The effect of carotenoids and flavones on oxidative stress in endothelial cells

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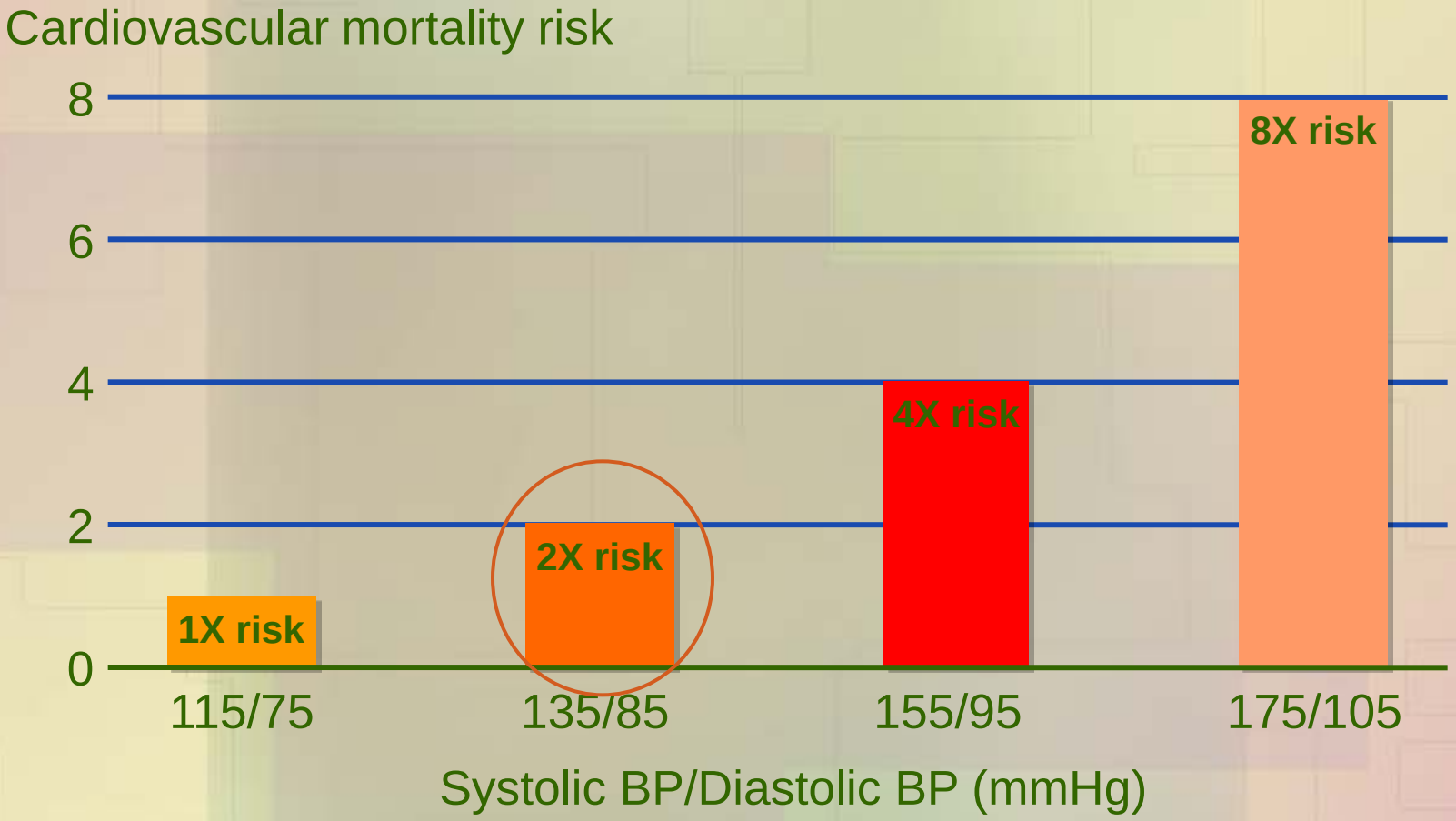
Cardiovascular Mortality Risk Doubles with Each 20/10 mmHg Increment in Systolic/Diastolic BP*



*Individuals aged 40–69 years

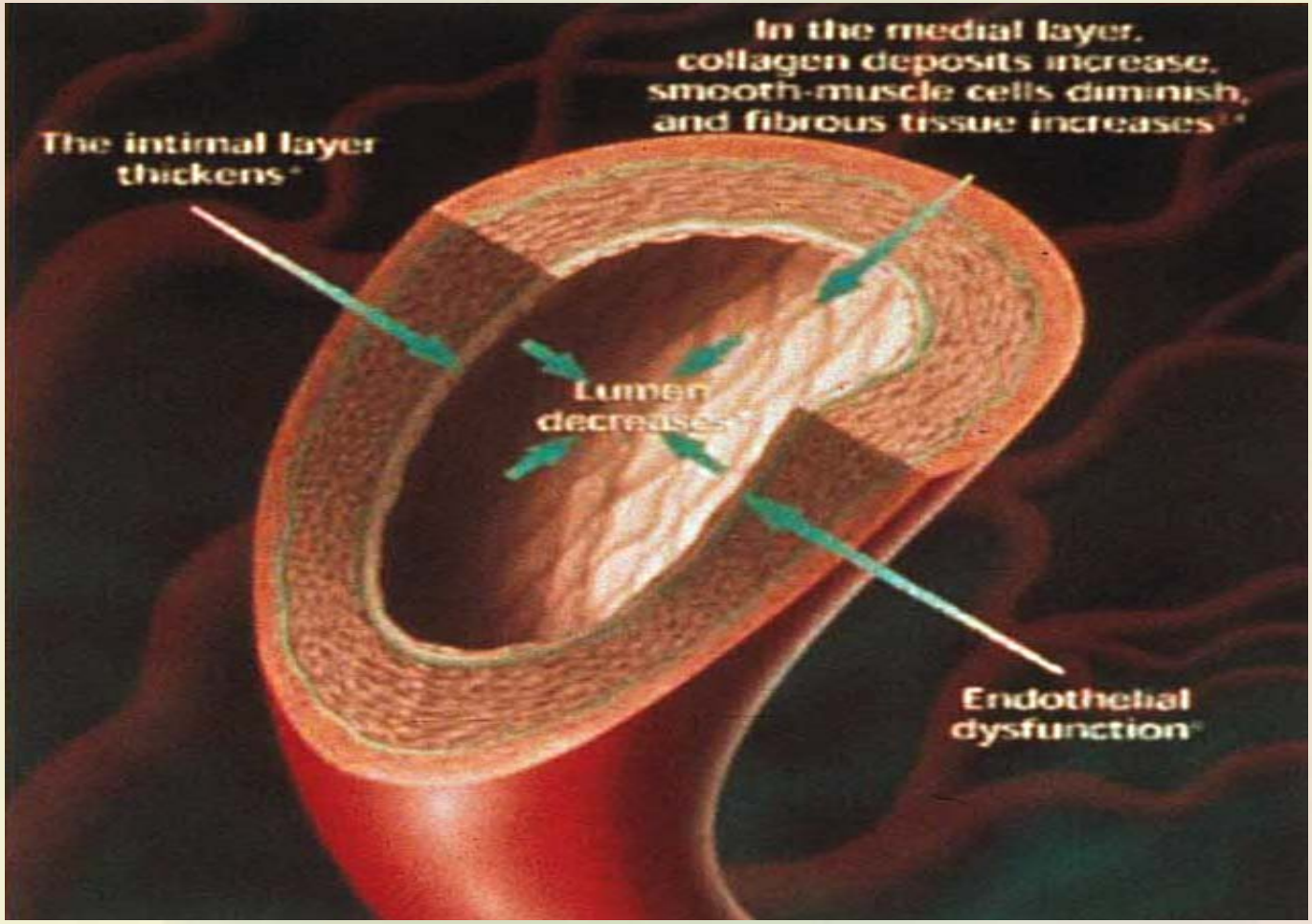
Lewington et al. Lancet 2002;360:1903–13

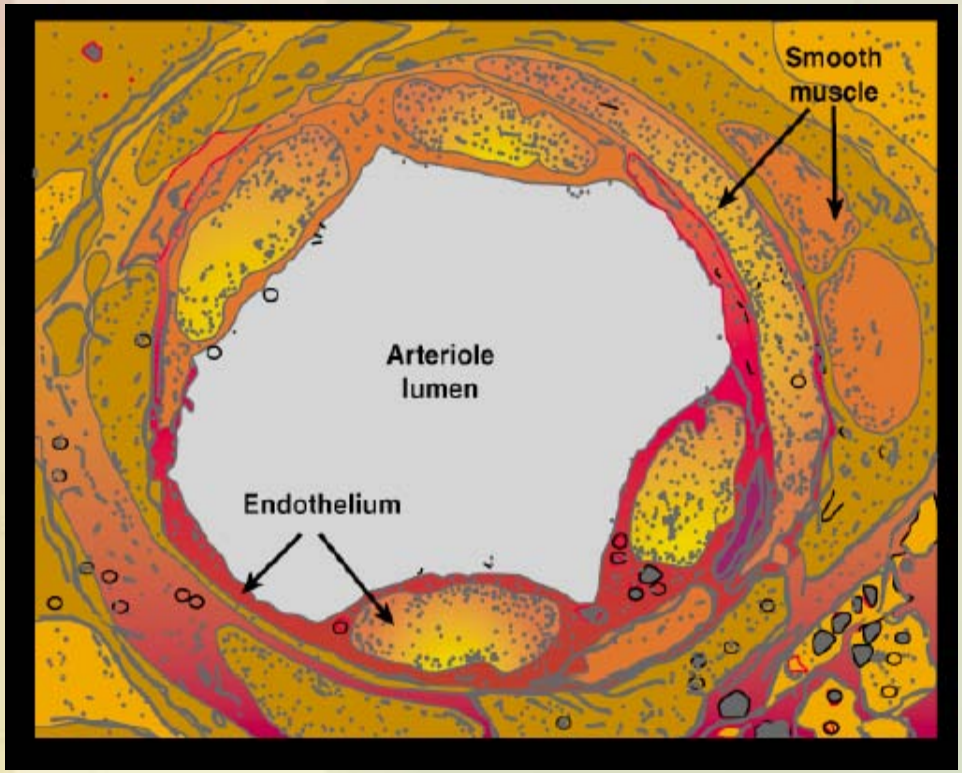
BP Reduction of 2 mmHg Decreases the Risk of Cardiovascular Events by 7–10%

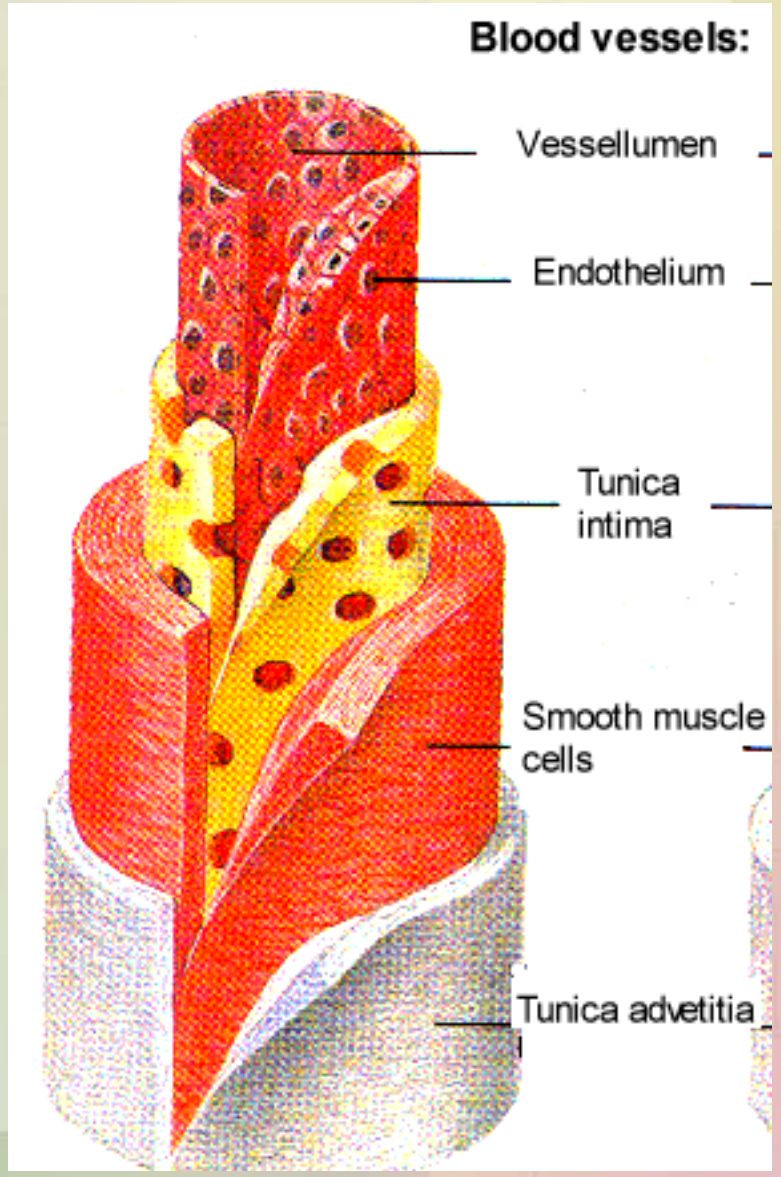
- Meta-analysis of 61 prospective, observational studies
- 1 million adults
- 12.7 million person-years

Lewington et al. Lancet 2002;360:1903–13

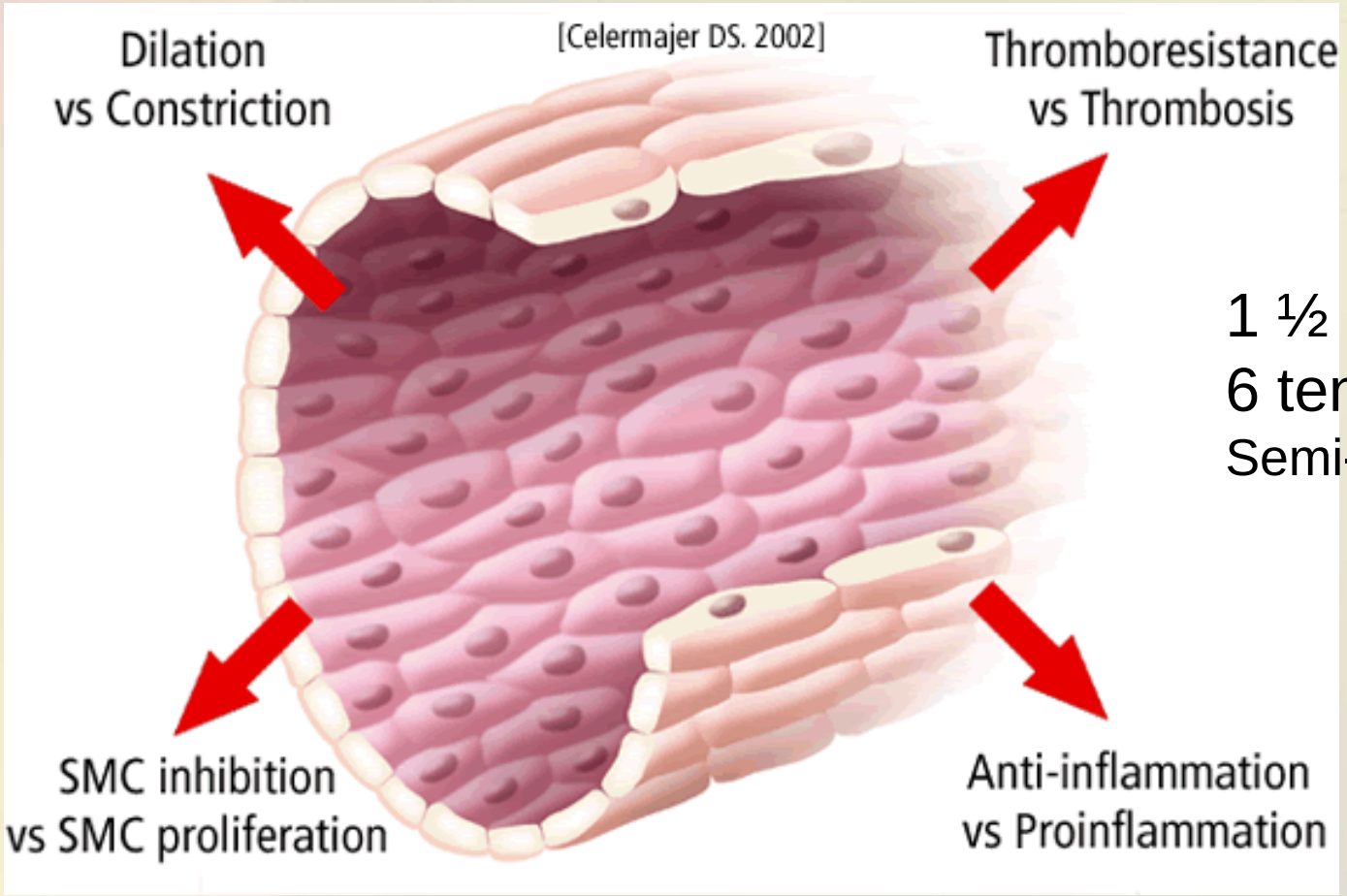
Vascular wall




Smooth muscle
Arteriole lumen
Endothelium


Blood vessels:
Vessellumen
Endothelium
Tunica intima
Smooth muscle cells
Tunica advetitia

