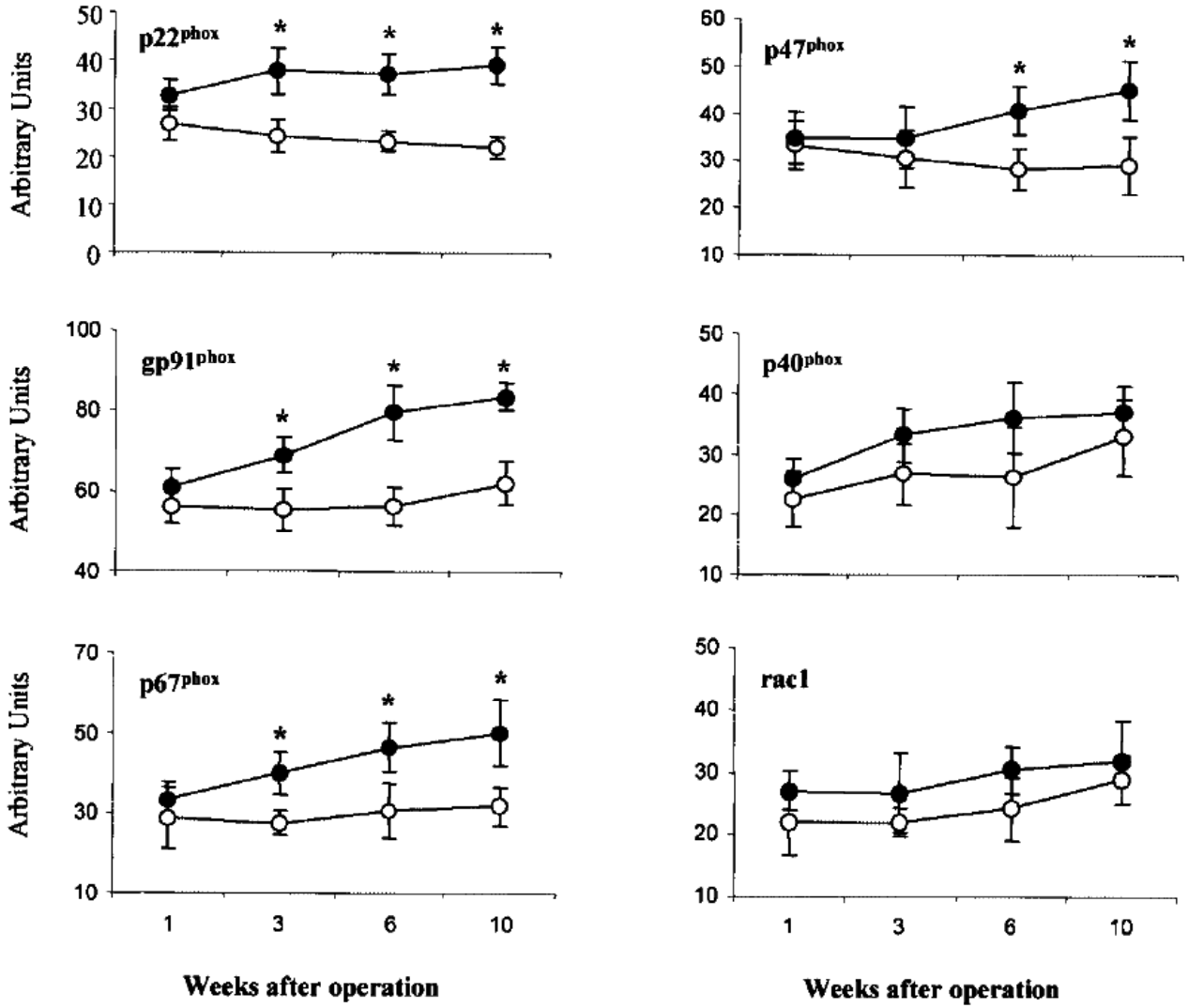


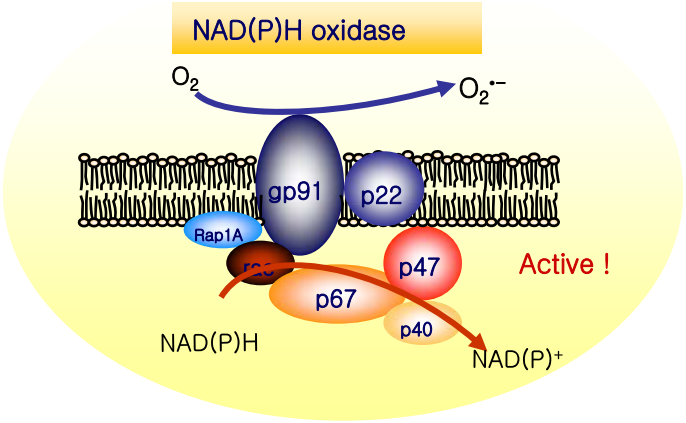
- BBRC 2004

SUMMRY: NAD(P)H oxidase and cardiac fibrosis in aldosterone-salt rats



Changes in protein expression of $p22^{phox}$, $gp91^{phox}$, $p67^{phox}$, $p47^{phox}$, $p40^{phox}$, and rac1 during LVH progression in guinea pig

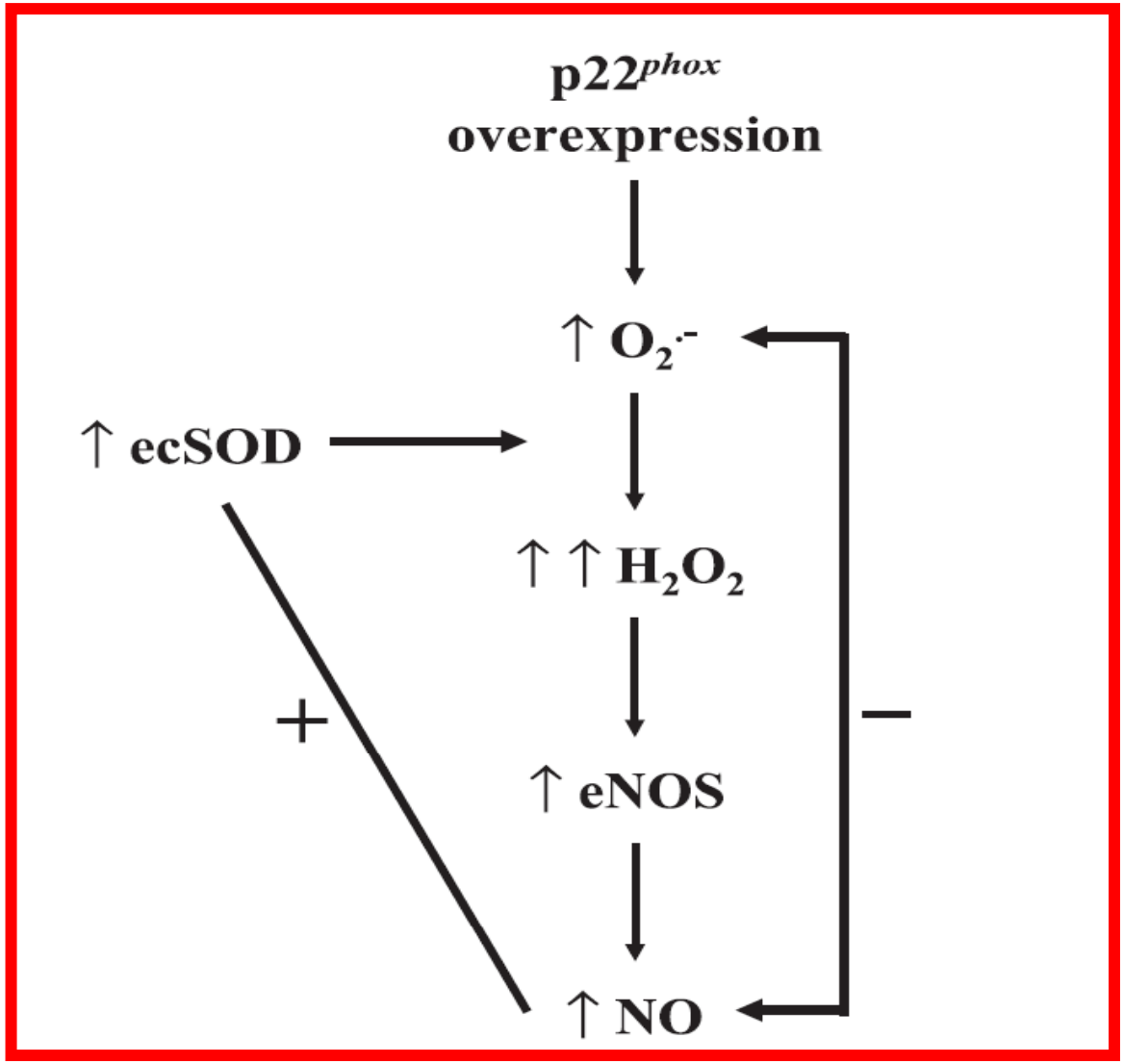




SHAM
LVH

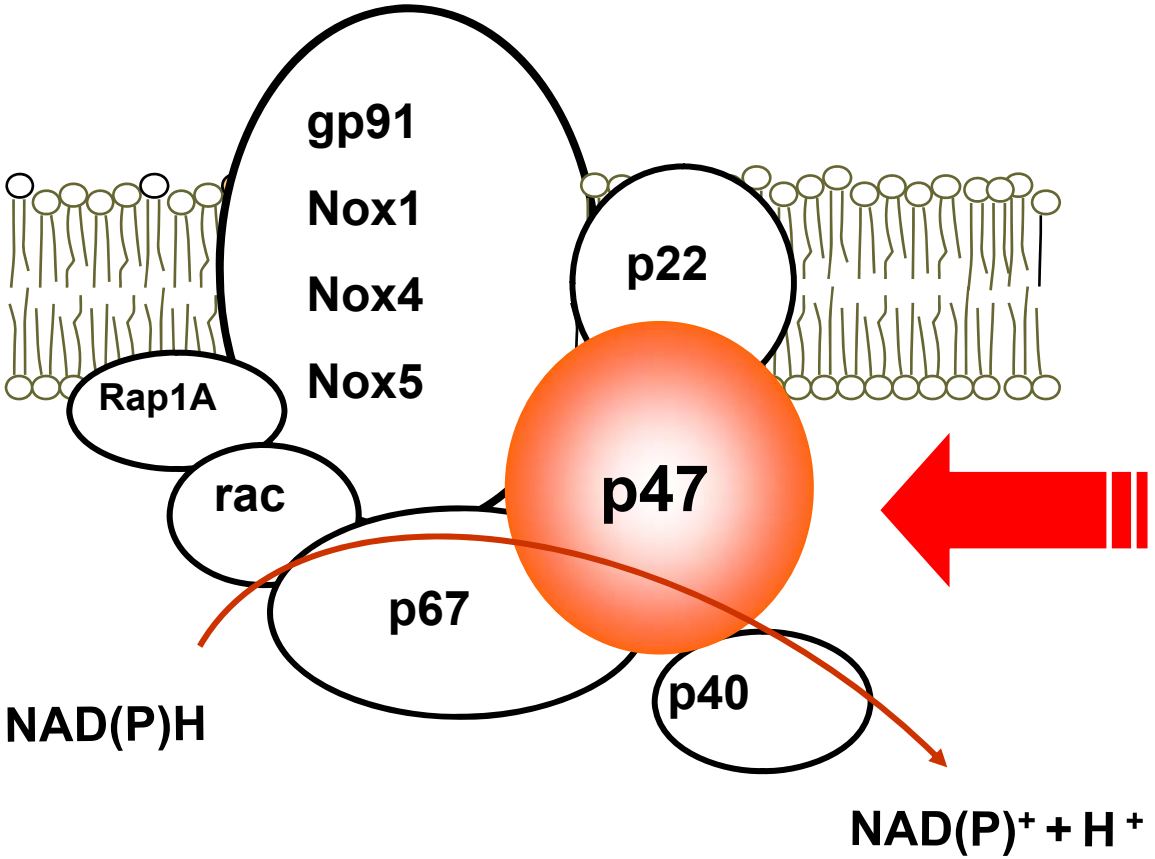
Li JM et al. Hypertension 2002

Proposed compensatory pathway in response to chronic vascular oxidative stress.



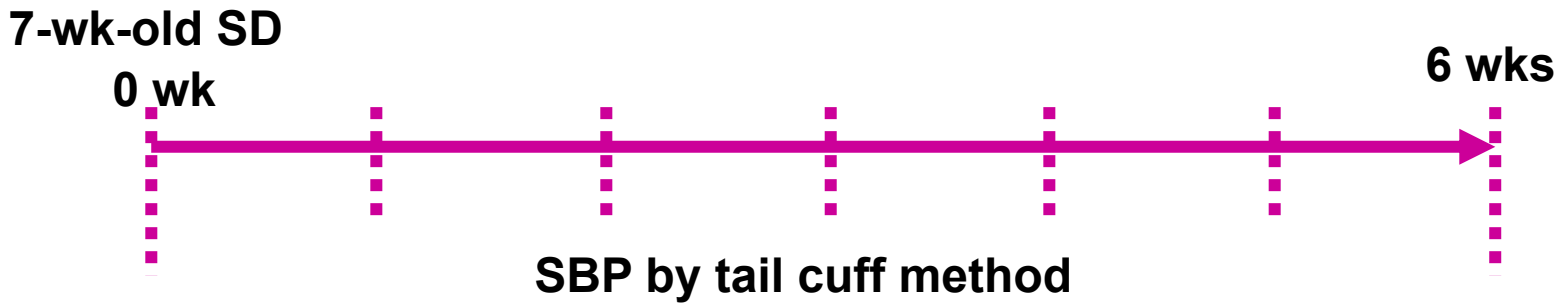
Laude K et al.
AJP Heart Circ Physiol 2005

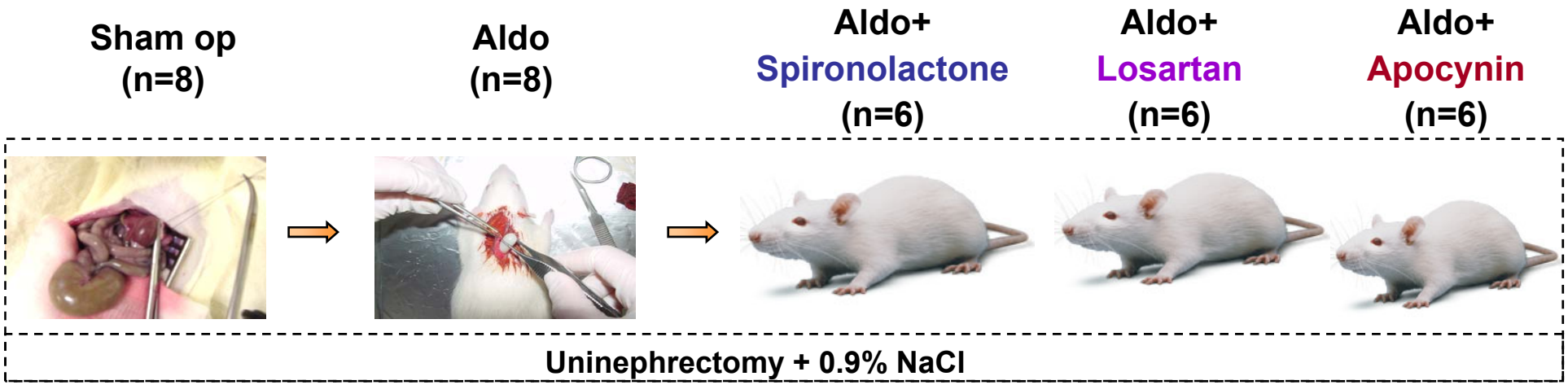
Association of aldosterone with NAD(P)H oxidase subunits ($p47^{phox}$)?



Role of NAD(P)H oxidase subunit p47^{phox} on hypertension and aortic, cardio-renal hypertrophy in aldosterone-infused rats

Young Mee Park and Jeong Bae Park





2005 AHA

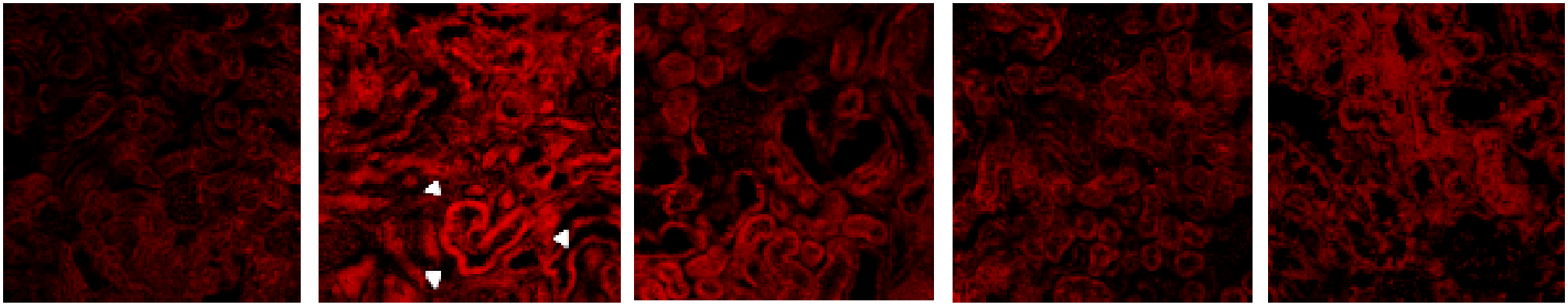


Fig 3. Aldosterone-indused superoxide (O2-) production is attenuated in kidney of spironolactone, losartan and apocynin treated rats. O2- levels were determined by fluorescent dihydroethidium (DHE[10μmol/L]) and visualized by confocal microscopy. Arrow indicates intense O2- production.