Endothelial function



1 ½ kg.
6 tennis courts
Semi-permeable

Imbalance in Factors Affecting Vascular Tone and Structure

Constrictors/ Growth Promoters

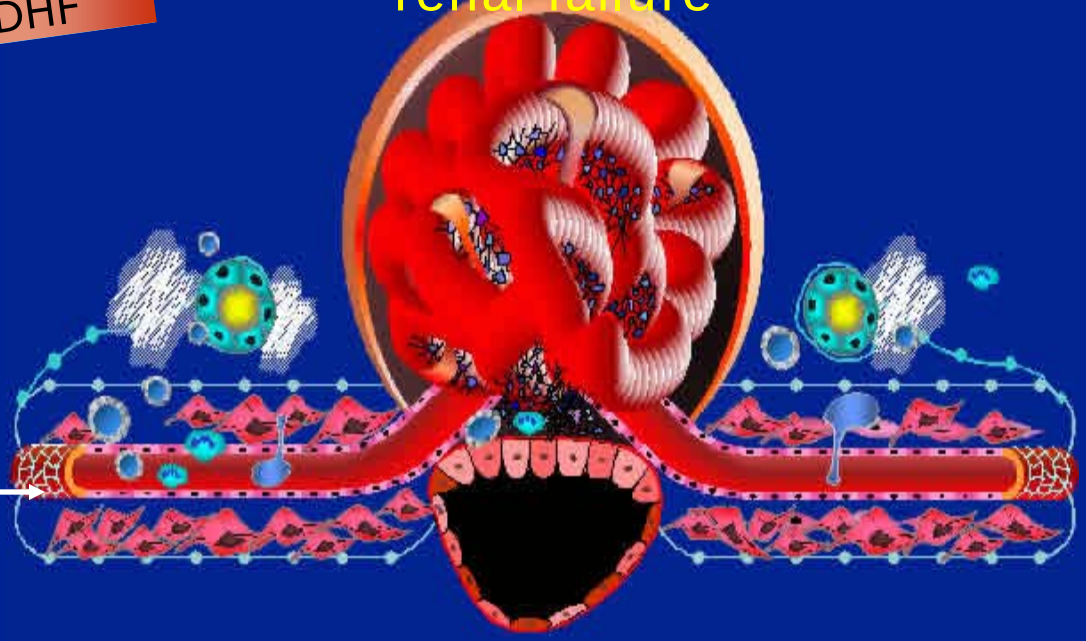
Angiotensin II
Catecholamines
Endothelin-1
ROS
Cytokines
EDCF

Dilators/ Growth Inhibitors

Nitric Oxide
Prostacyclin
Bradykinin
EDHF

Vascular tone and structure

Nephron destruction nd renal failure

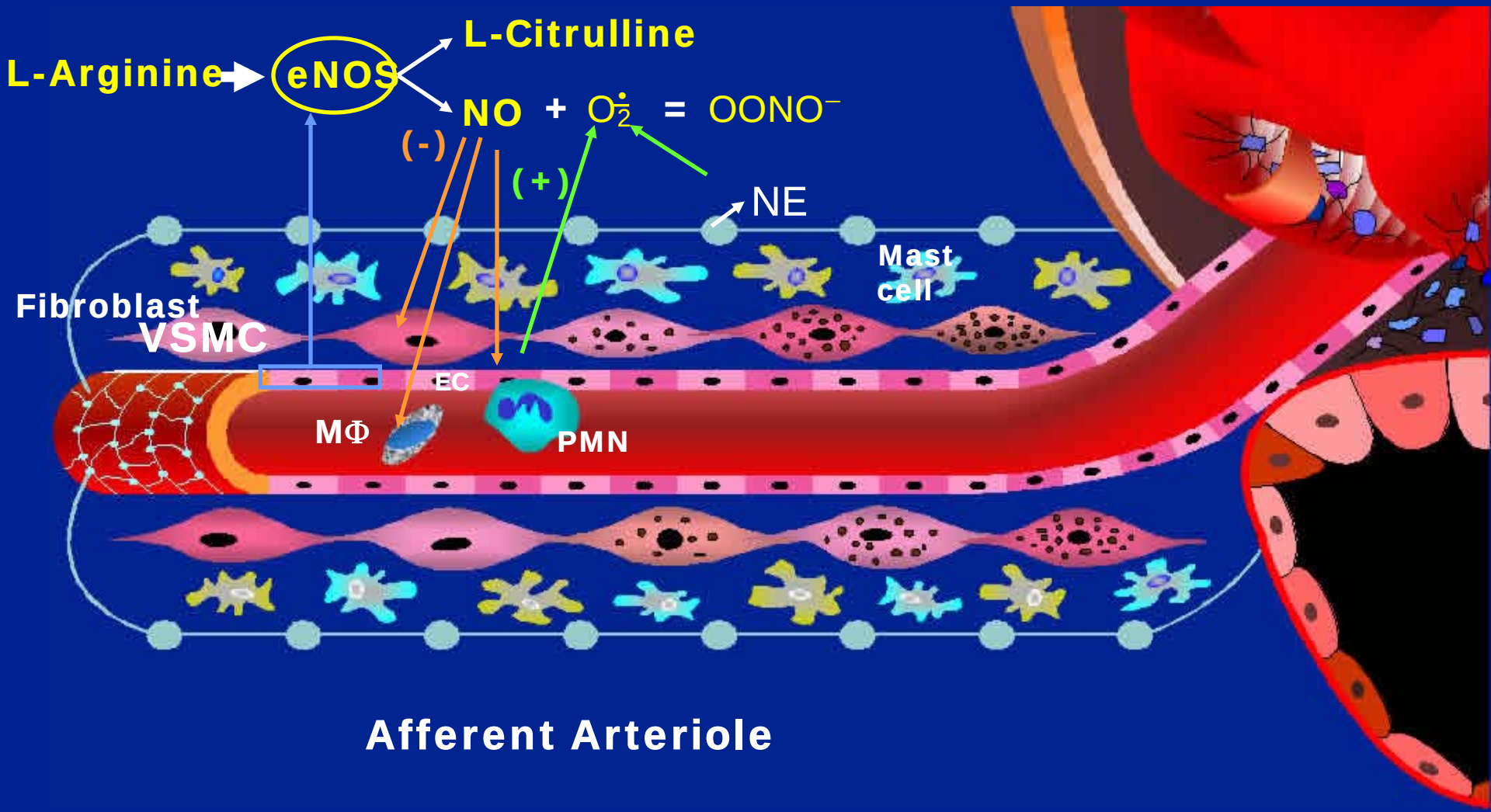


EDHF= endothelium-derived hyperpolarizing factors

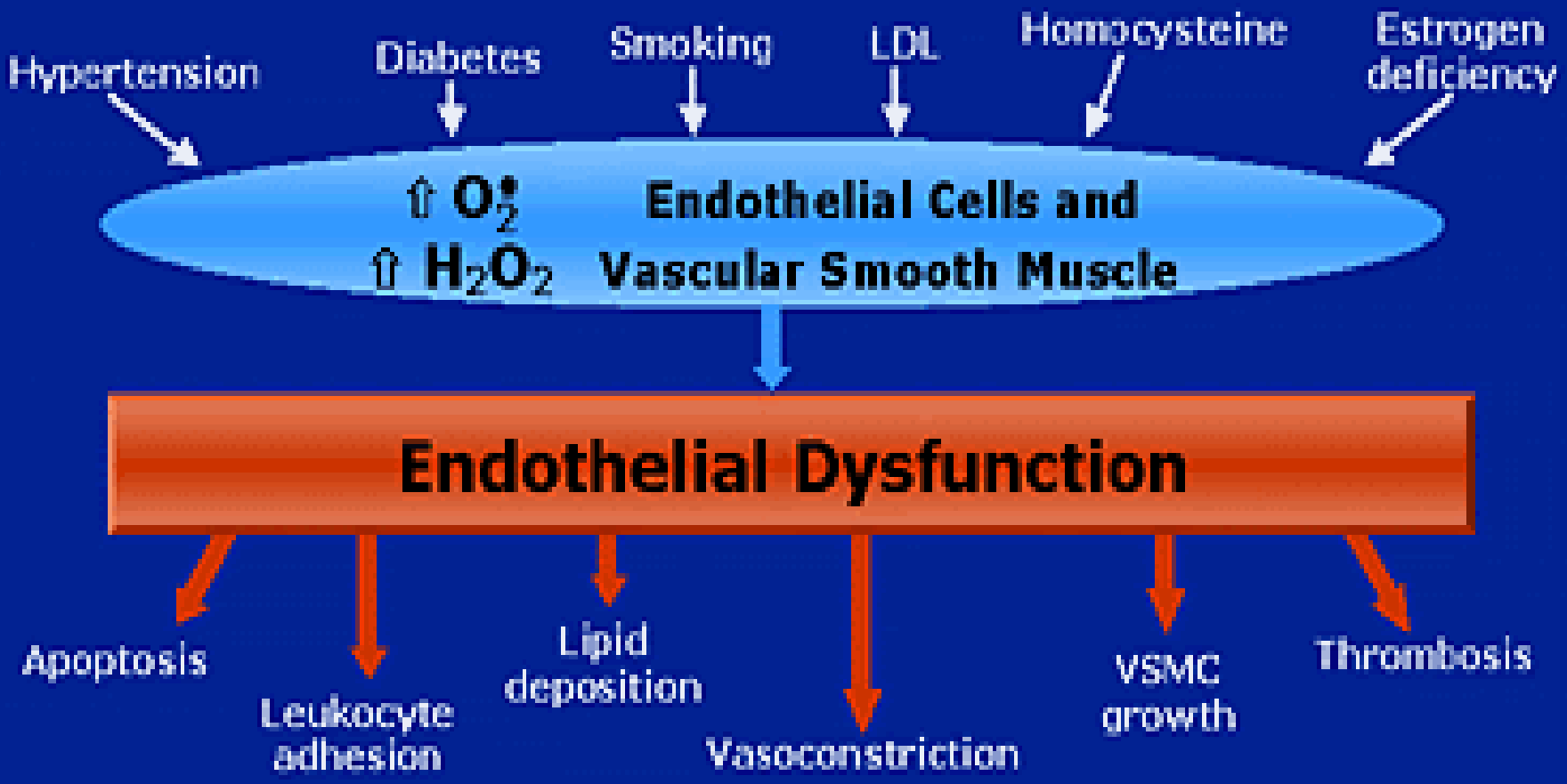
ROS= reactive oxygen species

EDCF= endothelium-derived constricting factors

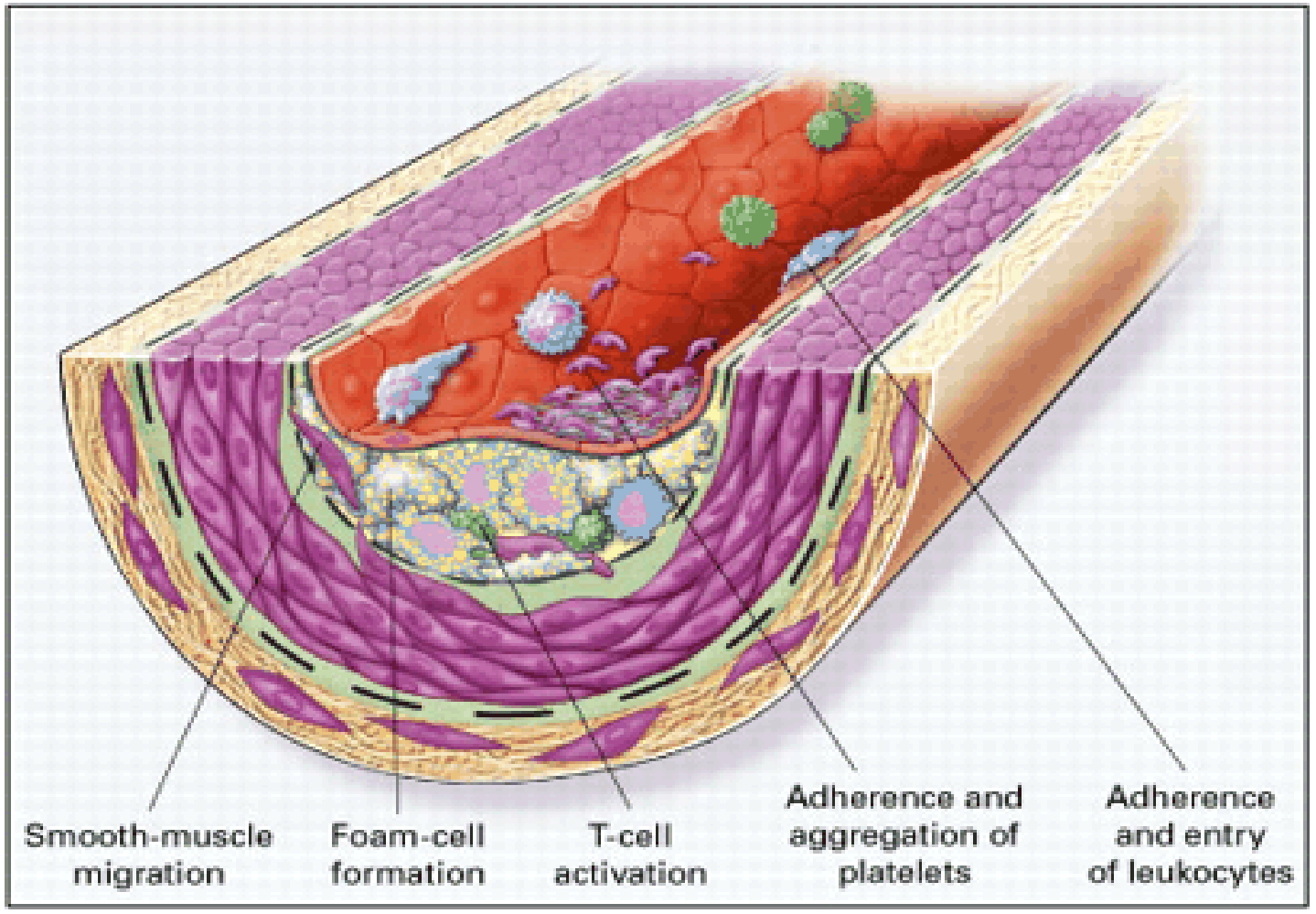
ROS Reduces the Biological Effects of NO



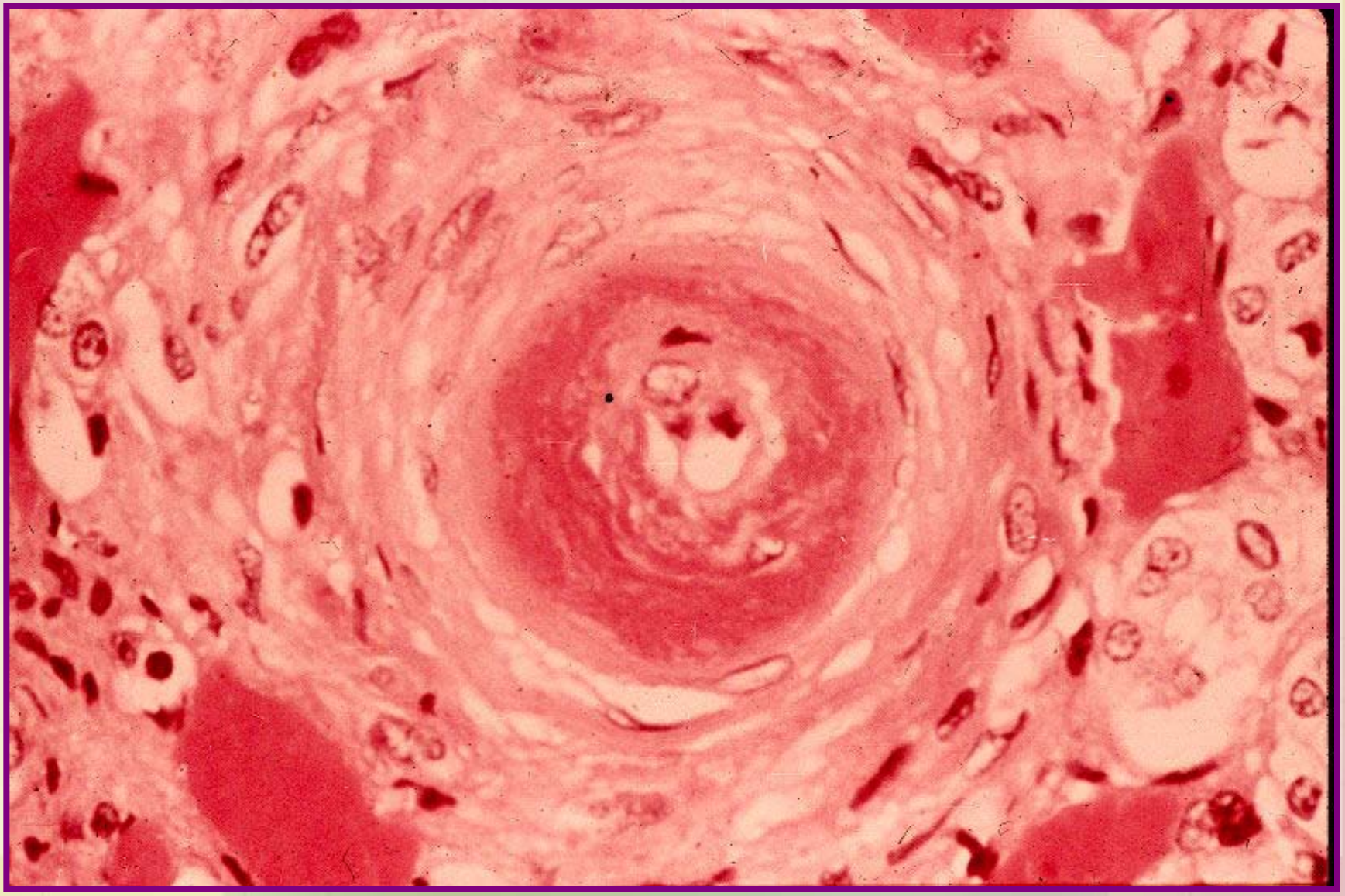
Oxidative Stress: Endothelial Dysfunction and CAD/Renal Risk Factors