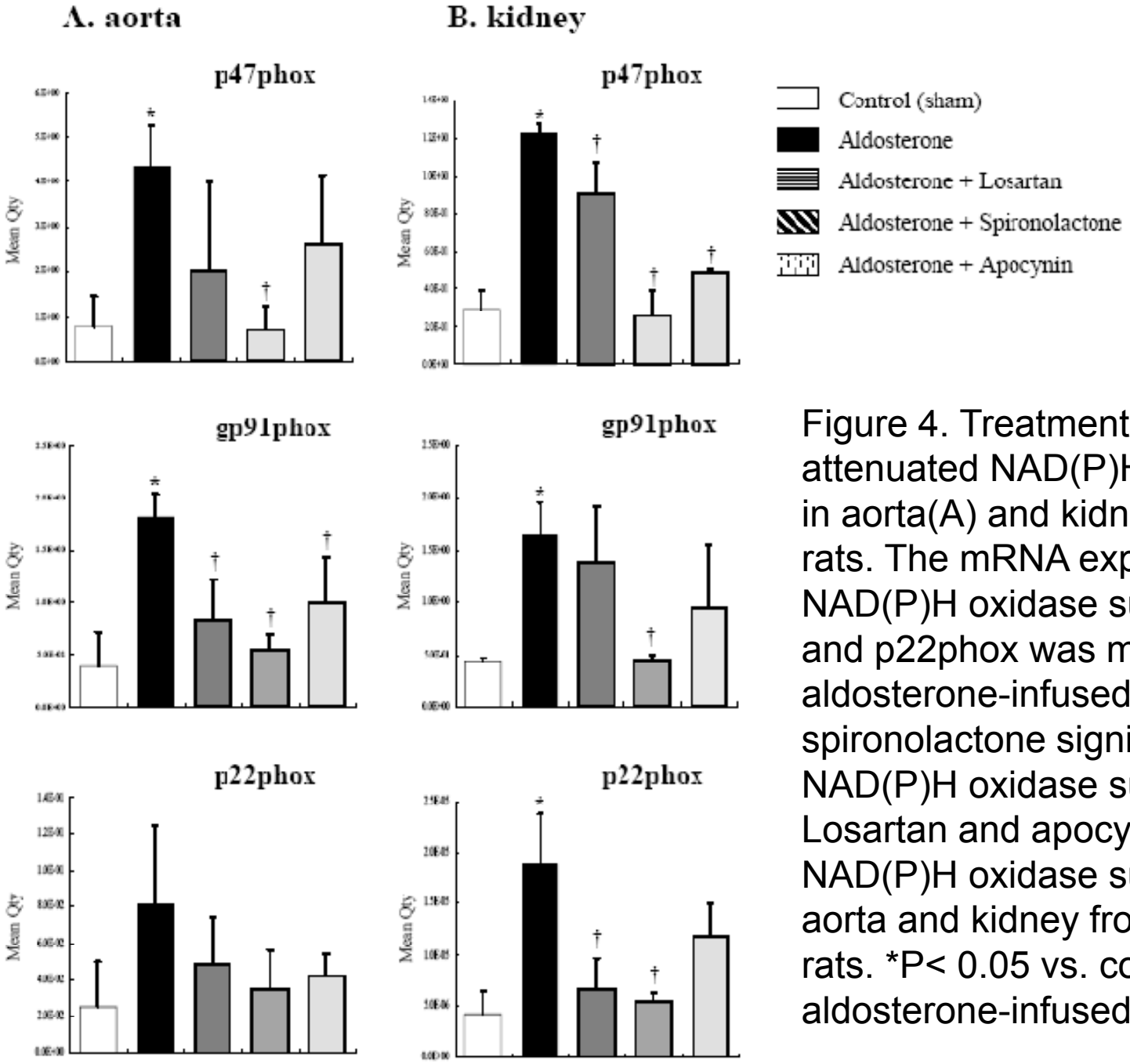


Figure 4. Treatment with spironolactone attenuated NAD(P)H oxidase mRNA expression in aorta(A) and kidney(B) of aldosterone-infused rats. The mRNA expression of the NAD(P)H oxidase subunits p47phox, gp91phox and p22phox was markedly increased in aldosterone-infused rats. The treatment with spironolactone significantly reduced NAD(P)H oxidase subunits mRNA expression. Losartan and apocynin decreased on NAD(P)H oxidase subunits mRNA expression in aorta and kidney from aldosteroneinfused rats. *P< 0.05 vs. control rats; †P<0.05, vs. aldosterone-infused rats.

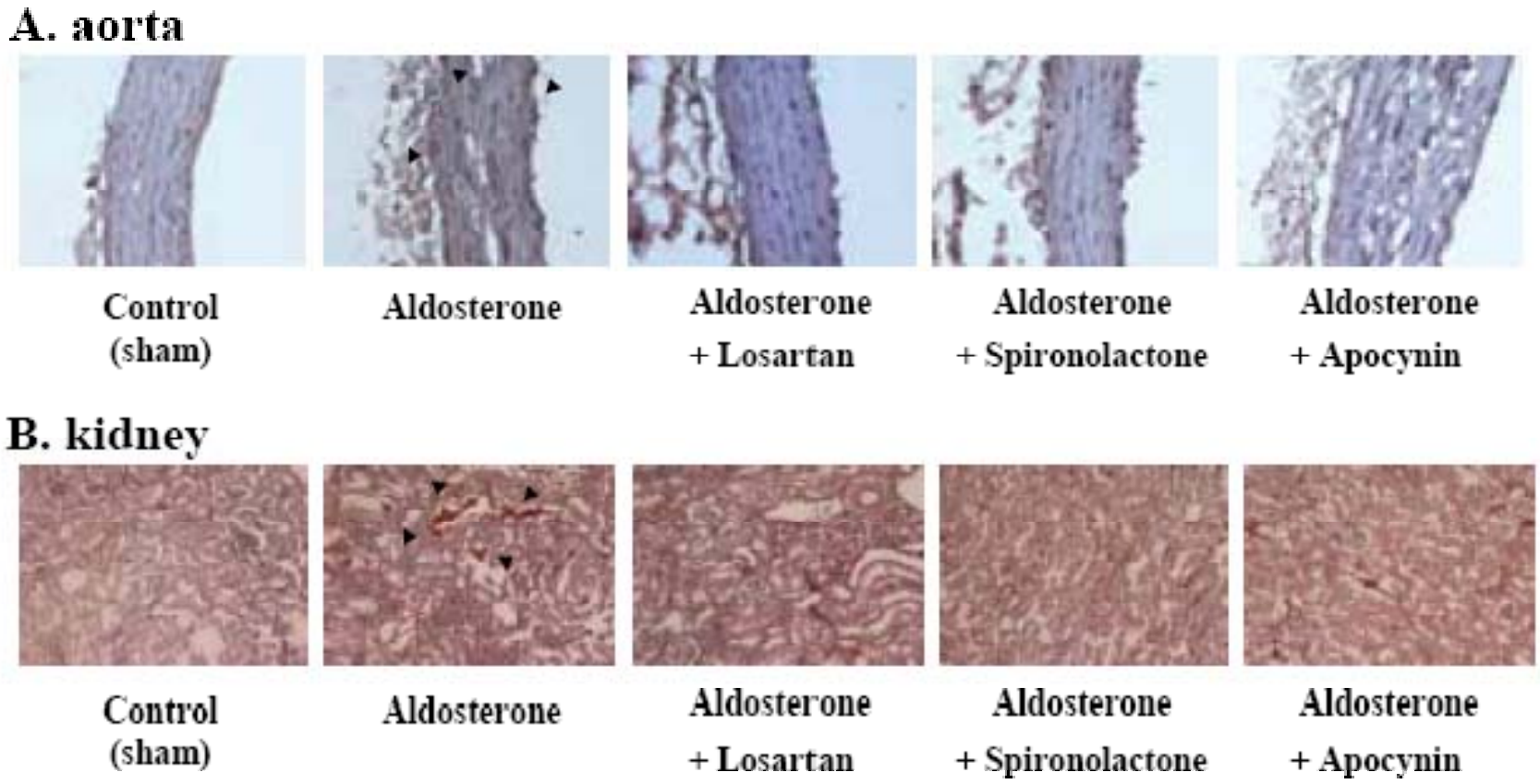
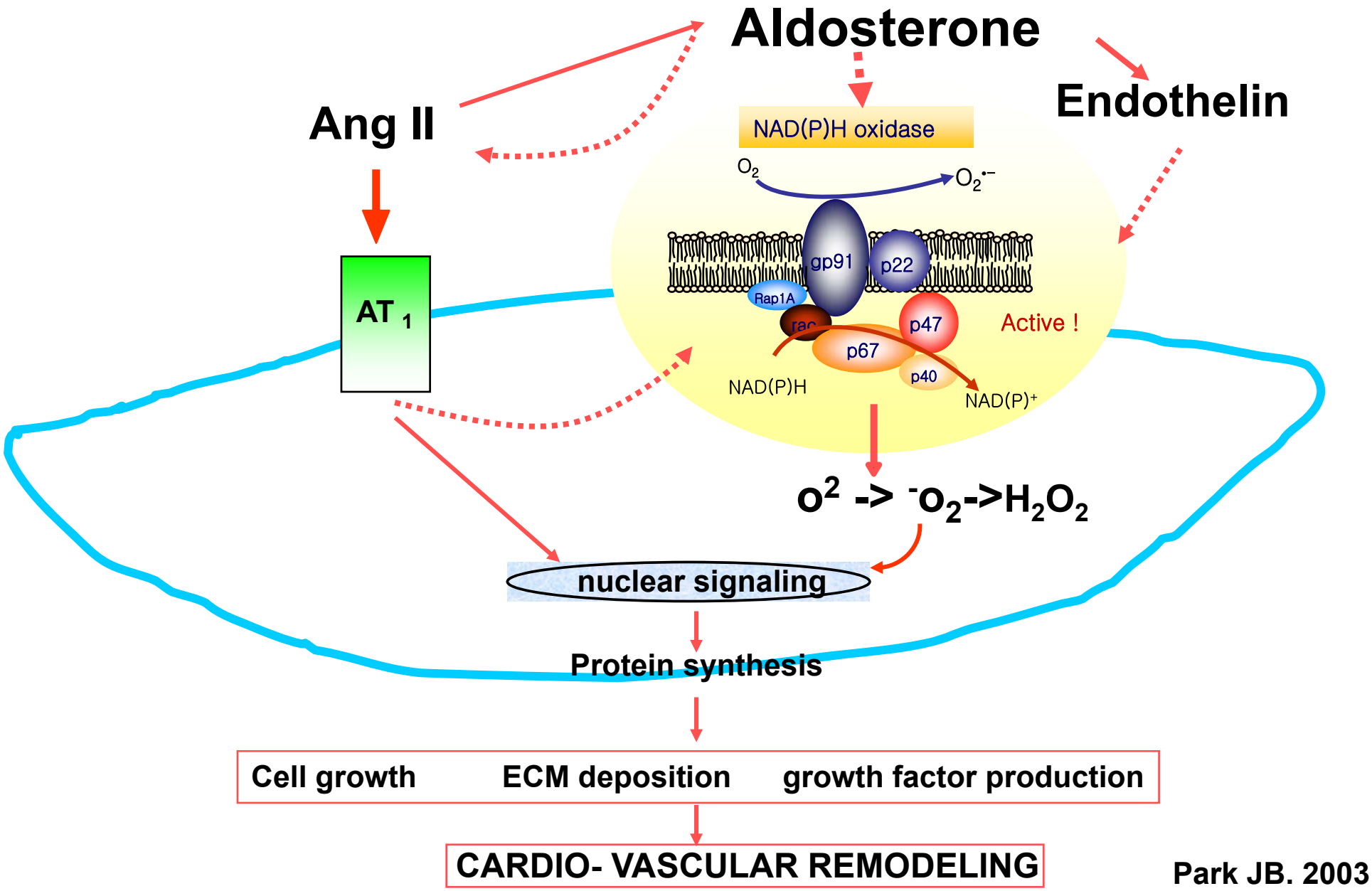


Fig 5. Immunostaining for NAD(P)H oxidase subunit, p47phox in aorta(A) and kidney(B) of control rats, aldosterone-salt rat, aldosterone-salt rat treated with losartan, spironolactone and apocynin. A. The expression of p47phox was increased in aorta of aldosterone-infused rats. Spironolactone significantly decreased p47phox expression in aorta of aldosterone-infused rats. B. The expression of p47phox was increased in kidney aldosterone-infused rats. Spironolactone significantly decreased p47phox expression in aorta of aldosterone-infused rats.


Aldosterone
Ang II
Endothelin
NAD(P)H oxidase
O_2
$O_2^{\bullet-}$
gp91
p22
Rap1A
rac
p47
p67
p40
Active !
NAD(P)H
NAD(P)+
AT 1
o^2 -> $^{-}o_2$->H_2O_2
nuclear signaling
Protein synthesis
Cell growth
ECM deposition
growth factor production
CARDIO- VASCULAR REMODELING

Park JB. 2003

Summary. Aldosterone and NAD(P)H oxidase

1. Aldosterone infusion into normotensive rat significantly increased systolic BP and cardio-renal hypertrophy.
2. Aldosterone increased NADPH oxidase activity ≈ 56% in aorta

→ Aldosterone increases blood pressure and aortic, cardiac, and renal hypertrophy through activation of NAD(P)H oxidase in rat

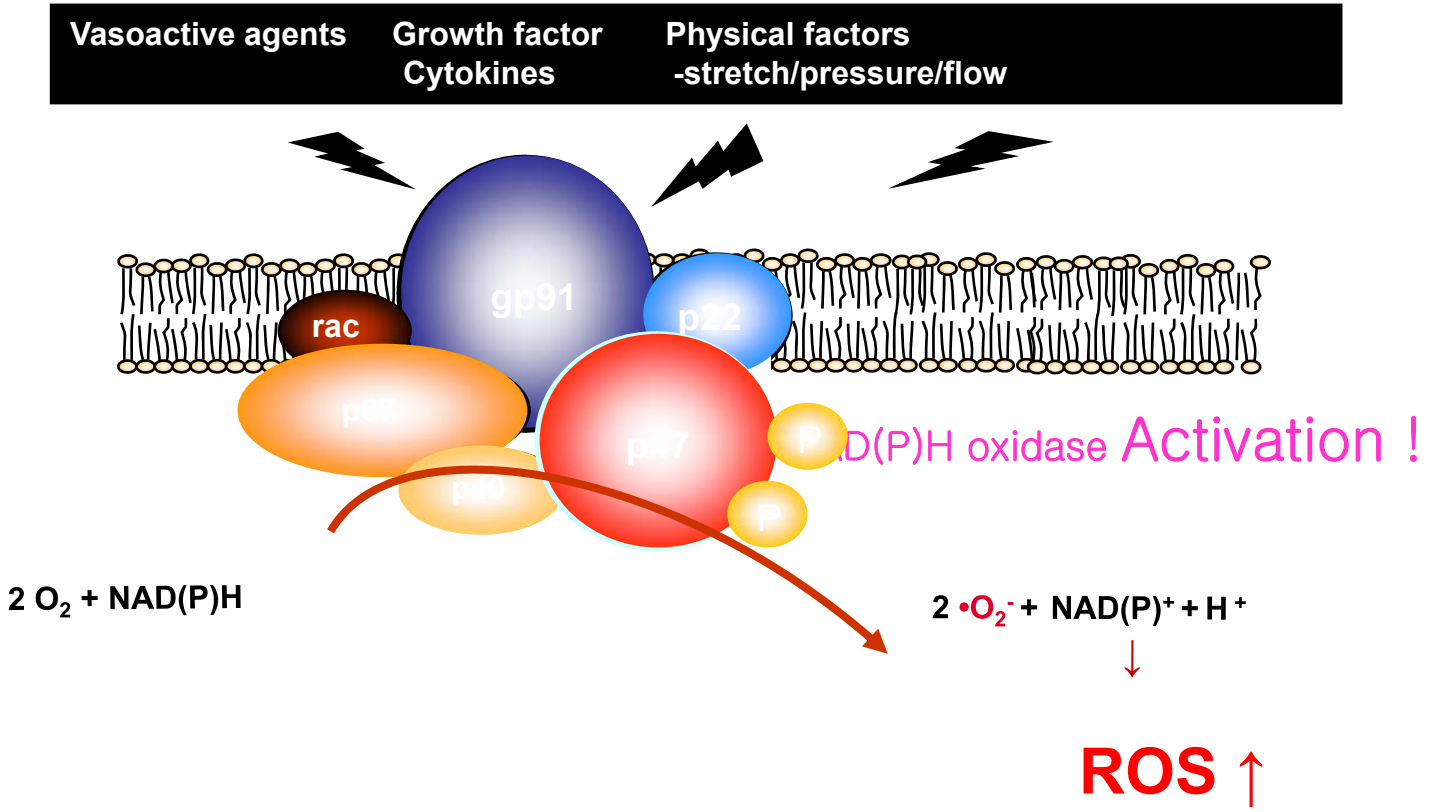
3. Aldosterone significantly increased the expression of $p22^{phox}$, $p47^{phox}$ mRNA of aorta and kidney. NAD(P)H oxidase inhibitor *trended to decrease*.