Endothelial dysfunction



Fibrinoid necrosis



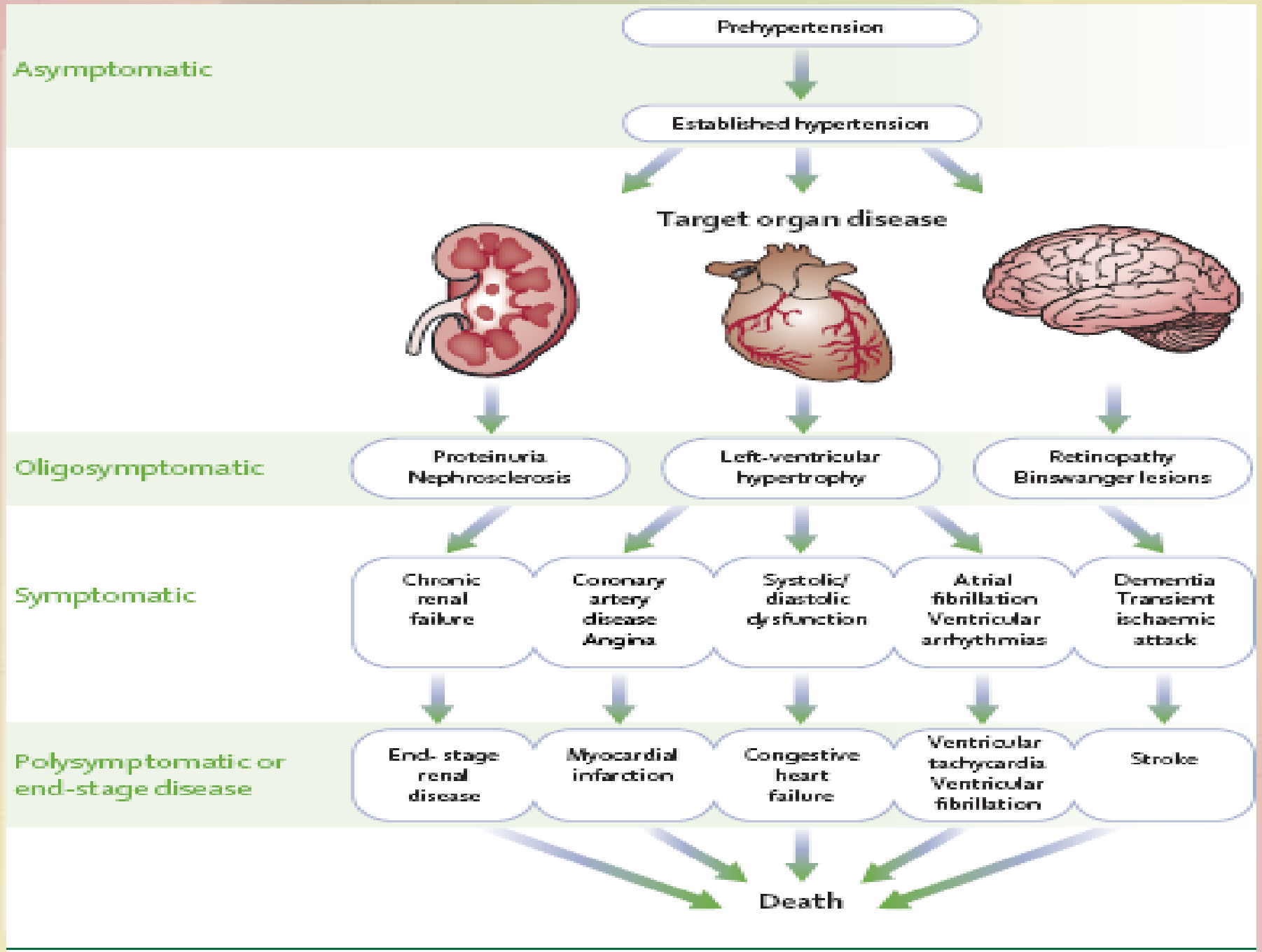
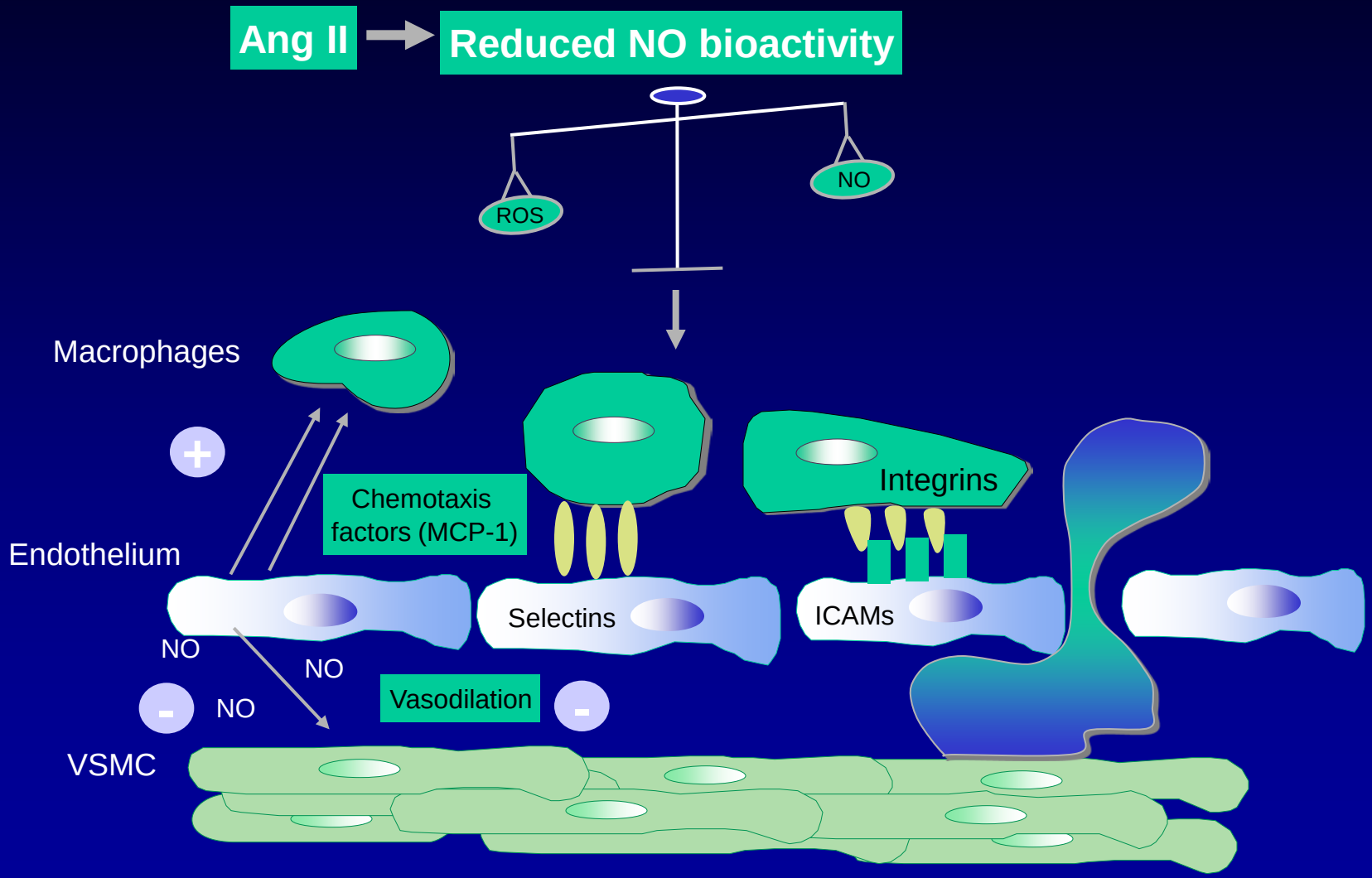


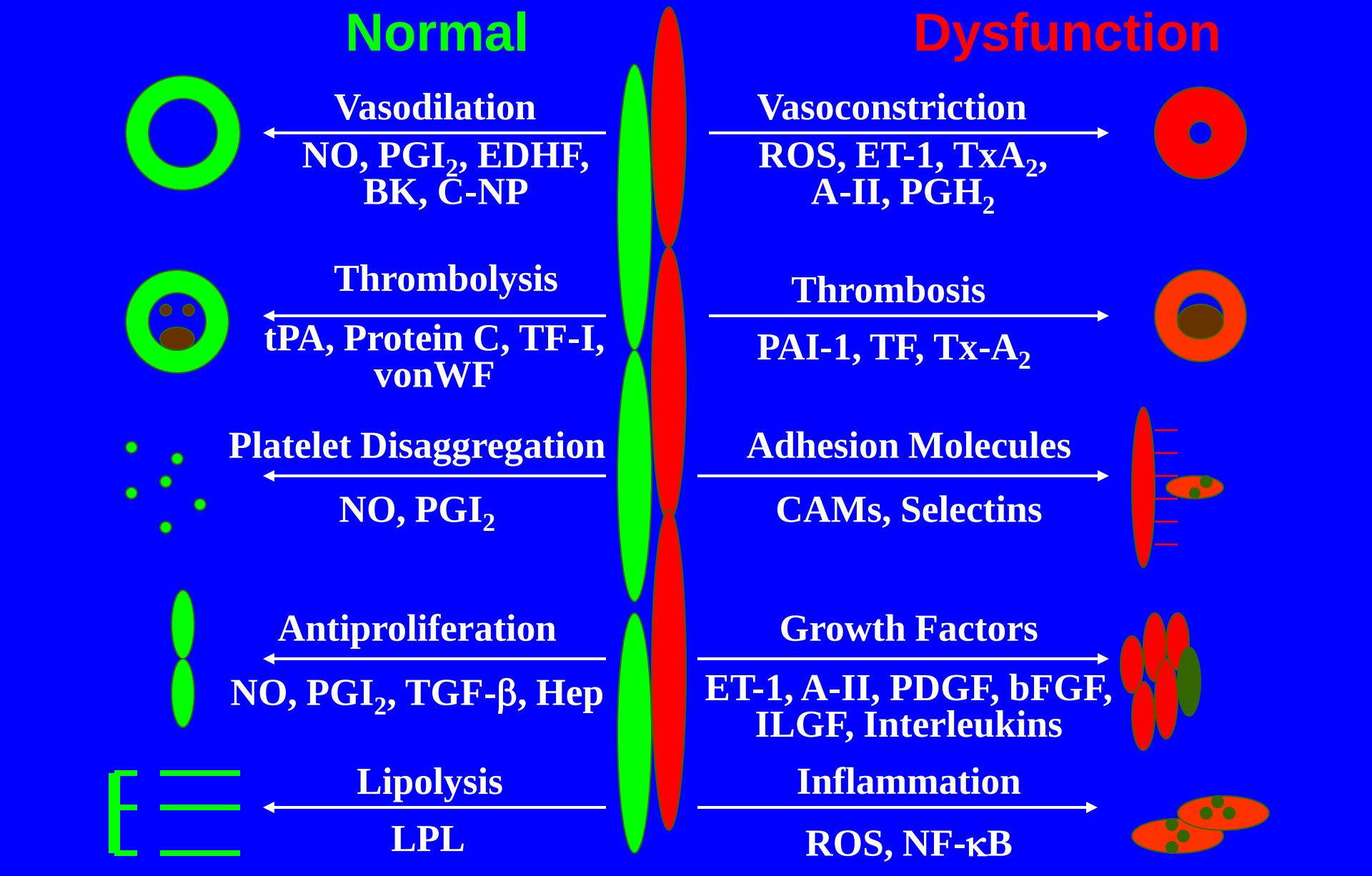
Figure 1: Range of hypertensive cardiovascular disease from prehypertension to target-organ damage and end-stage disease

Reactive oxygen species and endothelial dysfunction



Werner N, Nickenig G. *Eur Heart J.* 2003; 5(suppl A): A9-A13

Regulatory Functions of the Endothelium



How to Assess ED

- Endothelium-dependent vasodilation
  - Acetyl choline or post-ischaemic FMD*
  - Coronary or forearm arteries
- Intima-media thickness (IMT)
- Microalbuminuria
- Plasma markers
  - ADMA, CRP, adhesion molecules
- Clinical diagnosis

• flow-mediated dilatation (FMD**)**
• asymmetric dimethylarginine (**ADMA**)

- Can we prevent endothelial dysfunction?
- Are there treatments for endothelial dysfunction?
- Is it a reversible process?

Prehypertension =

endothelial dysfunction?

Correcting Endothelial Dysfunction

- Risk factor modification (BP, DM, Smoking)
- Exercise and weight loss
- **Blockade of the RAS- ACE Θ / ARB**
- **LDL reduction**, HDL augmentation.
- **PPAR-γ agonists**
- Antioxidants
- Reducing homocysteine levels
- Improving insulin sensitivity
- Lowering CRP
- L-arginine.

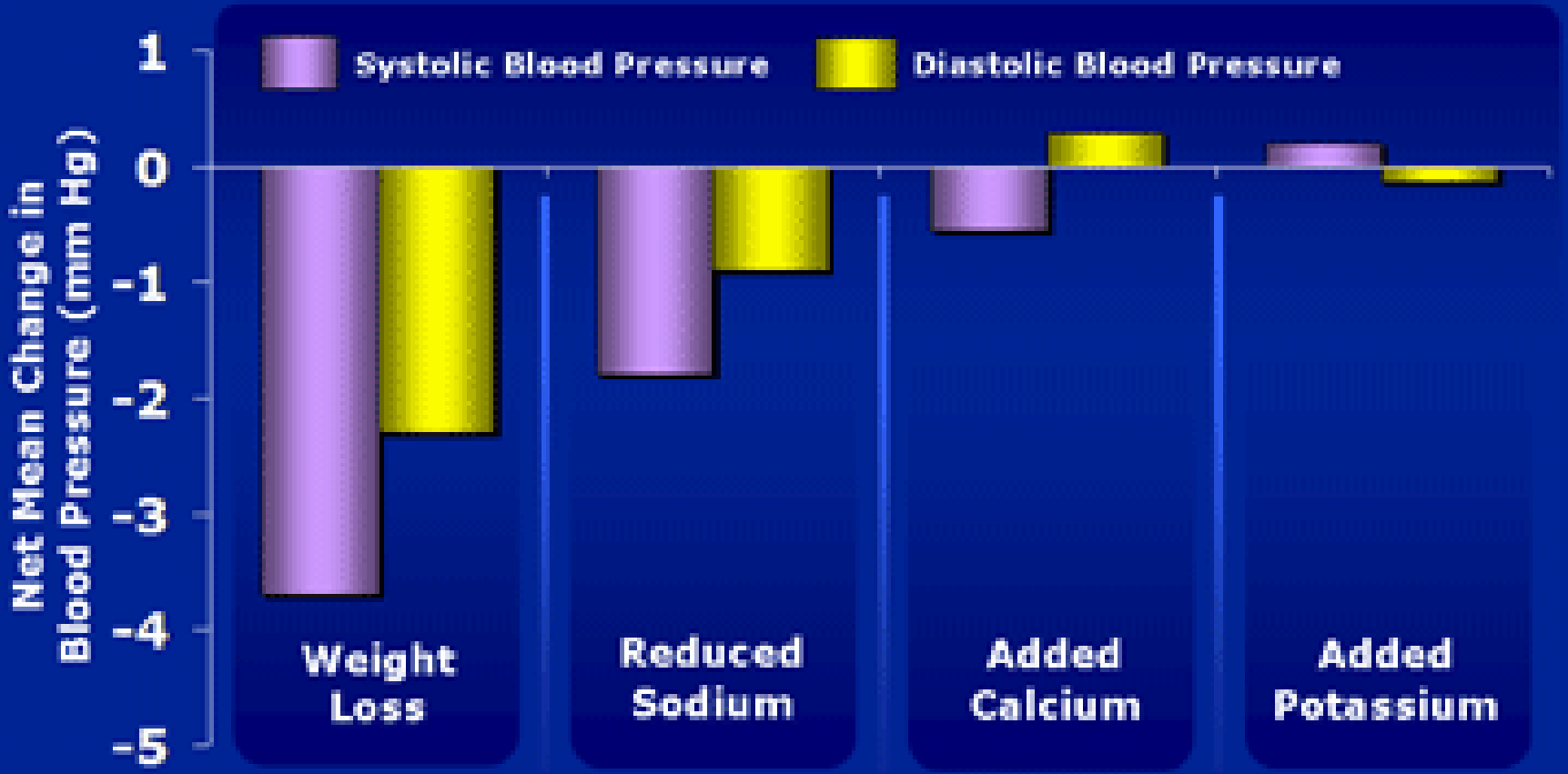
Life style modifications

- Lose weight, if overweight
- Limit alcohol intake
- Increase physical activity
- Reduce salt intake
- Stop smoking
- Limit intake of foods rich in fats and cholesterol: DASH Diet

JNC VII and ESH recommendation

Blood Pressure Reductions Resulting from Various Lifestyle Modifications

Trials of Hypertension Prevention – Phase I



Measures*	−5.67 kg	−58.45 mmol/24 h	1.22 mmol/24 h	44.4 mmol/24 h

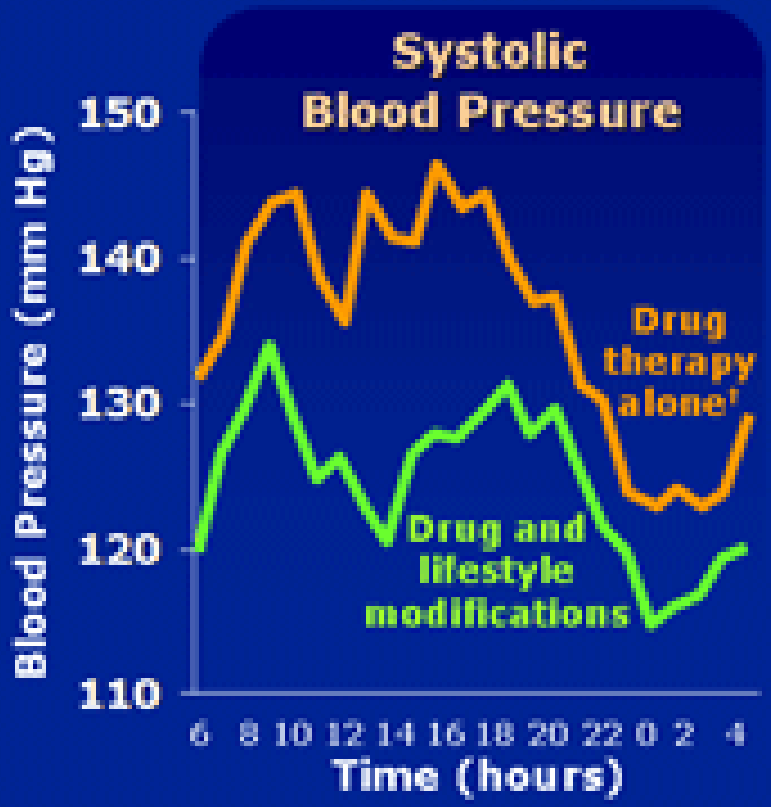
*All values are averages and are statistically significant at $P < 0.01$.