→ NAD(P)H oxidase inhibitor suggests a good treatment model for prevention or reversal of TOD in CV disorder.

Time course changes of blood pressure, cardio-renal hypertrophy and vascular changes in p47^{phox}-deficient mice

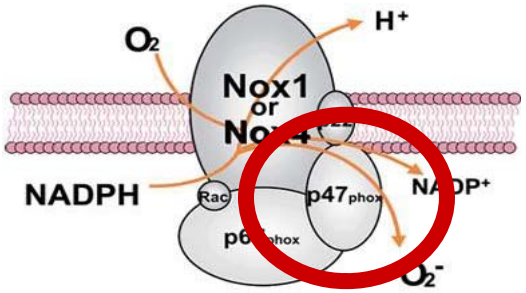
Young Mee Park, Bong-Hee Lim, Jeong Bae Park

Cheil Hospital and Sungkyunkwan University School of Medicine



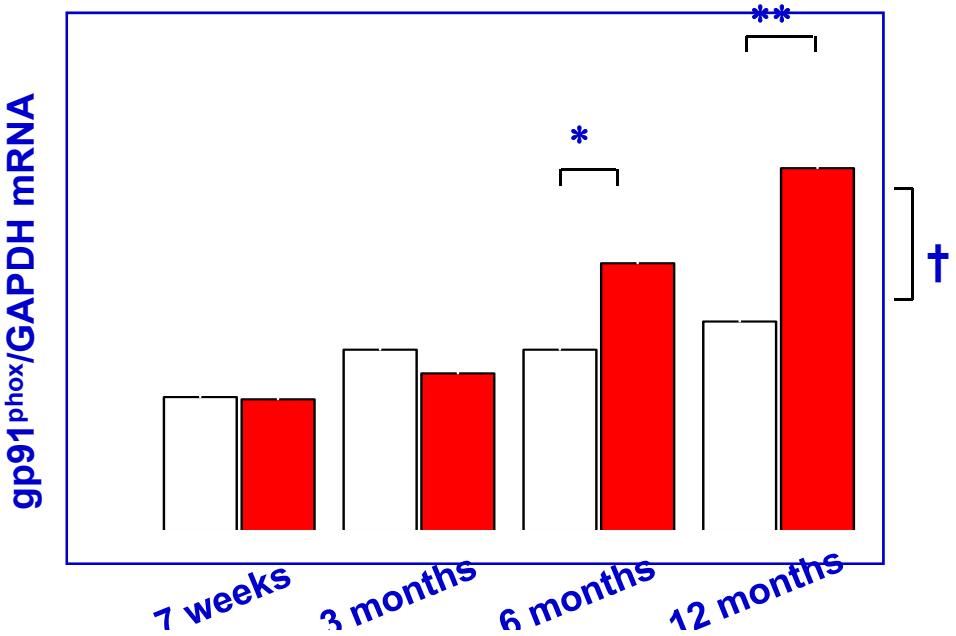
SUMMARY

Natural changes of P47$^{phox-/-}$ (vs. WT mice)



- ✓ On BP;
- ✓ On cardio-renal hypertrophy;
- ✓ On vascular function ;
- ✓ On NADPH oxidase activity and mRNA expression

gp91$^{phox-/-}$



Oxidative Stress in Clinical Study

Oxidative stress marker

1. Oxidative damage;

- Oxidative DNA Biomarkers
  - Urinary 8-OHDG (8-hydroxy-2'-deoxyguanosine) ELISA : most sensitive
  - 8-OHDG ELISA
- Oxidative Lipid Biomarkers; Oxidative damage to lipids (lipid peroxidation)
  - Lipid Hydroperoxide (LOOH) Assay
  - Acrolein (ACR) -Lysine ELISA
  - Hexanoyl-Lysine (HEL) ELISA
  - Malondialdehyde (MDA) Assay
  - 8-Isoprostane ELISA Assay
  - Urinary 8-Isoprostane Assay
  - Oxidized Low Density Lipoprotein ELISA (Ox-LDL-EIA Sandwich Format).
  - Oxidized Low Density Lipoprotein ELISA (Ox-LDL-EIA Competitive Format)
- Oxidative/Nitrosative Protein Biomarkers
  - Protein Carbonyl ELISA
  - Nitrotyrosine ELISA