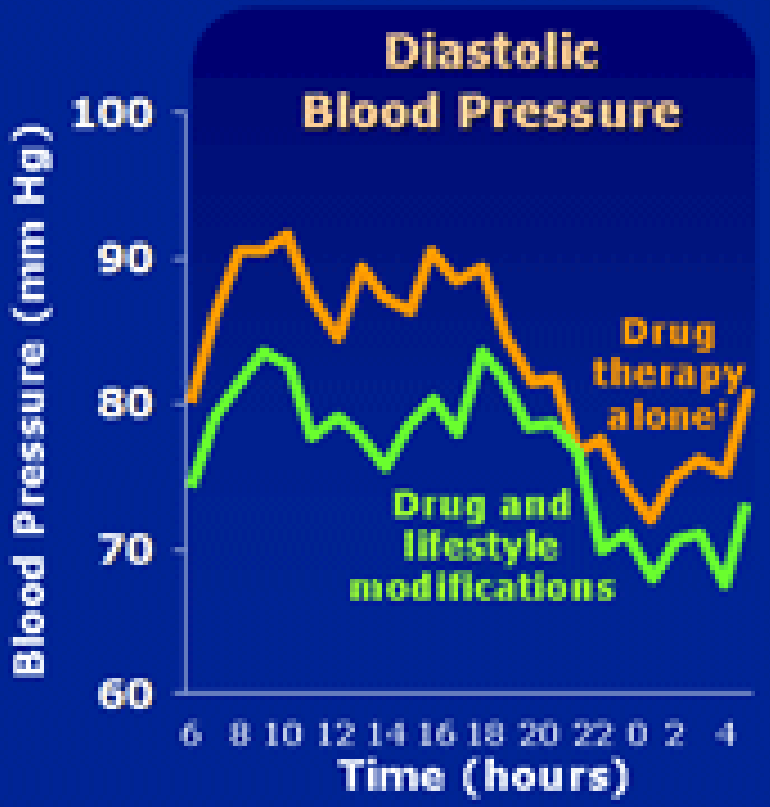
Trials of Hypertension Prevention Collaborative Research Group. *JAMA*. 1992;267:1213-1220.

Slide Source
Hypertension Online
www.hypertensiononline.org

Effect of Antihypertensive Monotherapy Is Augmented by Lifestyle Modifications*

Diet-Exercise-Weight Loss Intervention Trial





*Low-calorie, low-sodium diet and exercise.
†A single antihypertensive drug.

Reprinted from Miller ER III, et al. *Hypertension*. 2002;40: 612-618, with permission from Lippincott Williams & Wilkins.













Health benefit of carotenoids

- Epidemiological studies
- Clinical data
- Clinical studies
- Animal experiments
- In vitro models

Epidemiologic evidence

High serum values of carotenoids such as α-carotene, β-carotene, and lycopene were found to be significantly associated with low hazard ratios for cardiovascular disease mortality.

Japan 3061 people; 12 years follow-up

Ito et al J Epidemiol, 2006. 16, 154-60

The Kuopio Ischaemic Heart Disease Risk Factor Study

725 middle-aged men free of CVD at the study baseline

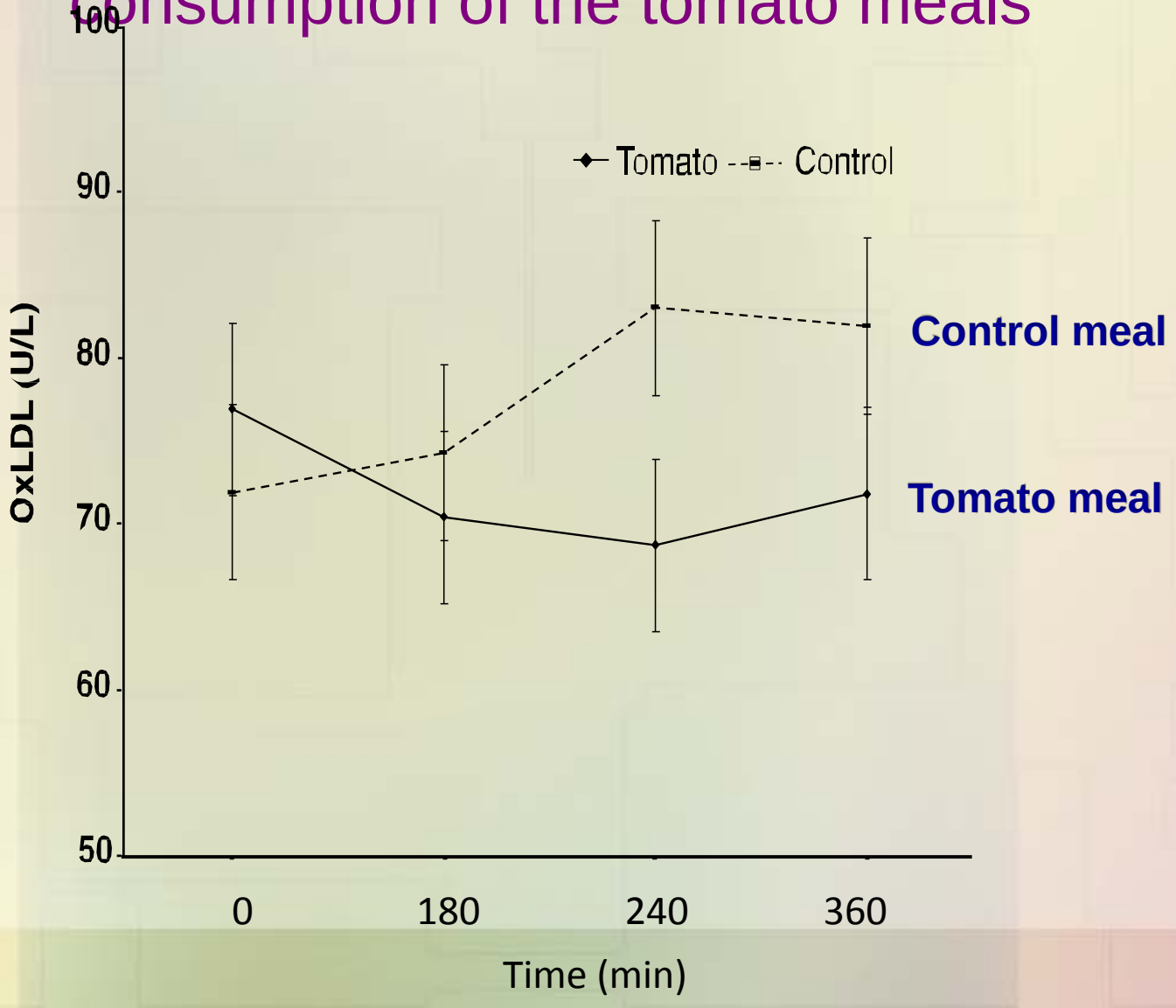
Low plasma lycopene concentration is associated with **increased intima-media** thickness of the carotid artery wall and excess incidence of acute coronary events and stroke:

Men in the **lowest quartile** of serum levels of lycopene had a **3.3-fold** ($P < 0.001$) risk of the acute coronary event or stroke as compared with others

Rissanen et al
1.Arterioscler Thromb Vasc Biol, 2000. 20 2677-811
2.Br J Nutr, 2001 85, 749-54
3.Am J Clin Nutr, 2003 77, 133-8

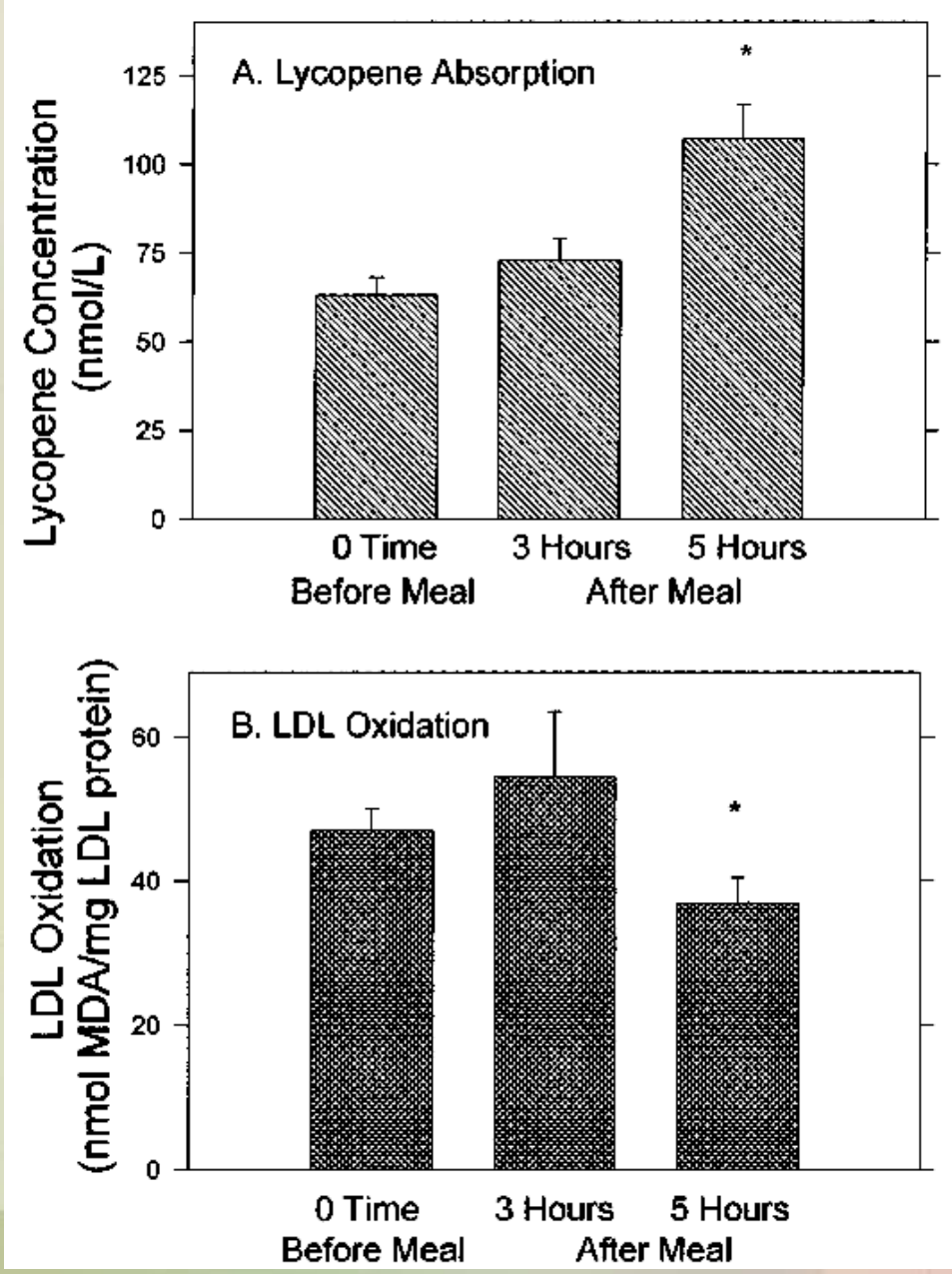
Clinical Data

Decreased Oxidized LDL concentrations after consumption of the tomato meals



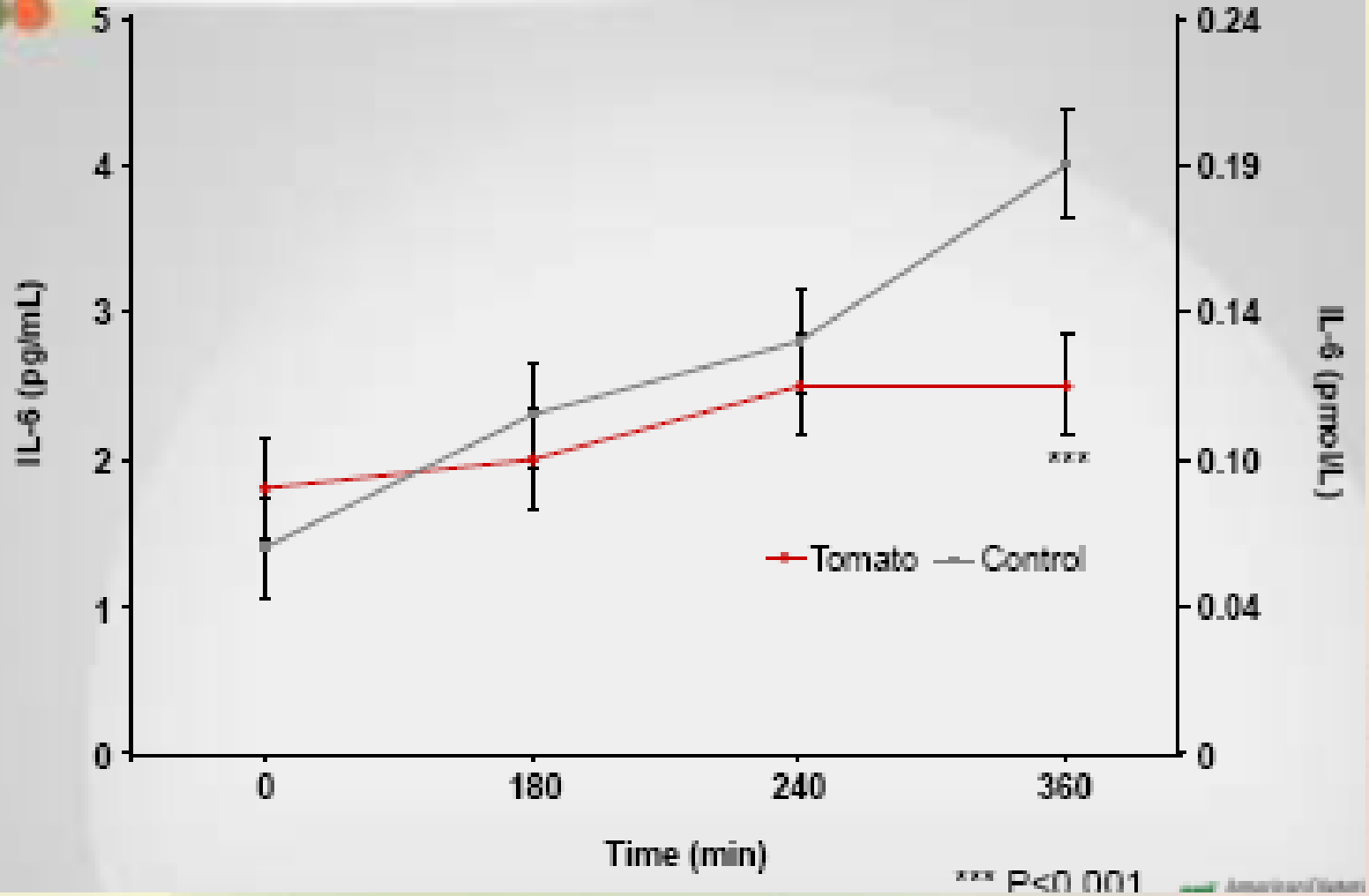
Decreased LDL oxidation after tomato ext consumption

Fatty meal containing 30 mg lycopene in the form of tomato oleoresin (LycoMato)