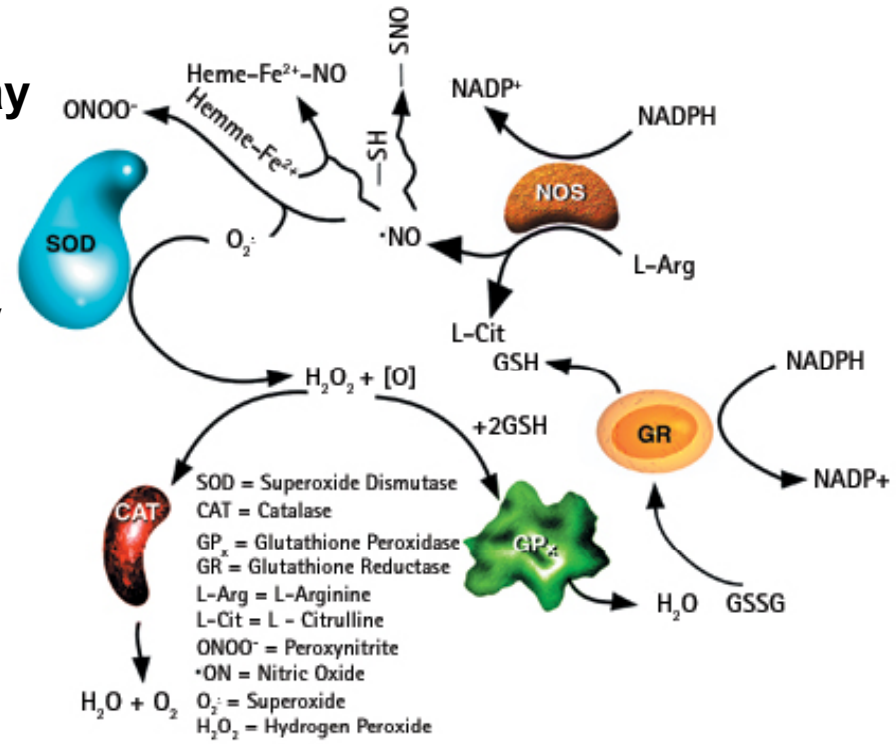
Oxidative stress marker

2. Antioxidant Biomarkers

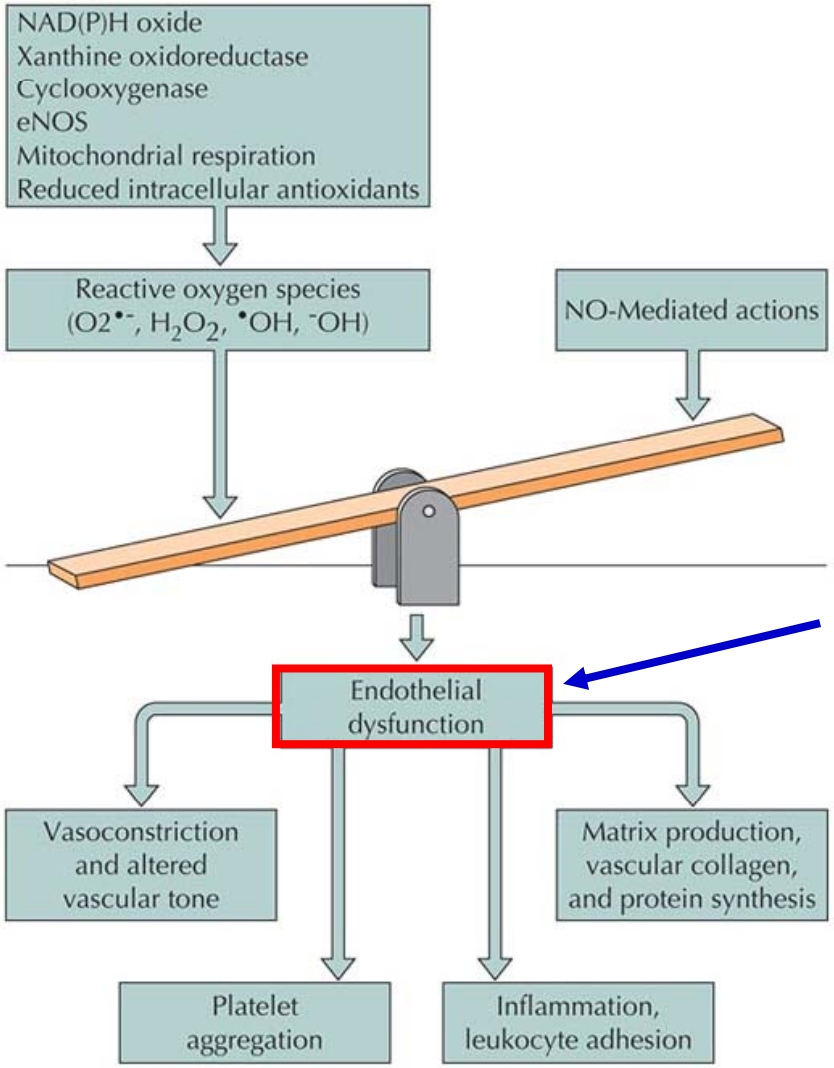
- Total Antioxidant Capacity against Peroxyl Radical (TAC-Peroxyl) Assay
- Antioxidant Reductive Capacity (Antioxidant Status) Assay
- Glutathione Assay
- Glutathione Peroxidase (GPx) Assay
- Glutathione Reductase (GR) (Microplate) Assay
- Glutathione Reductase (GR) (Cuvette) Assay
- Human Cu/ZnSOD ELISA
- Superoxide Dismutase Enzyme Activity Assay
- Catalase Enzyme Activity Assay

3. Inflammatory Biomarkers

- Human Myeloperoxidase (MPO) ELISA
- Myeloperoxidase Activity Assay
- Hydrogen Peroxide Assay



Oxidative stress marker and endothelial function



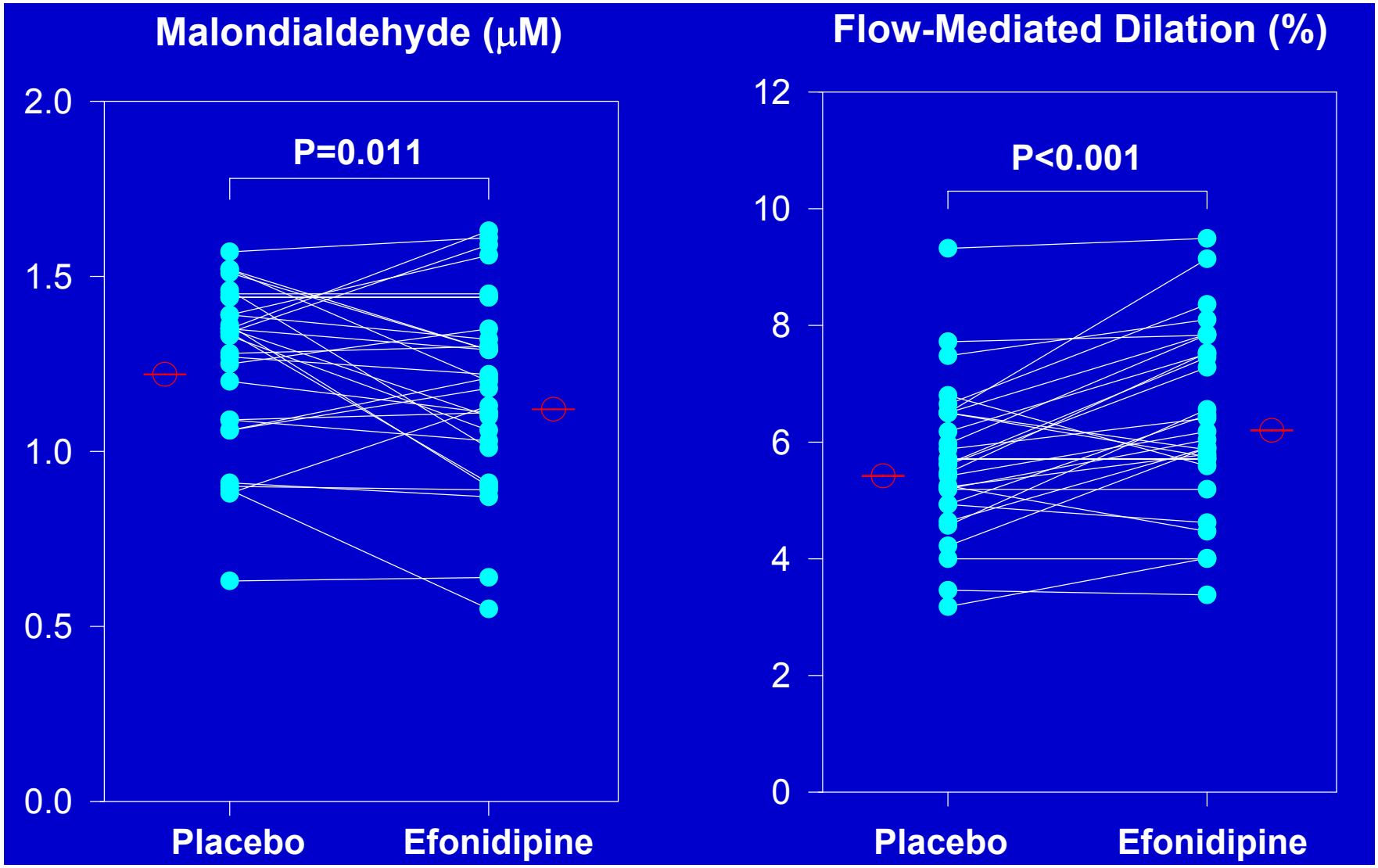
- **brachial arterial flow-mediated dilatation**
- **strain gauge plethysmography**
- **peripheral pulse waveform analysis**
- **LASER-Doppler fluximetry**
- **digital thermal monitoring**
- **invasive intracoronary provocation tests.**

Efonidipine simultaneously improves blood pressure, endothelial function, and metabolic parameters in nondiabetic patients with hypertension.

Diabetes Care. 2007 Jun;30(6):1605-7

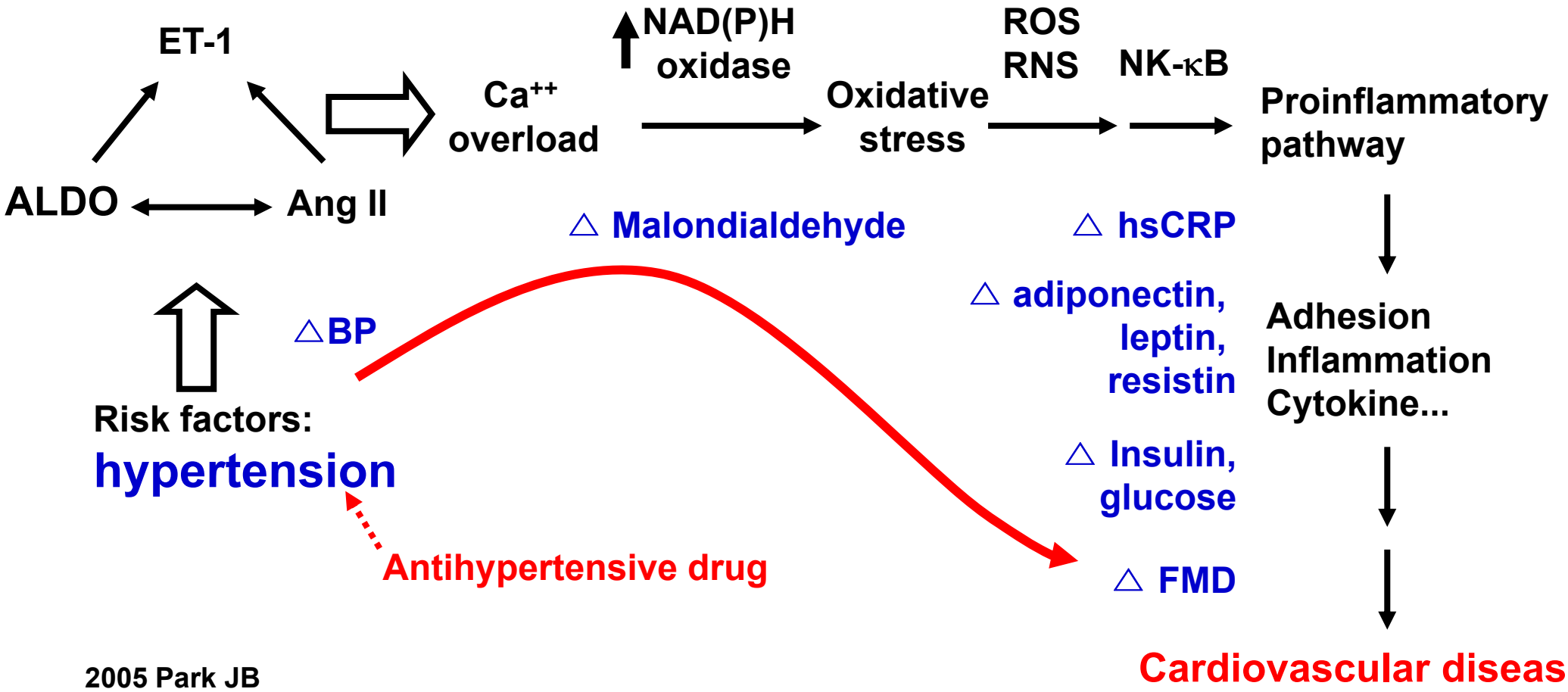
Koh KK, Quon MJ, Lee SJ, Han SH, Ahn JY, Kim JA, Chung WJ, Lee Y, Shin EK.

Effects of Efonidipine on Oxidation and FMD



Koh,.. Han.. et al. Diabetes Care. 2007

Characteristics or pleotrophic effects of antihypertensive drug

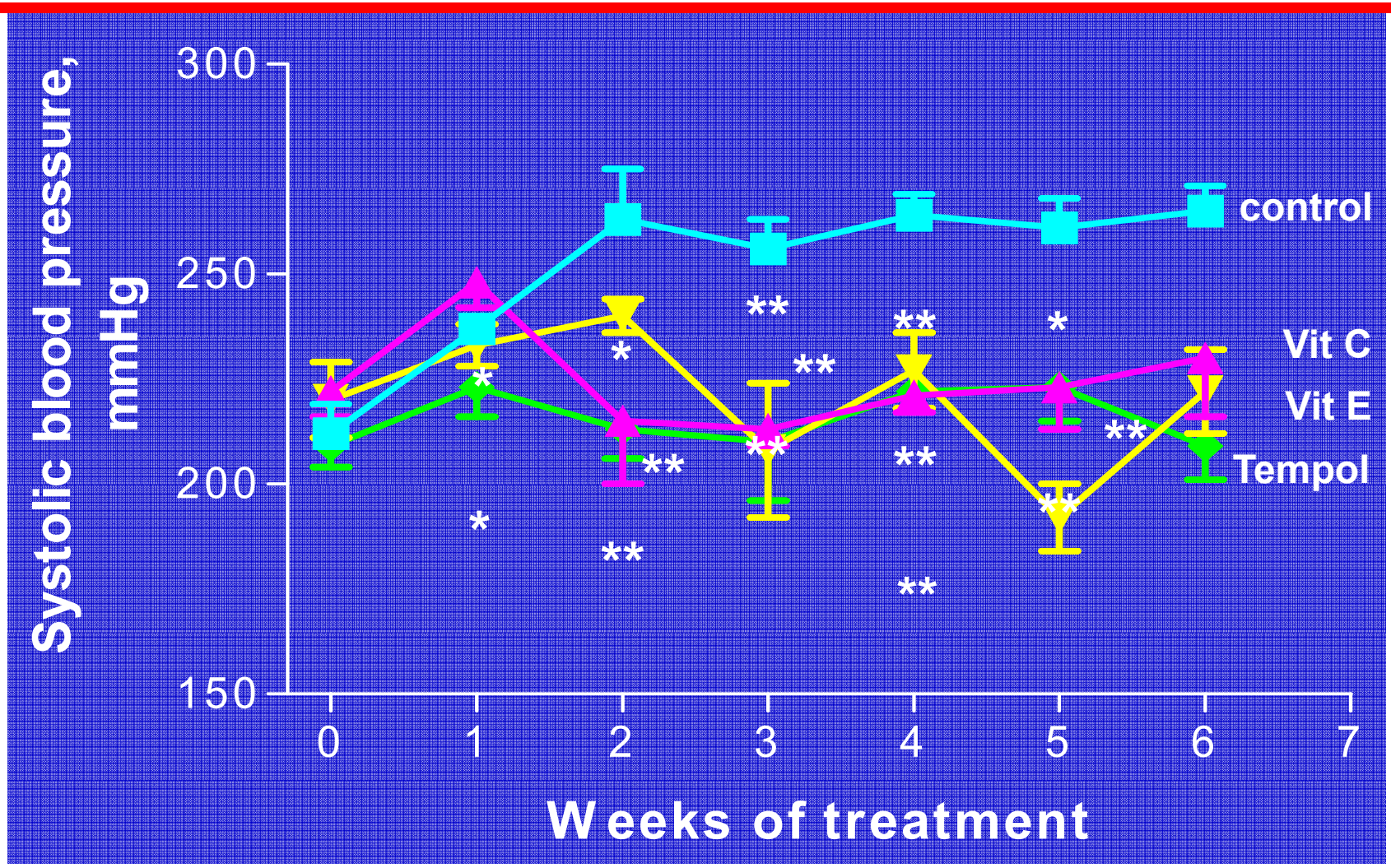


2005 Park JB

Evidence linking ROS and human vascular disease

- ↑ markers of oxidative stress in hypertension and atherosclerosis
- ↓ plasma levels of ROS scavengers in essential hypertension.
- Oxidative stress is increased in patients with diabetes and CKD
- Antioxidants improve renal function and microalbuminuria in CKD
- Ang II ↑ $\bullet O_2^-$ via NAD(P)H oxidase in human arteries and veins
- Association between p22phox polymorphisms and BP stronger in hypertensive vs normotensives

Antioxidants Attenuate Progression of Hypertension in SHRSP.



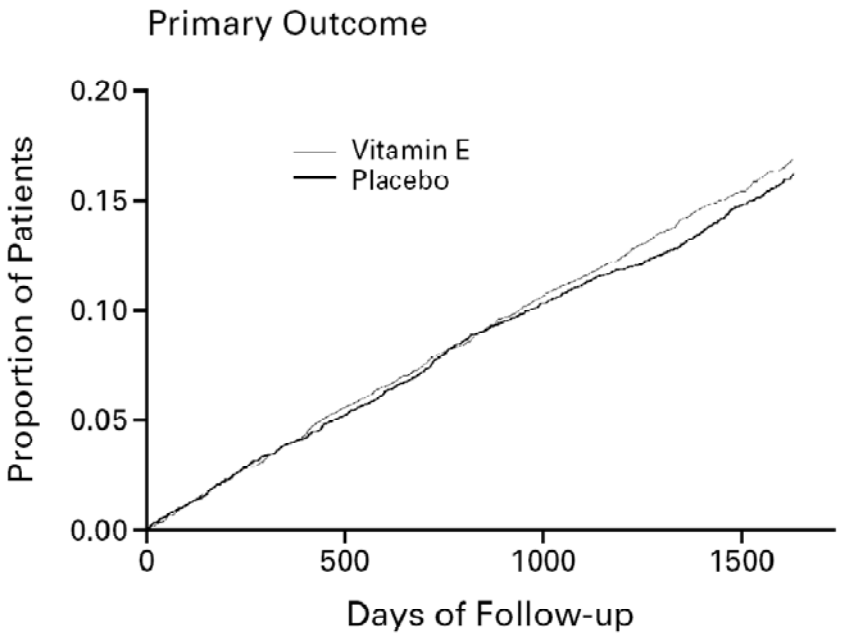
Park et al. Hypertension. 2001;38(2);606-611