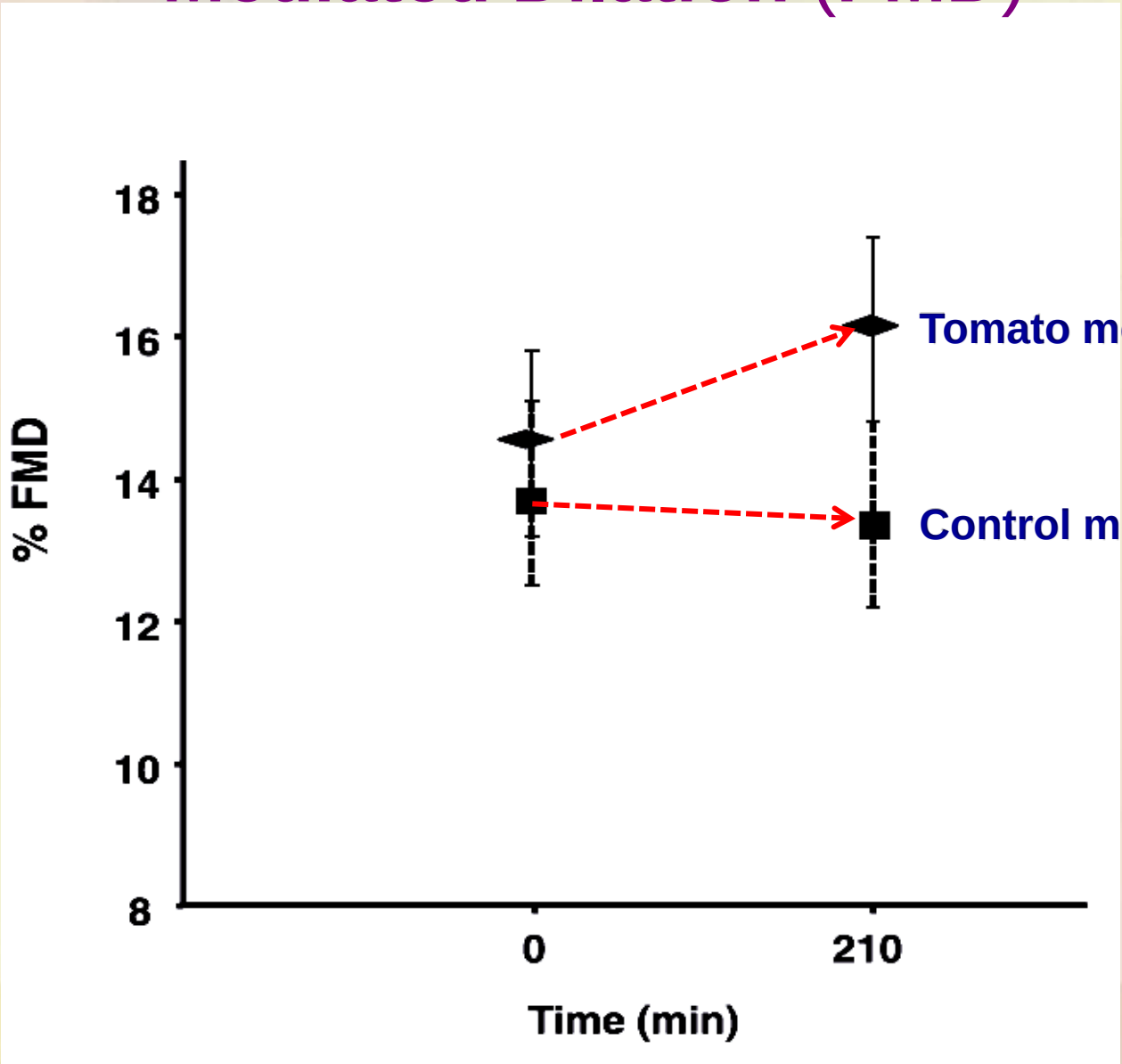


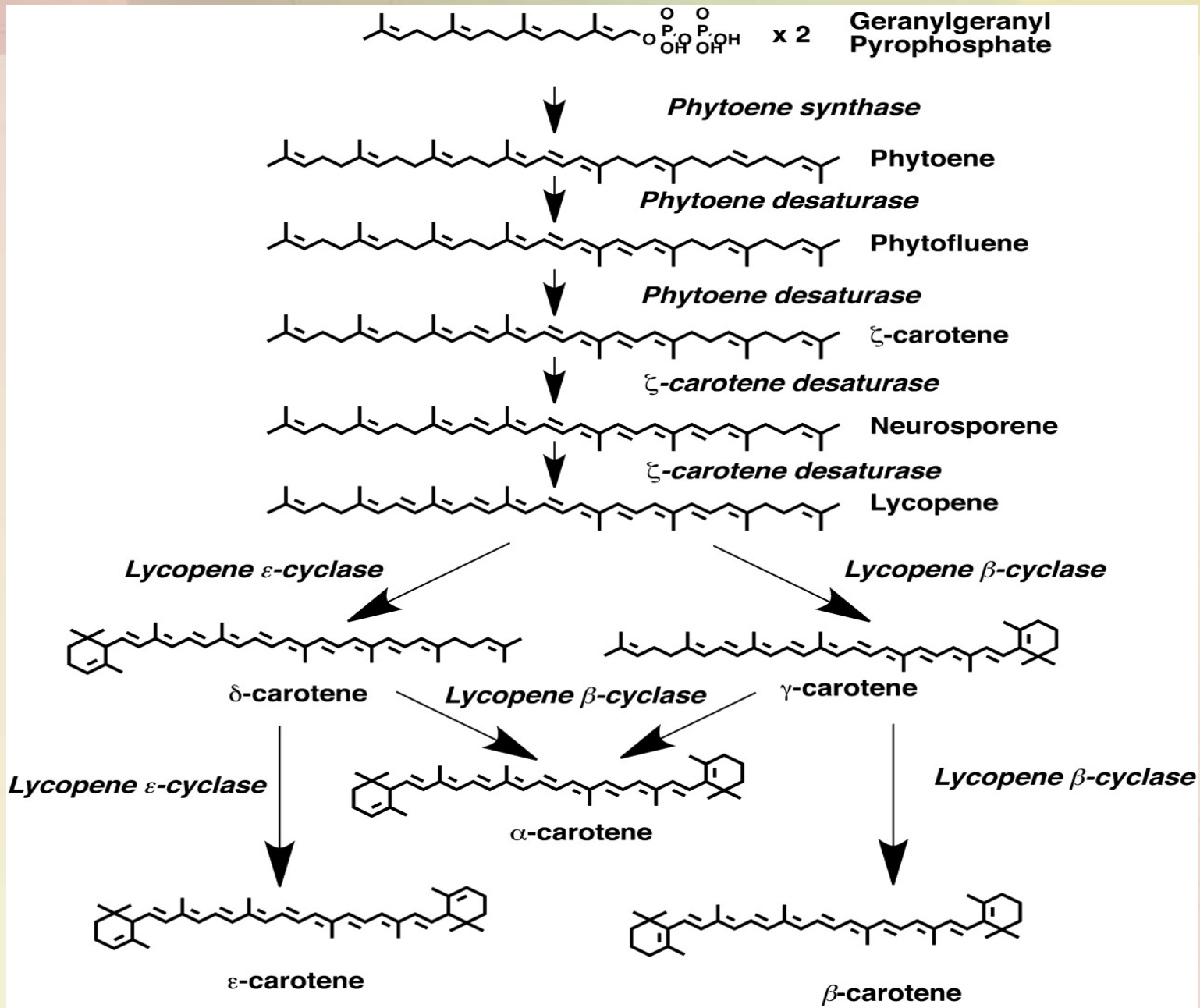
Aviram et al. Antioxidants & Redox Signaling 2, 492, 2000

IL-6 concentrations at baseline & after consumption of tomato and control meals

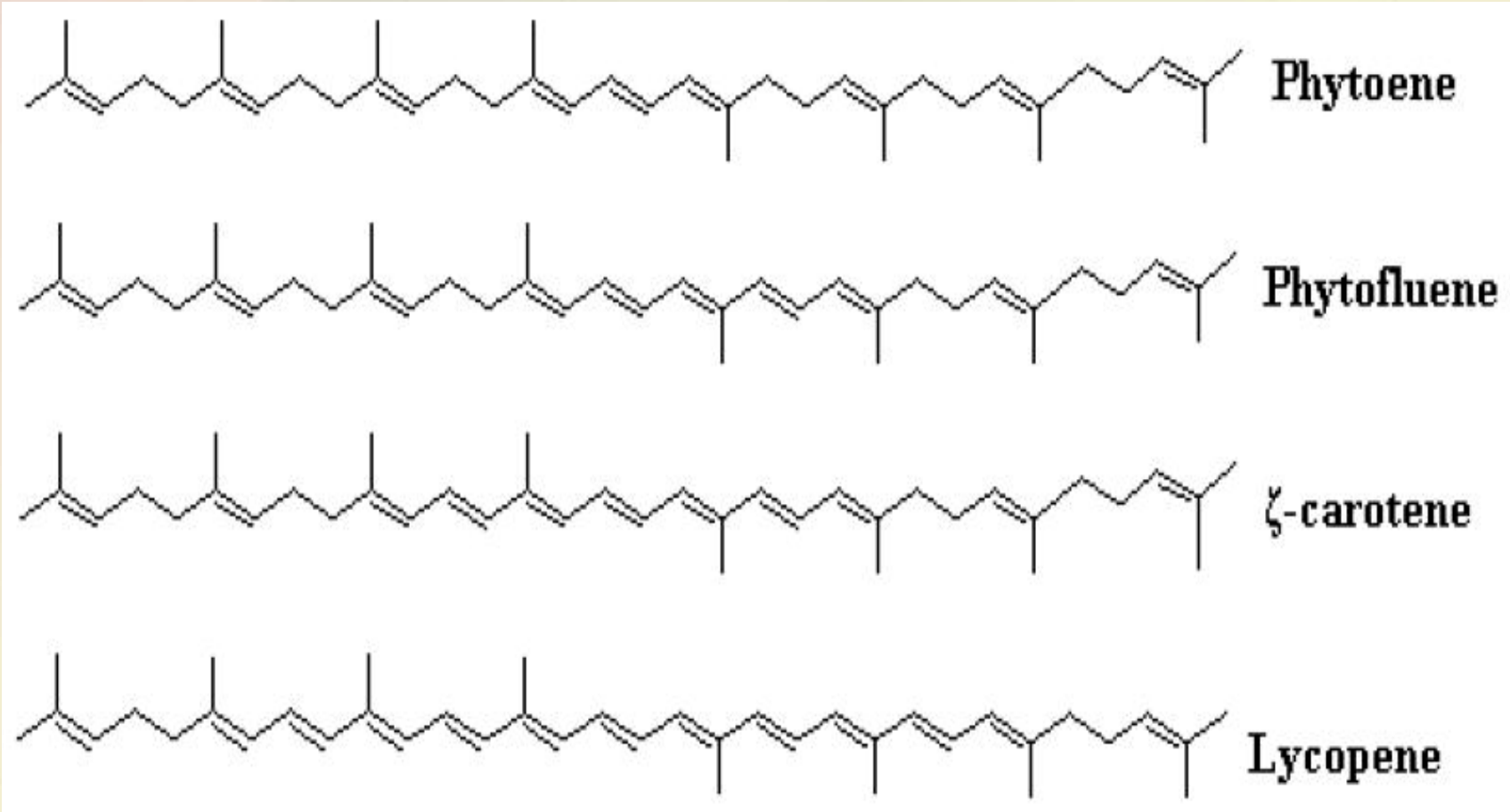


Consumption of tomato meal increased **Flow-Mediated Dilation (FMD)**

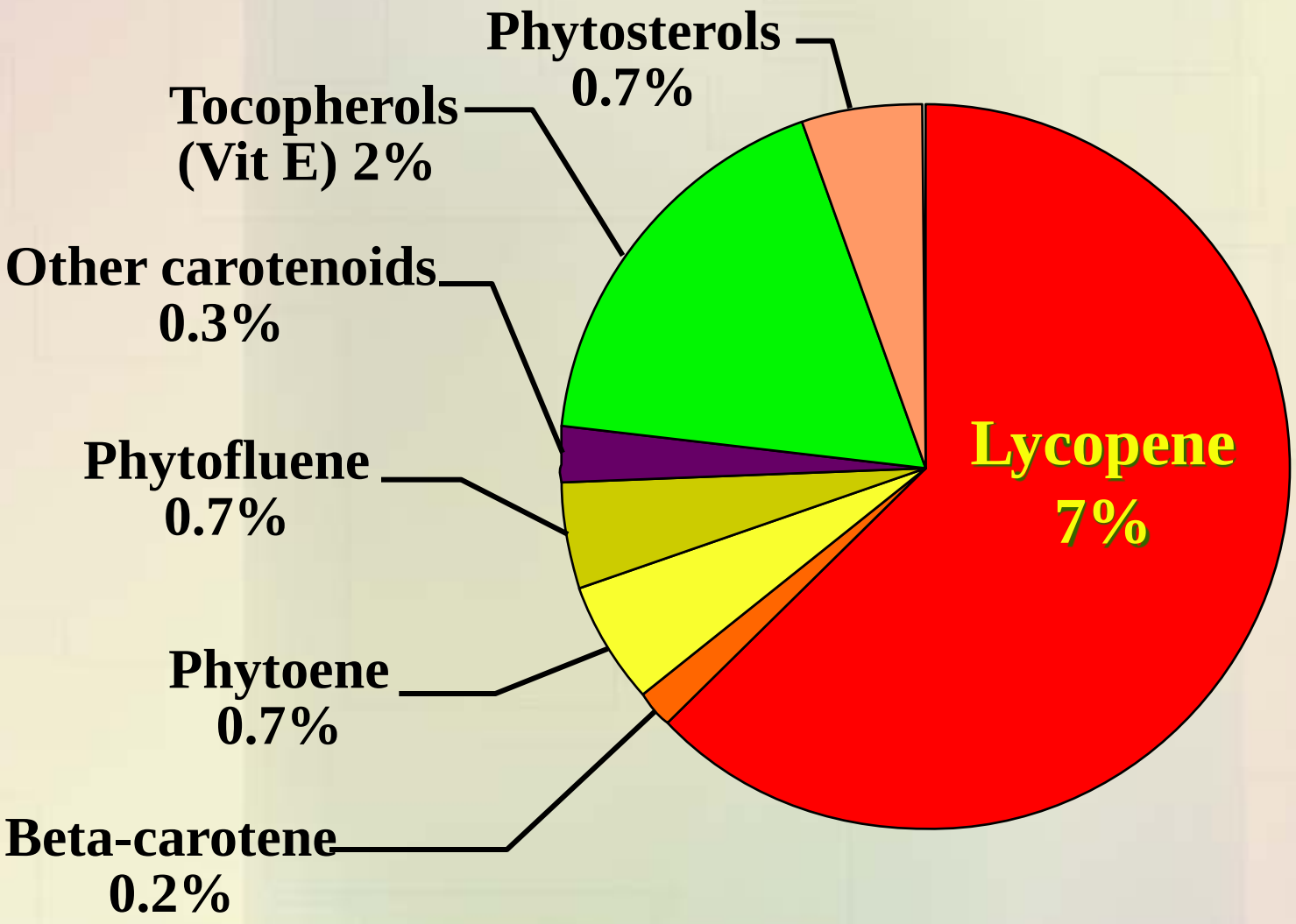




Engelmann N J et al. Adv Nutr 2011;2:51-61


Phytoene
Phytofluene
ζ-carotene
Lycopene

תכולת חומרי המזון השומניים במיצוי מעגבניות LYC-O-MATO®



Clinical studies
Aims

Examining the effects of tomato lycopene on:

- Systolic and diastolic blood pressure
- Biochemical parameters:

  Serum lipids and lipoproteins

- Oxidative stress markers

Results

Systolic blood pressure

Significant reduction of SBP has been achieved as early as the sixth week of Cardi-O-Mato administration, -4.7 mm Hg and 10 mmHg on the eight week

Engelhard Y. Paran E. Am. Heart J 2006;151: 100

Results

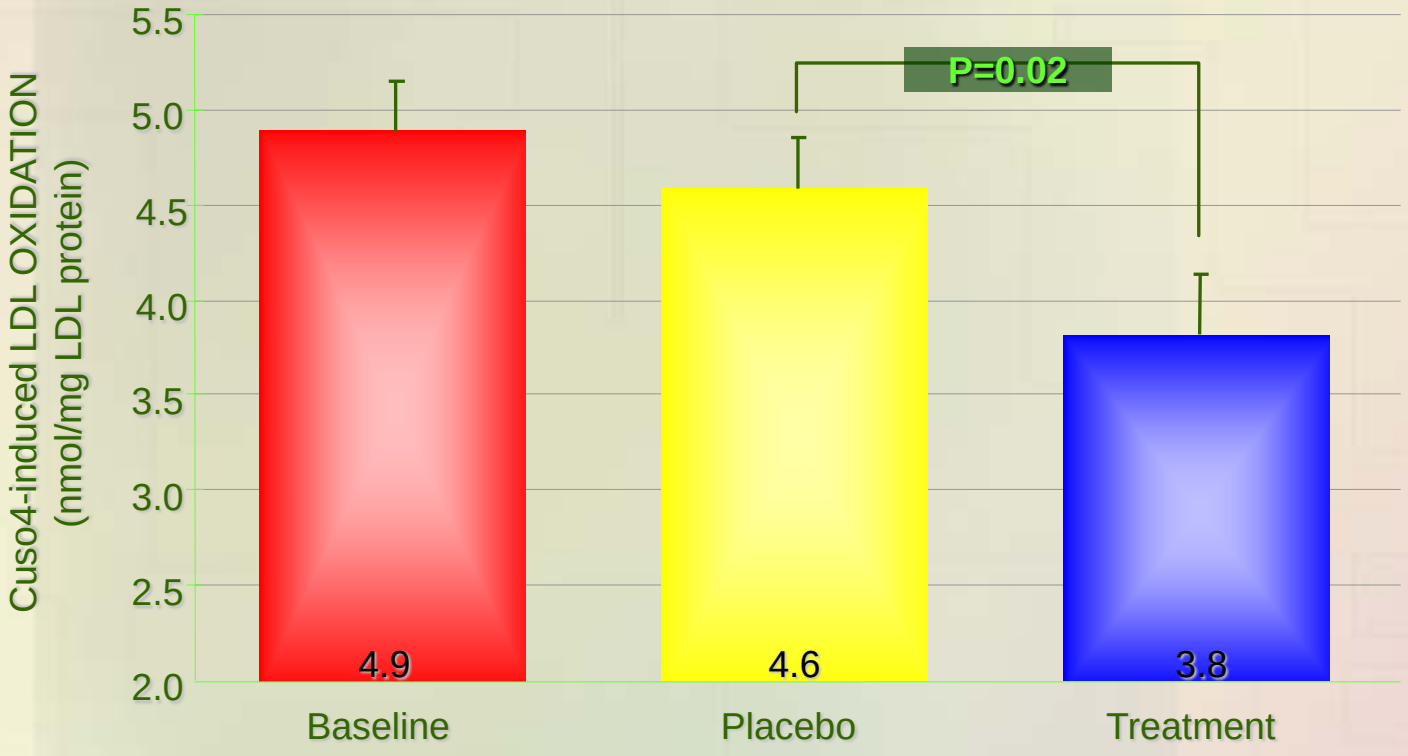
Diastolic blood pressure

Significant reduction in DBP was demonstrated as early as the fourth week (-1.27 mm Hg, p=0.029)and 5 mmHg on the 8th week

Engelhard Y. Paran E. Am.Heart J 2006,151: 00

Results

Thiobarbituric acid reactive substances (TBARS)



Engelhard Y. Paran E. Am .Heart J 2006,151: 100

Conclusions

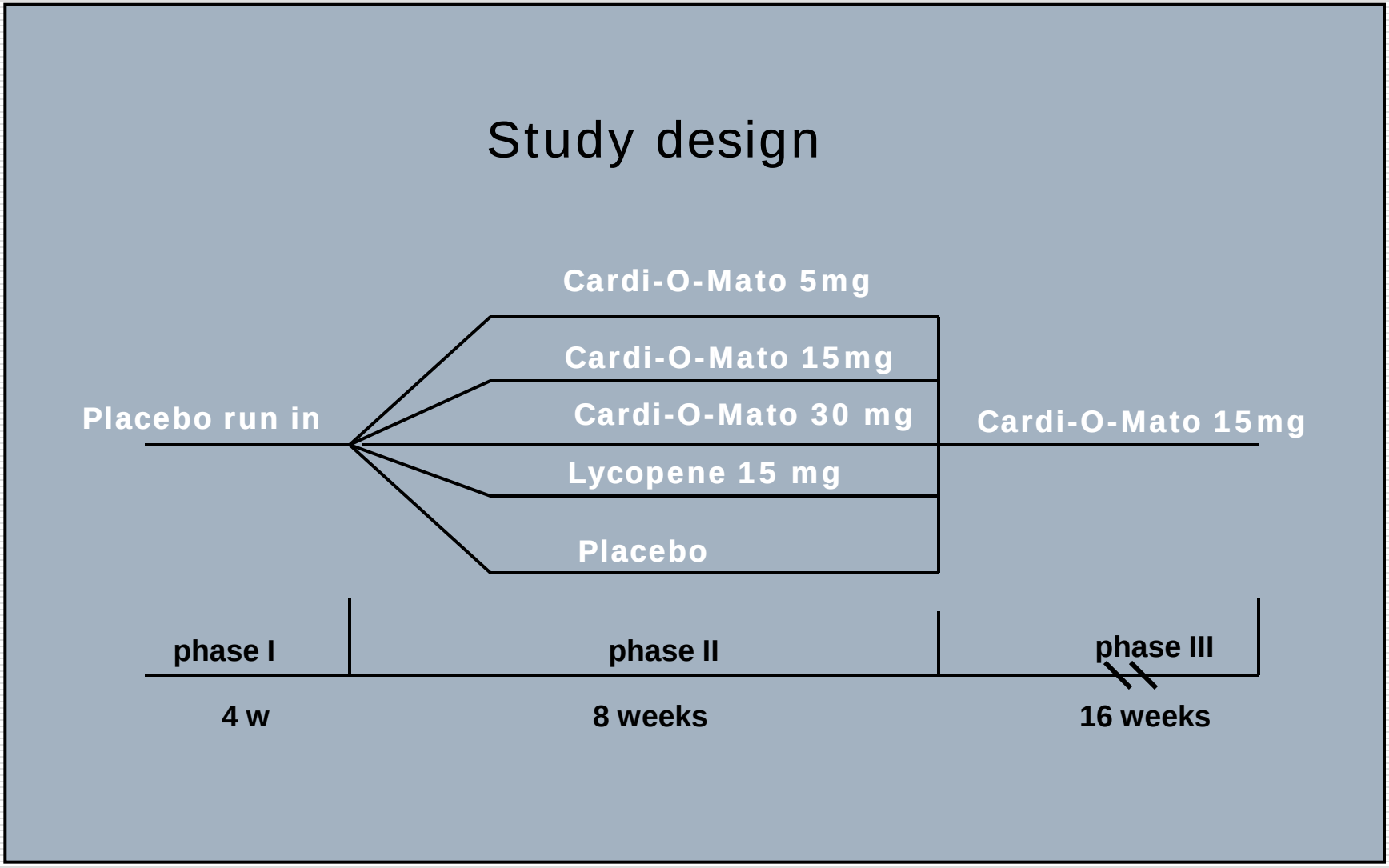
- Tomato lycopene can reduce systolic and diastolic blood pressure significantly in newly diagnosed never treated mild hypertensives
- The same effect of tomato lycopene was recorded in mild to moderate treated patients
- Patients were compliant with the treatment at least for the relatively short term of these studies (12-16 weeks)
- No side effect was observed in any of the patients in all studies

Study design Prehypertensives

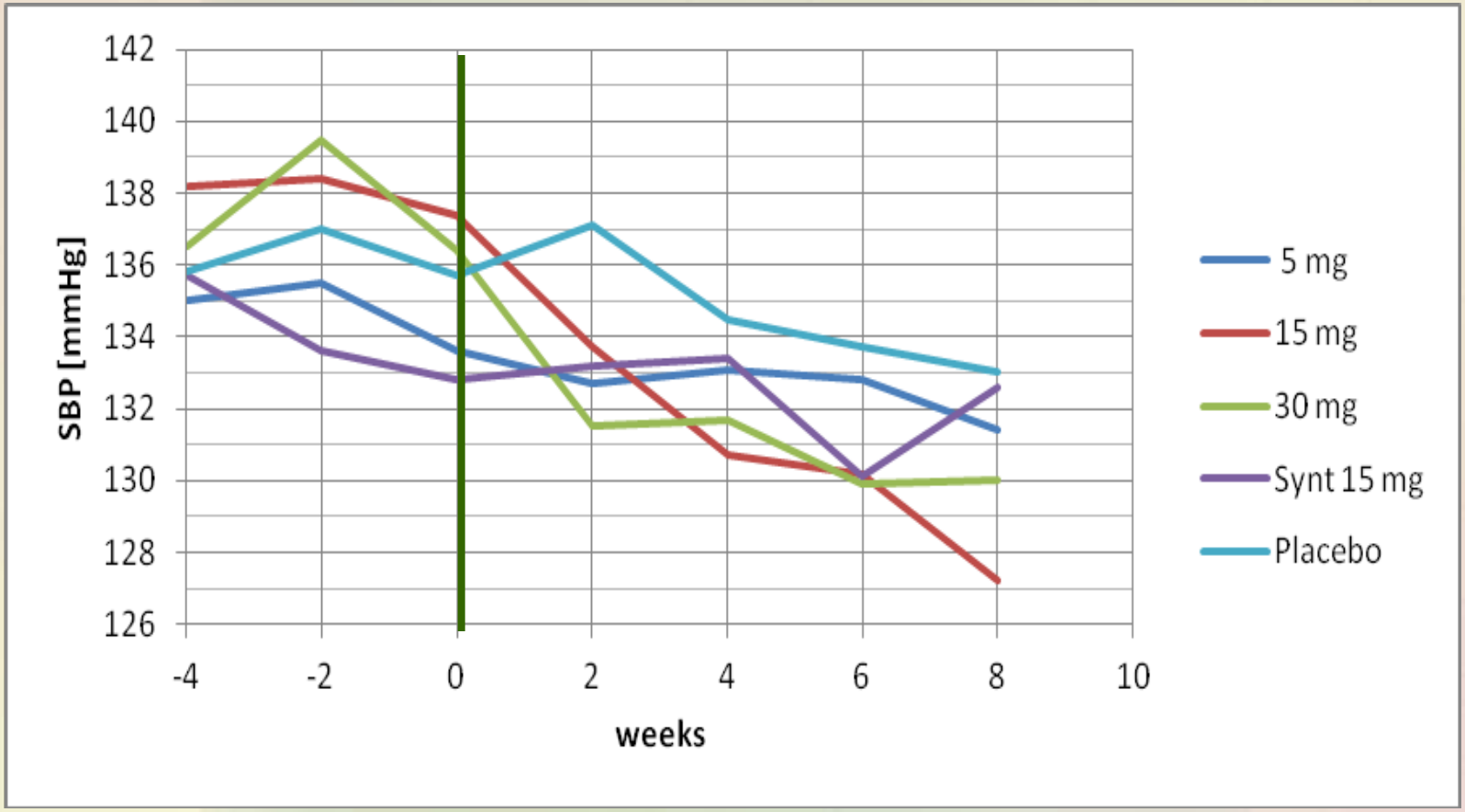
The study consists of three phases:

- Phase I Single-blind *placebo* run–in period for 4 weeks.
- Phase II Double-blind 5 arm parallel group for 8 weeks of:
  1. *Cardi-O-Mato 5mg*
  2. *Cardi-O-Mato 15mg*
  3. *Cardi-O-Mato 30mg*
  4. *Lycopene 15 mg*
  5. *Placebo*
- Phase III Long term treatment for 3 months with Cardi-*O-Mato 15mg*

Dose-response study in prehypertensive subjects



Systolic blood pressure changes during phase I-II of the study



Significant reduction of SBP after 4 weeks already with Cardi-O-Mato 15 and 30 mg

Conclusions

- Tomato lycopene (Cardi-O-Mato) in doses 15mg and 30 mg reduced
  - SBP by 9 and 7mmHg compared to 1.97 mmHg by placebo or synthetic lycopene
  - DBP was reduced by 4.15 and 3.8 mmHg compared to 0.7 and -0.9 mmHg with synthetic lycopene and placebo
- The dose of 5mg caused very mild non-significant reduction in SBP and DBP

What is the mechanism for the antihypertensive effect of the tomato lycopene?

- At what point the anti-oxidant property is interferes with the vasoconstrictive forces?
- Are these actions restricted to the endothelium?
- Is there **one** active component that does all the work or is there a unique combination of micronutrients that responsible for the effect?

Effect of Lyc-O-Mato® Supplementation on Endothelial Function and Oxidative Stress

Clinical trial setting

- Healthy frequent smoking men (n=126) were randomized to receive placebo, Lyc-O-Mato® pills (6 or 15 mg lycopene) daily for 8-week

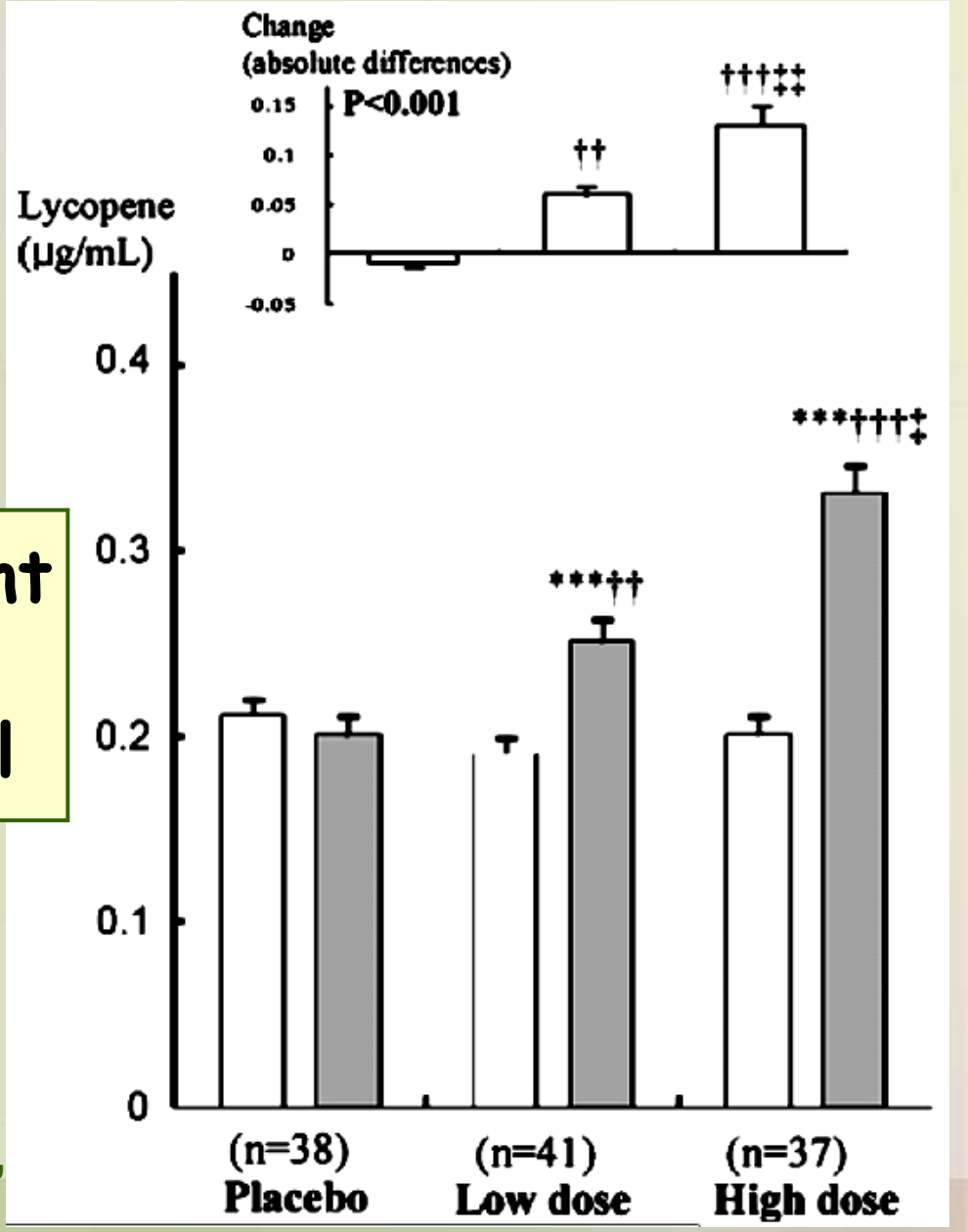
Endpoints:

- **Endothelial function as measured by reactive hyperemia peripheral arterial tonometry (RH-PAT)**
- Oxidative stress measured by plasma superoxide dismutase (SOD) activity; Alkaline comet assay for DNA damage in circulating lymphocytes; Plasma sVCAM-1, sICAM-1 and LDL particle size

Kim et al. Atherosclerosis, 2011. 215(1): p. 189-95.

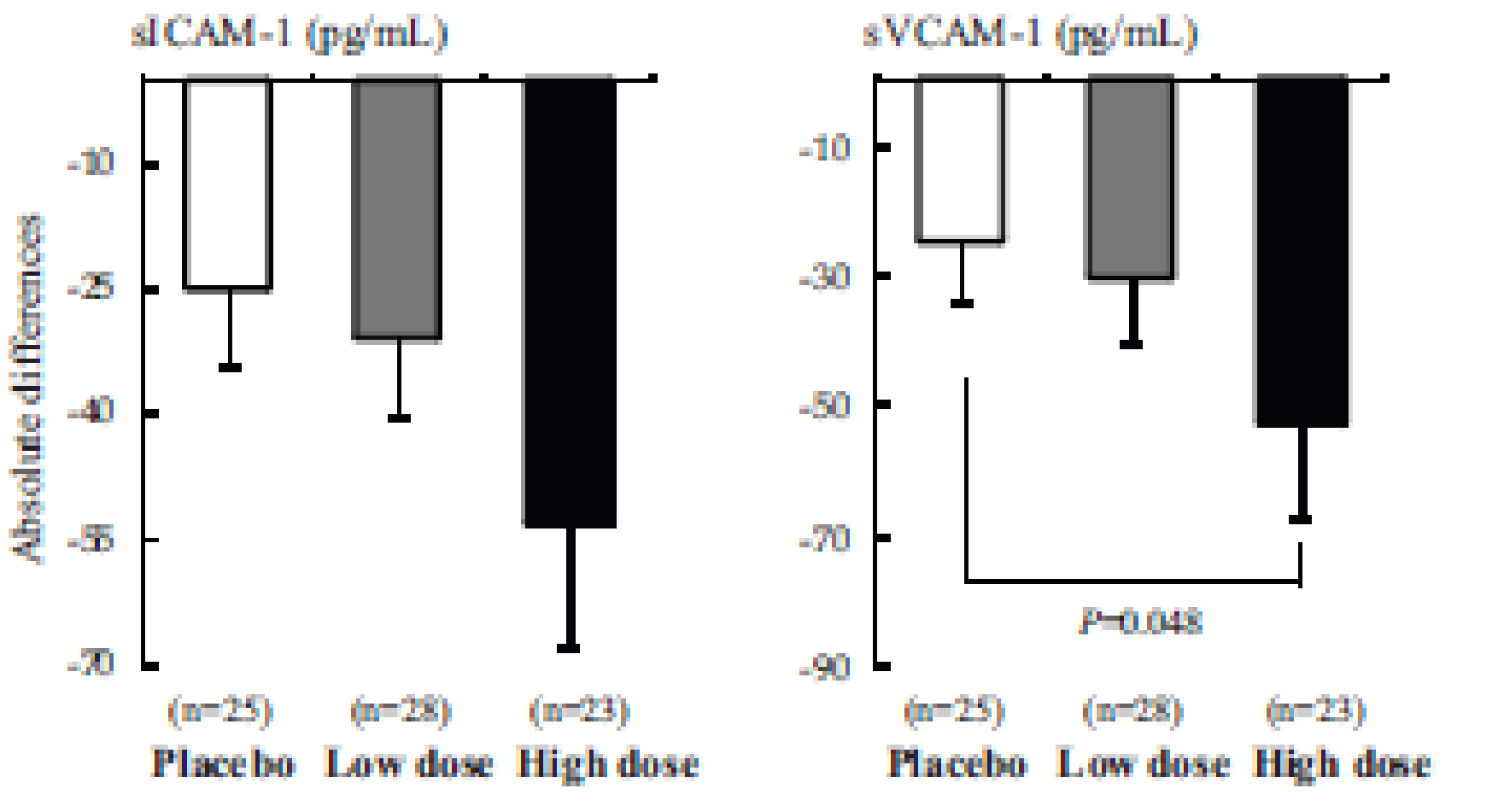
Lyc-O-Mato® Increased lycopene blood level

Dose dependent increase in lycopene level



Kim et al Atherosclerosis, 2011. 215(1): p. 189-95.

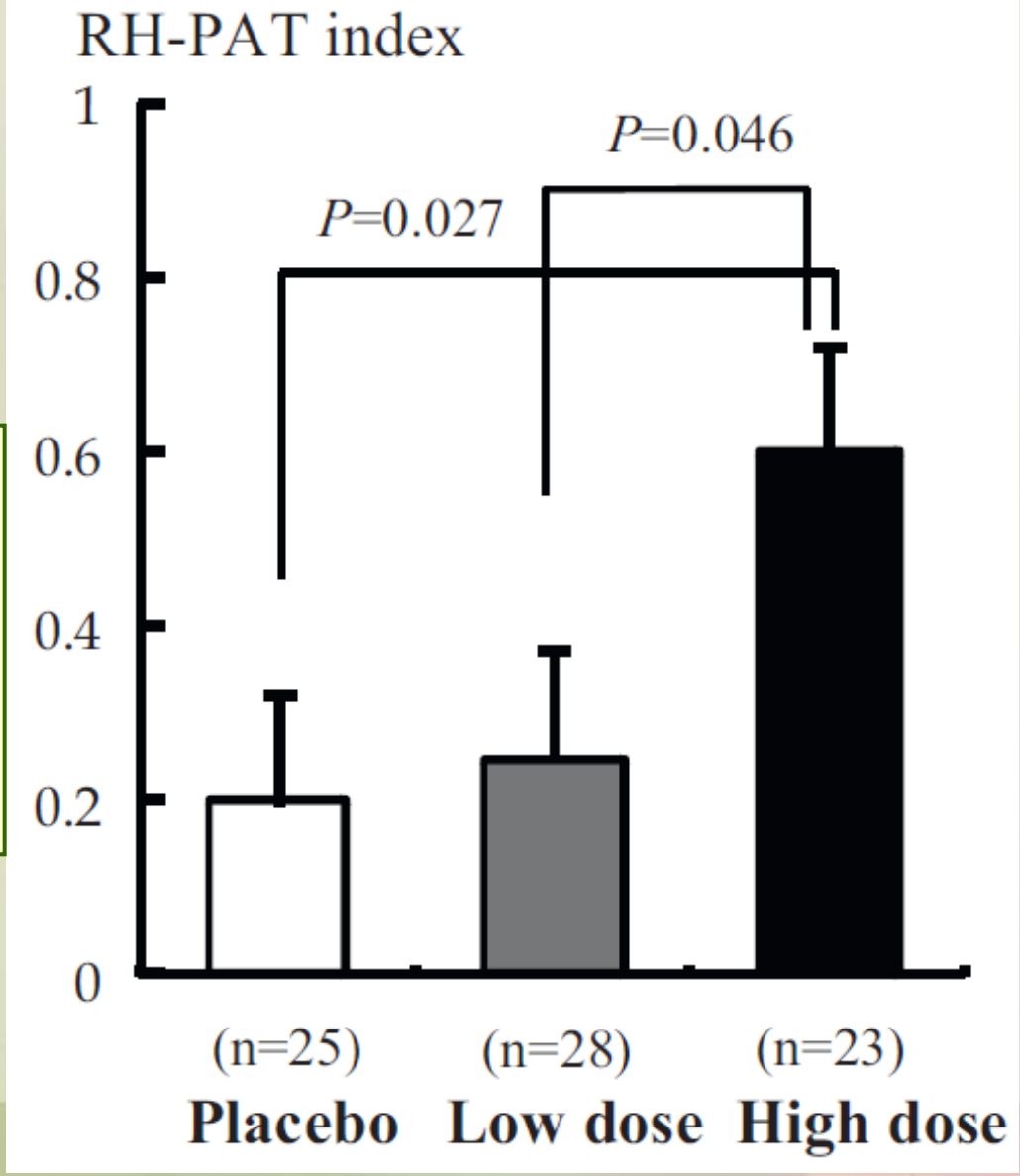
Changes in adhesion molecules ICAM and VCAM



Kim et al. Atherosclerosis, 2011. 215(1): p. 189-95.