Clinical trials

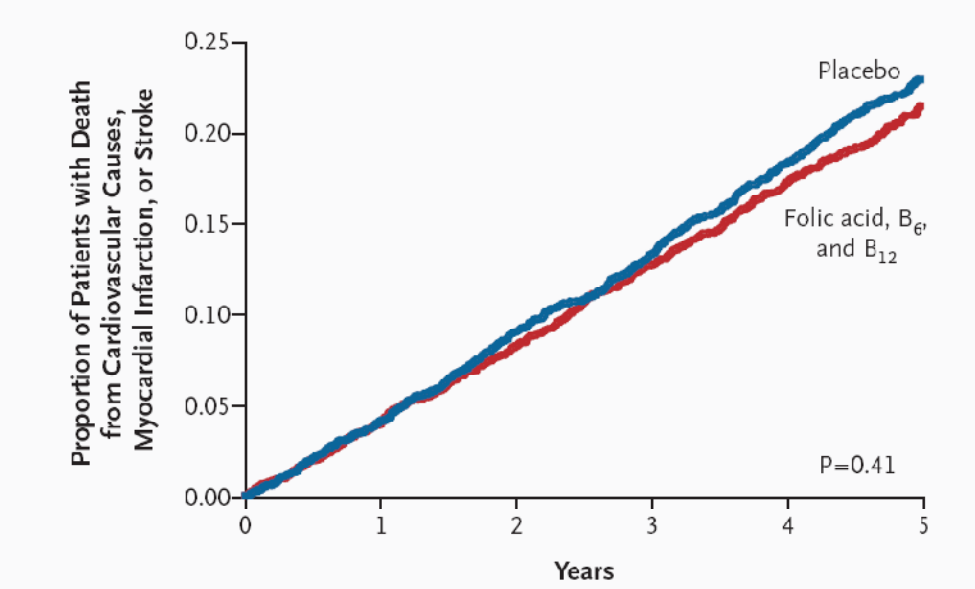
Strategies to Reduce Oxidative Stress in Cardiovascular Disease

- **Increase antioxidants**
- **Decrease ROS generation**

VITAMIN E / Folic acid + VITAMIN B6 and 12 SUPPLEMENTATION AND CARDIOVASCULAR EVENTS IN HIGH-RISK PATIENTS : HOPE study



New England Journal of Medicine 2000; 342(3): 154-160



New England Journal of Medicine 2006; 354: 1567-1577

Antioxidant Clinical Trials

- **Large clinical trials: antioxidant vitamins on risks of cardiovascular disease.**

  - Cambridge Heart Antioxidant Study (CHAOS).
  - α Tocopherol, β-Carotene Cancer Prevention Study (ATBC).
  - GISSI-Prevenzione trial.
  - Heart Outcomes Prevention Evaluation (HOPE and HOPE T00).
  - MRC/BHF Heart Protection Study.
  - Primary Prevention Project (PPP).
  -Supplementation en Vitamines et Minerax Antioxydants (SU.VI.MAX).

- **Most results were <u>negative.</u>**

- **Clinical studies, demonstrated, in large part, beneficial actions?**

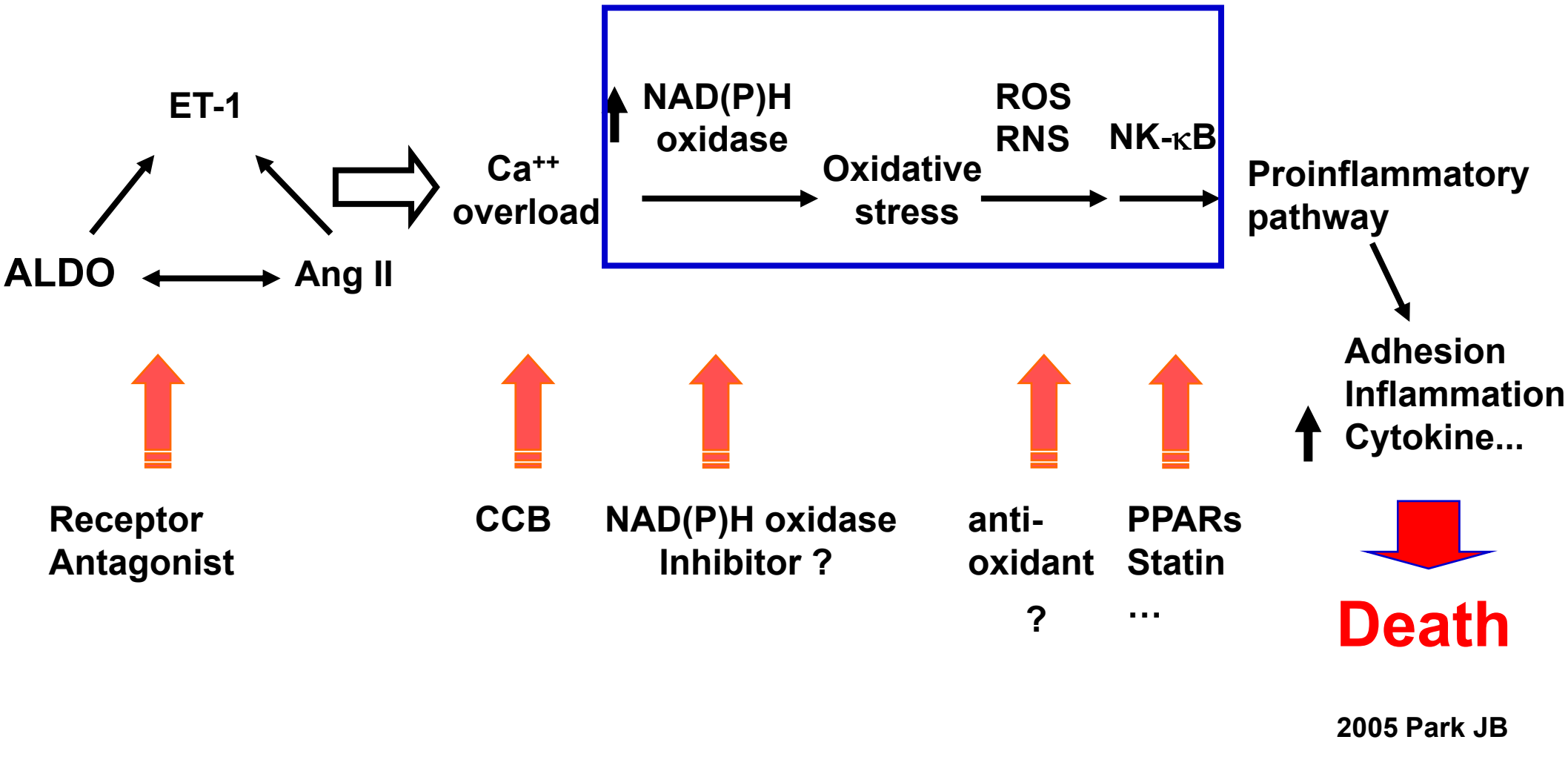
Why have Primary and Secondary Prevention Trials of Antioxidant Protocols Provided Negative Data?

- Pharmocokinetic & pharmacodynamic properties of antioxidants, eg oral antioxidants may be inactivated.
- Insufficient dosing/duration of antioxidant Rx.
- Harmful interactions between antioxidants.
- Compartmentalization of antioxidants. Vit E concentrates in lipoproteins and may not get to cytoplasmic ROS.
- Vitamins E and C act as pro-oxidants by converting into tocopheroxyl and ascorbyl radicals.

Why have Primary and Secondary Prevention Trials of Antioxidant Protocols Provided Negative Data?

- Trial patients have CVD - damaging effects of oxidative stress may be irreversible.
- Most patients were taking aspirin, which has intrinsic anti-oxidant properties. Additional antioxidants may be ineffective.
- Never proven that trial subjects had increased oxidative stress.

RAAS, NAD(P)H oxidase and Vascular damage and Potential intervention



2005 Park JB


Thank you!
2001 Summer at Lac Loise

Role of oxidative stress in essential hypertension

An alteration in the redox balance leads to increase of oxidative stress. This in turn not only exerts negative effects on vascular tone and remodeling, but is also able to activate important mechanisms with an established central role in the pathogenesis of hypertensive target organ damage (TOD).

As a consequence, a drug therapy able to restore ROS imbalance in hypertensive patients, in addition to targeting receptor, would probably exert additional benefits, as compared to blood pressure lowering per se, in terms of prevention of TOD and improved prognosis of these patients.