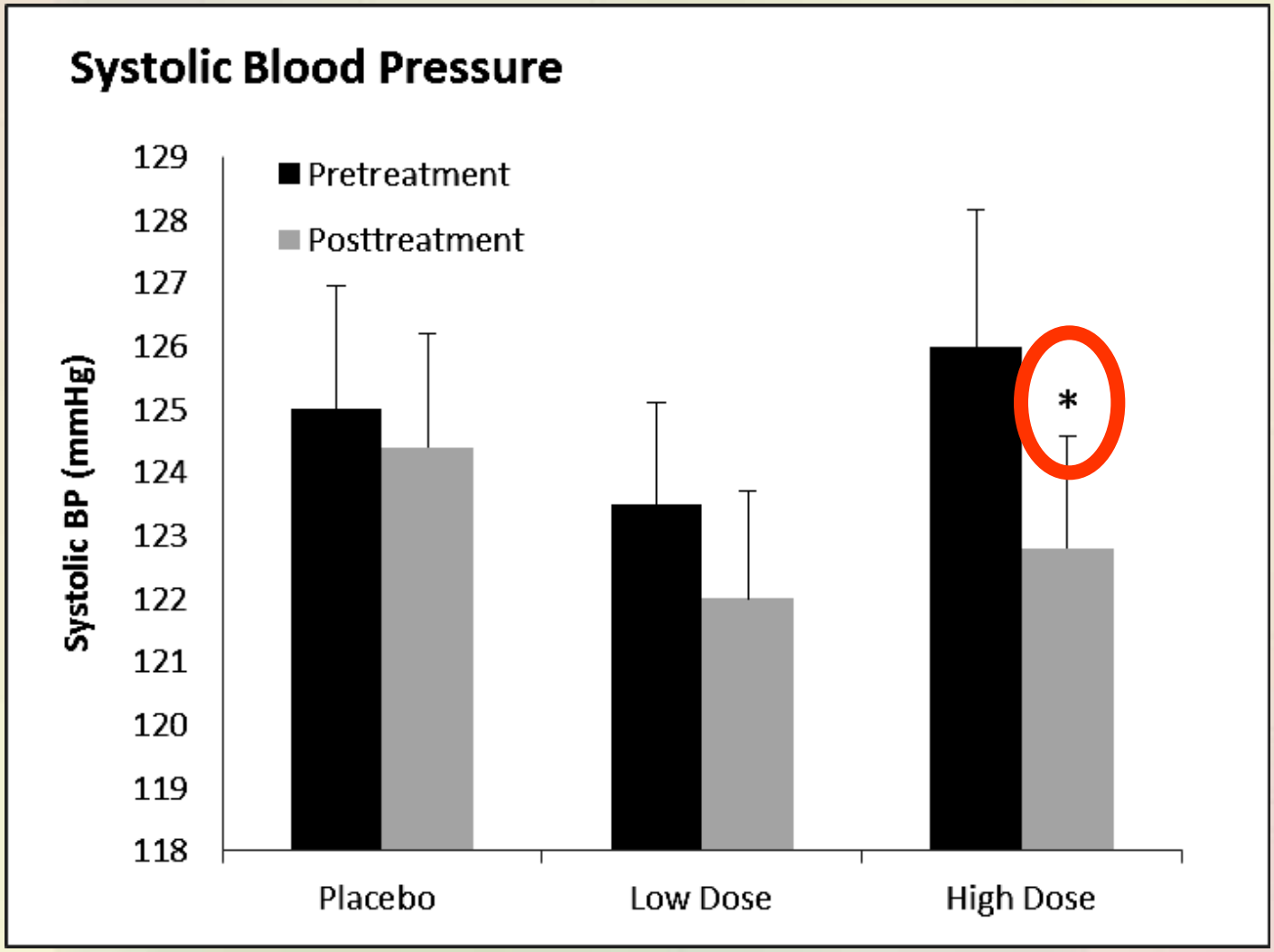
Endothelial Function improved after Lyc-O-Mato® consumption

Endothelial Function measured by Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT)



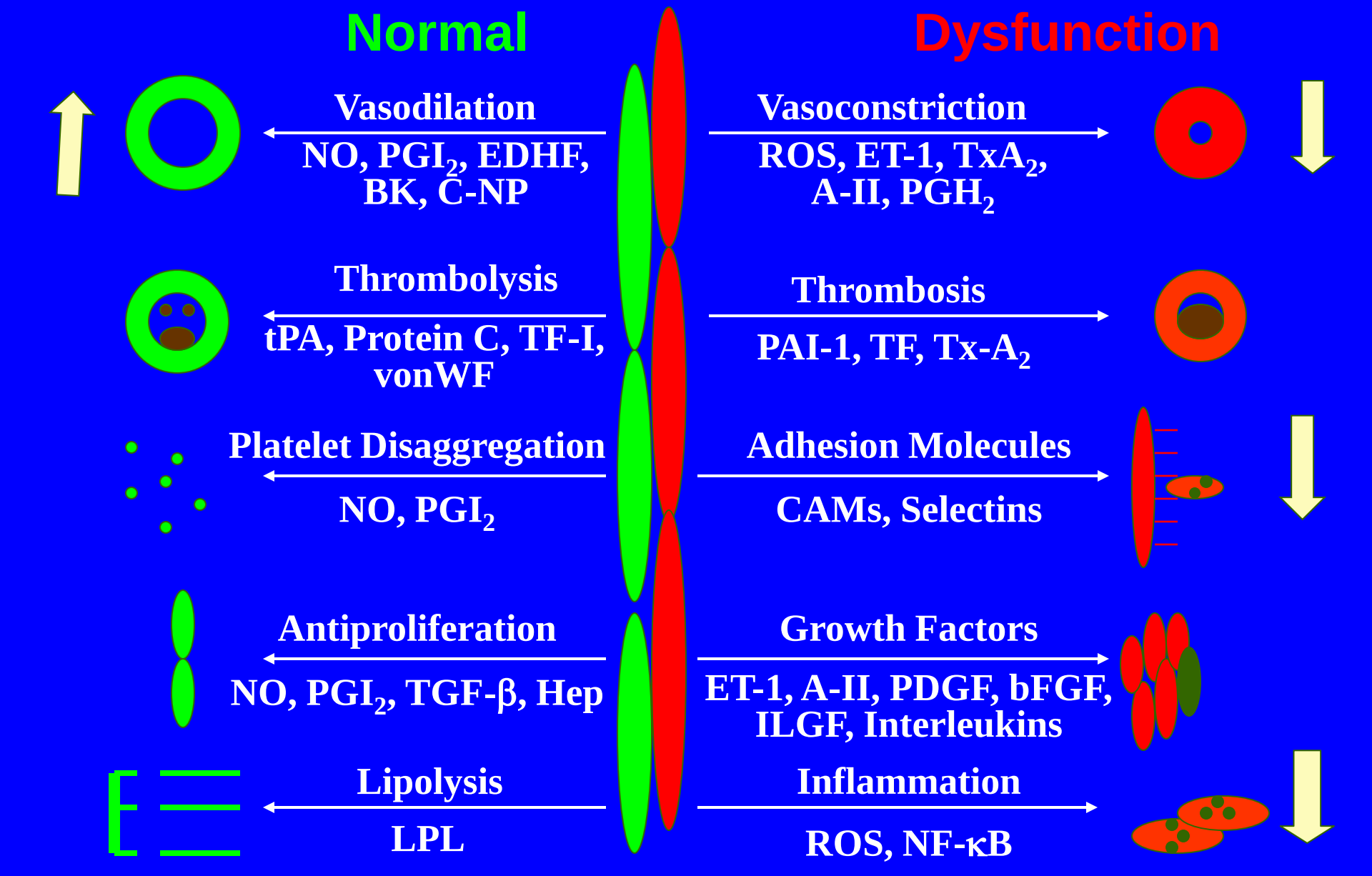
Kim et al Atherosclerosis, 2011. 215(1): p. 189-95.

Systolic blood pressure was reduced after Lyc-O-Mato® consumption



Kim et al Atherosclerosis, 2011. 215(1): p. 189-95.

Regulatory Functions of the Endothelium



Anti-inflammatory effect in animal models

studies carried out in:

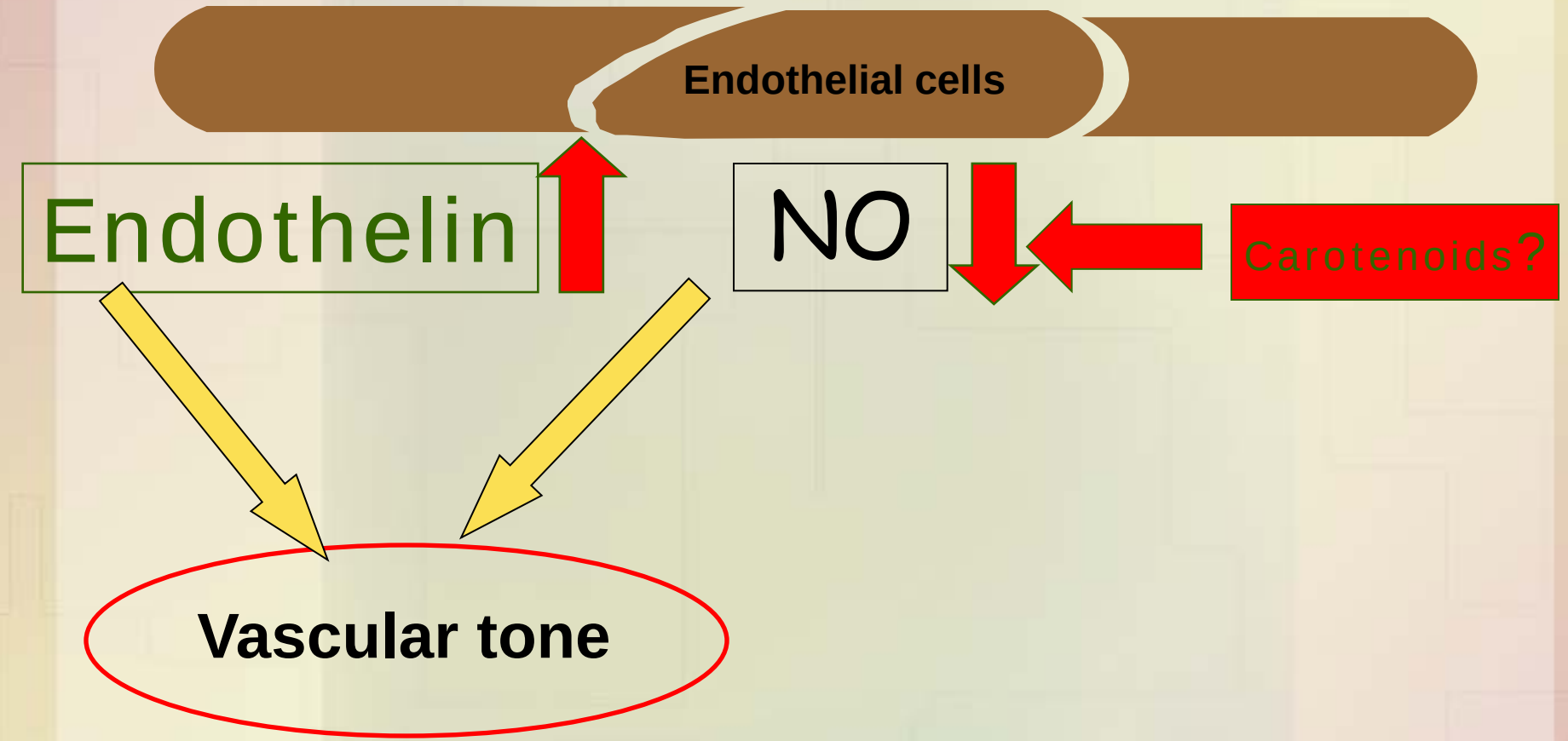
- in vitro – mouse macrophages
- in vivo – animal models:

  Paw edema in rats

  Peritonitis in mice

By Prof Rachel Levy
Ben-Gurion University

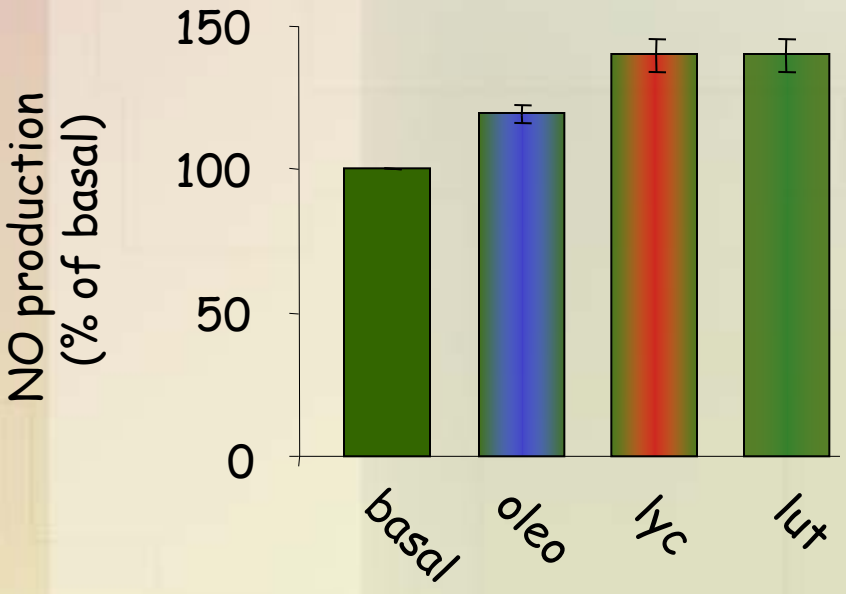
Endothelial dysfunction

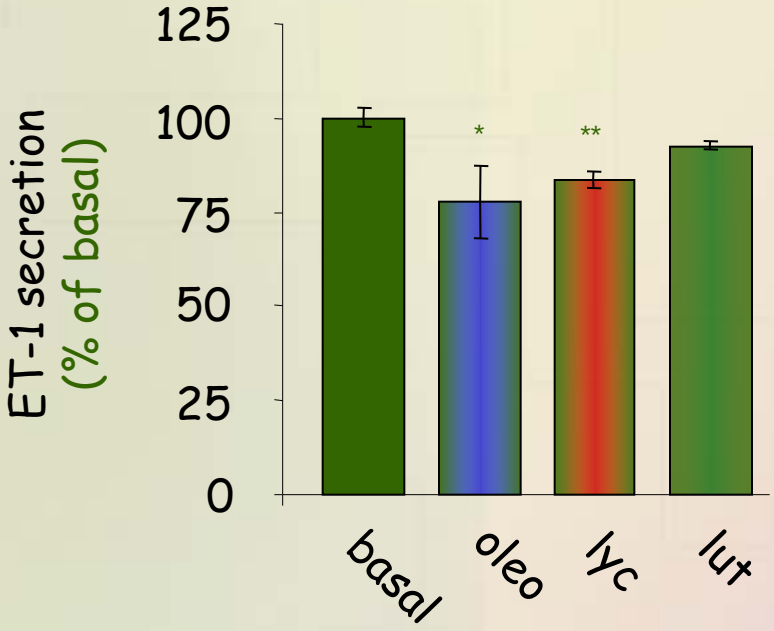


Effect of carotenoids on NO induction and ET-1 secretion

NO induction

ET-1 secretion





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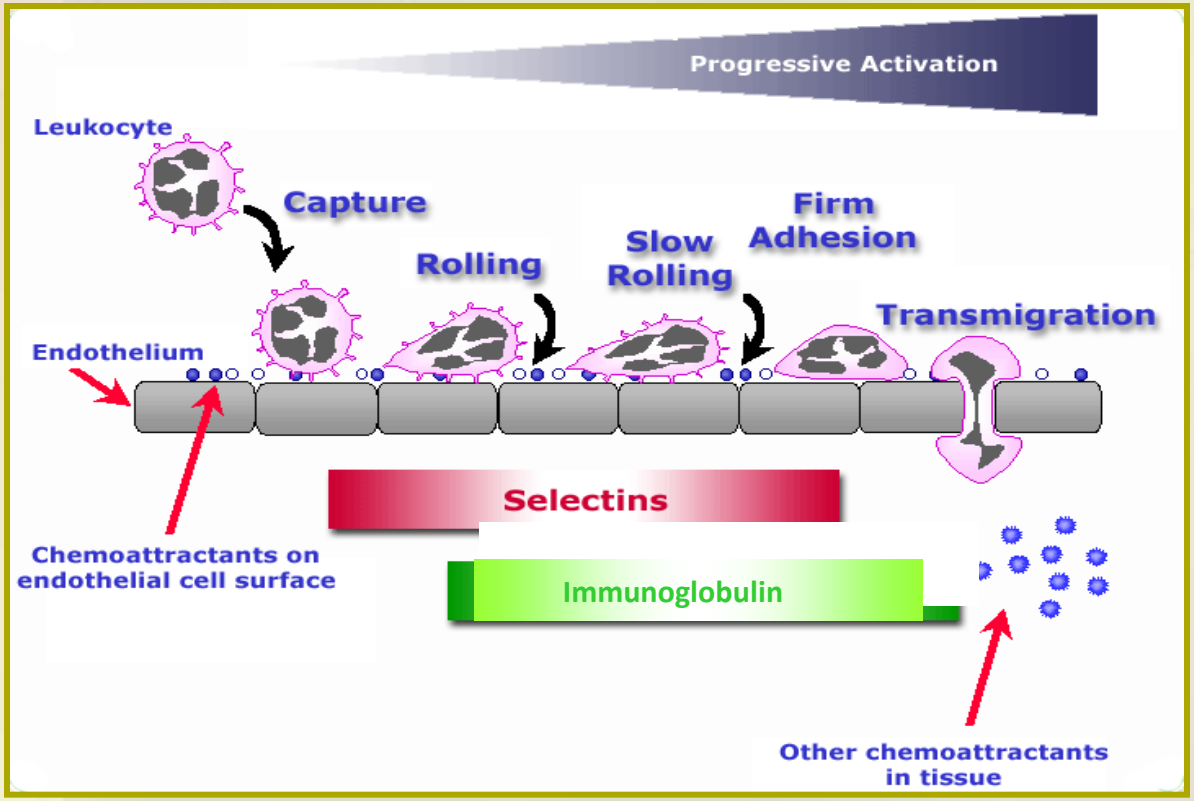
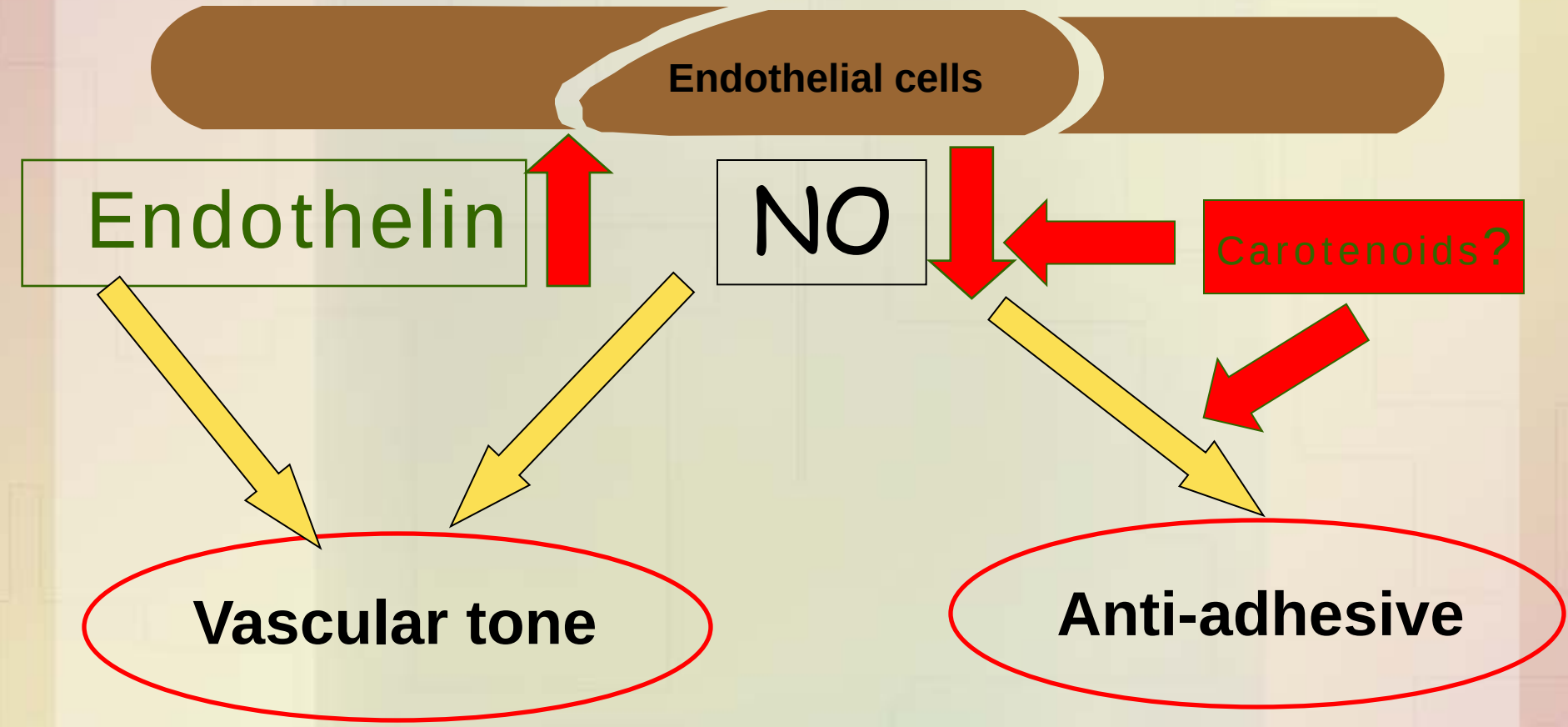
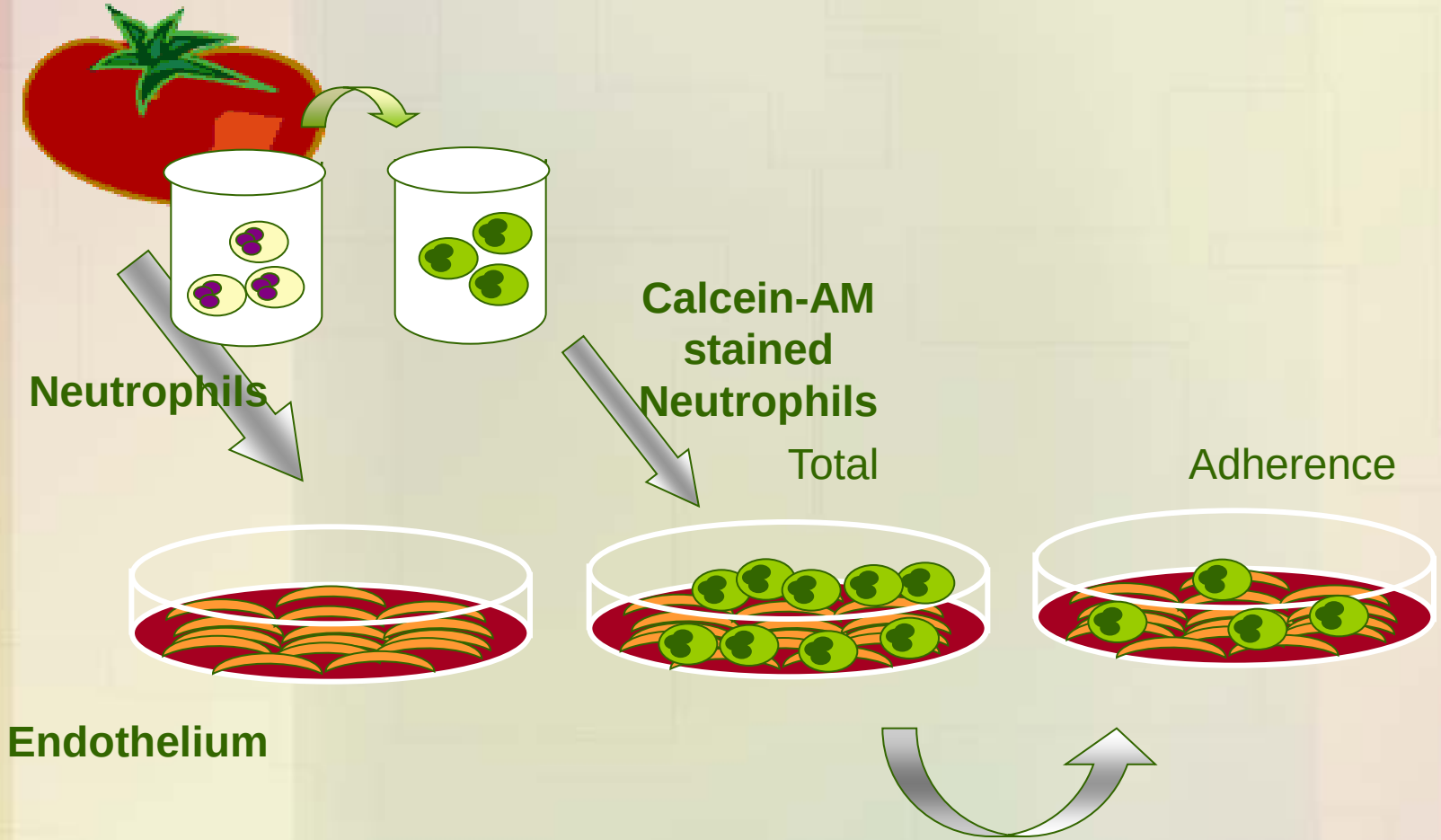
Adhesion of leukocytes



Endothelial dysfunction

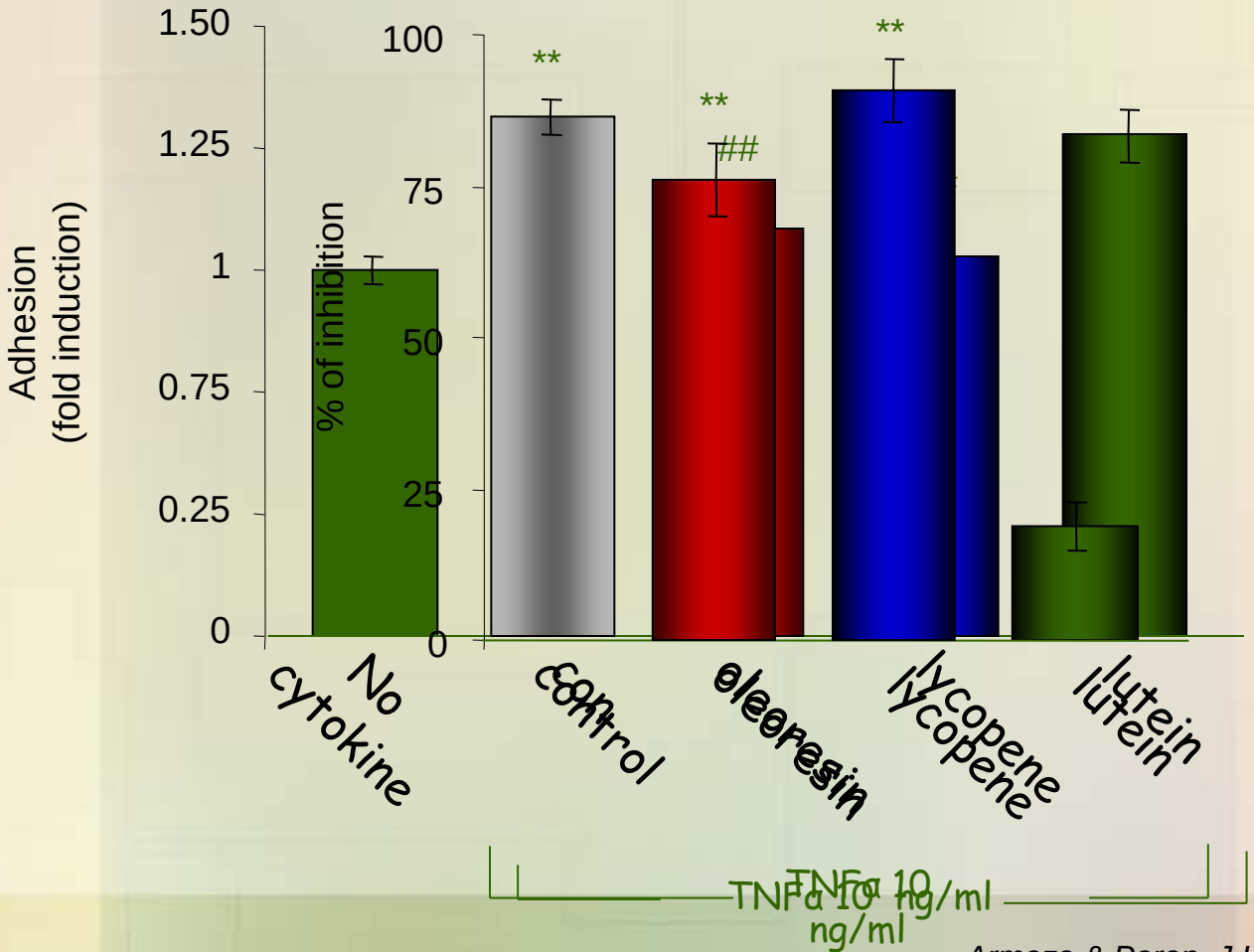


Adhesion assay



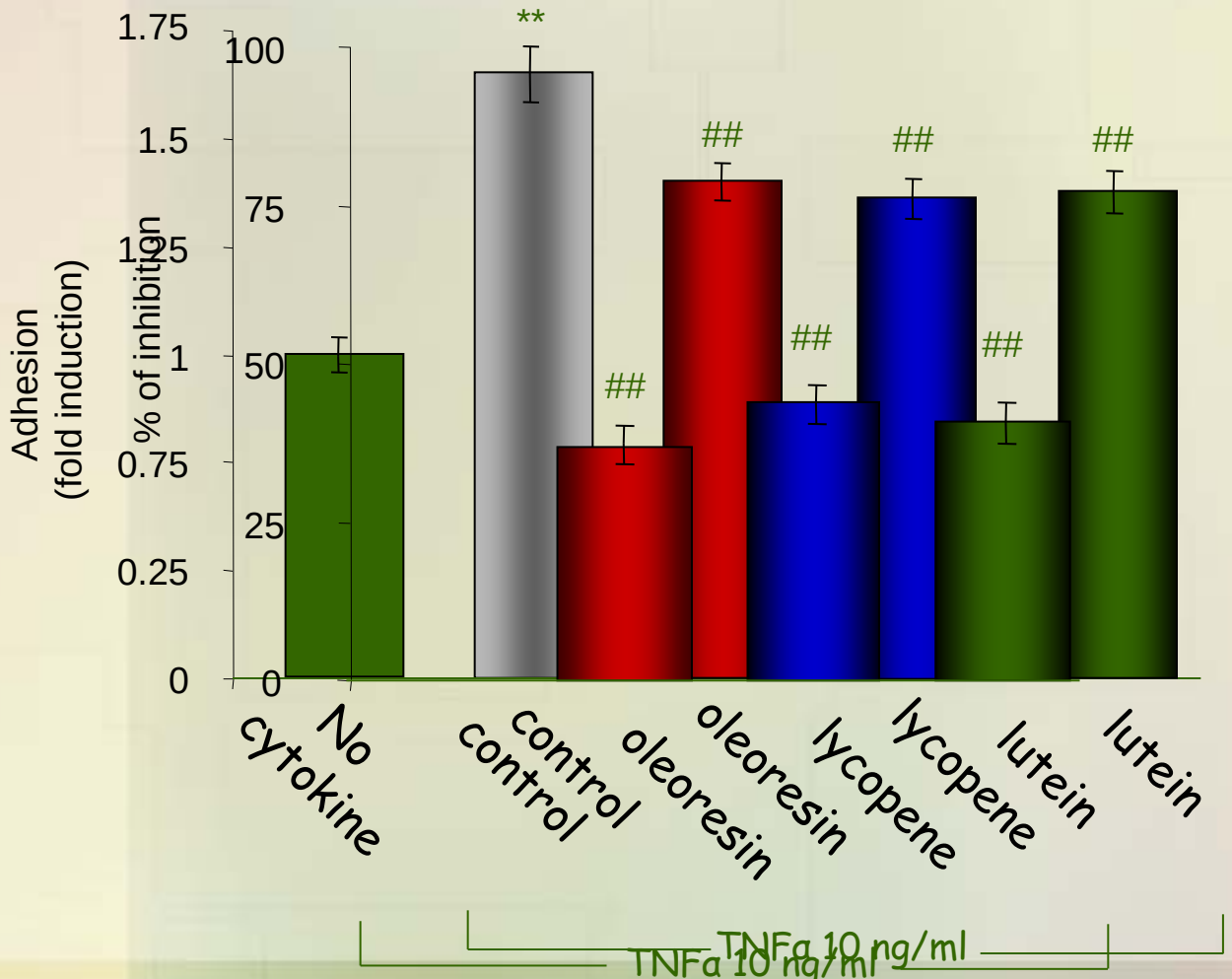
1. Lycopene, Lutein and tomato oleoresin inhibit the adhesion of white blood cells to stimulated EC

A **EA.hy 926** (cell-line)



.Armoza,&.Paran J Hypertension Dec 2012

B hUVEC (primary cell culture)



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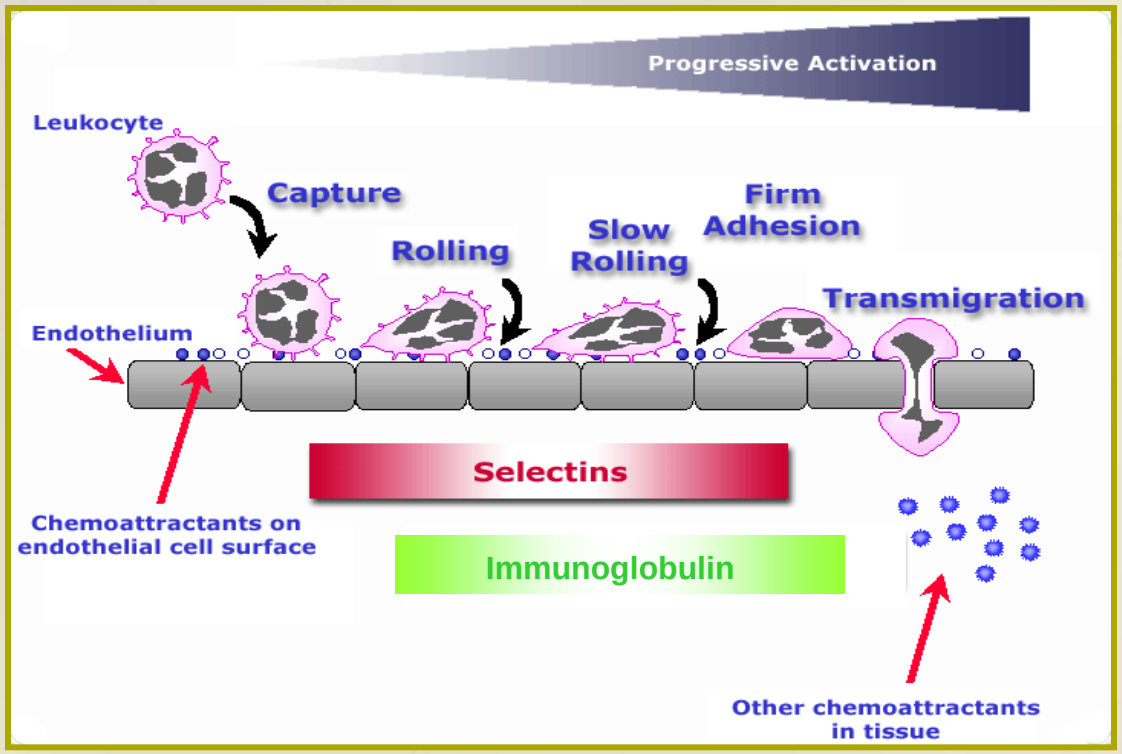
Adhesion of leukocytes



selectins

P-selectin (C,I)
E-selectin (I)

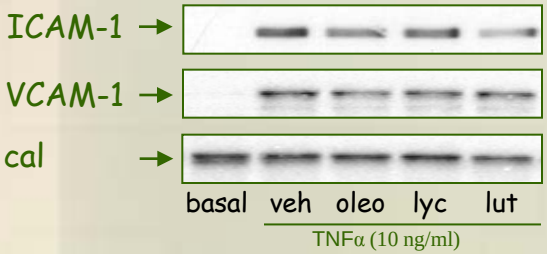
Immunoglobulin

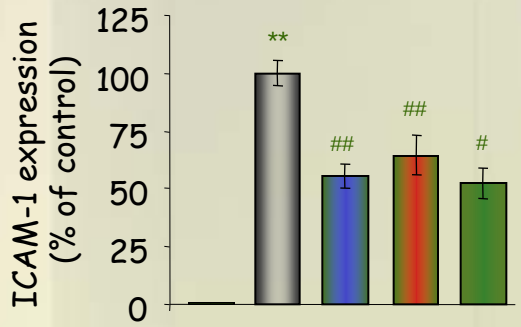
ICAM-1 (C,I)
ICAM-2 (C)
VCAM-1 (C,I)

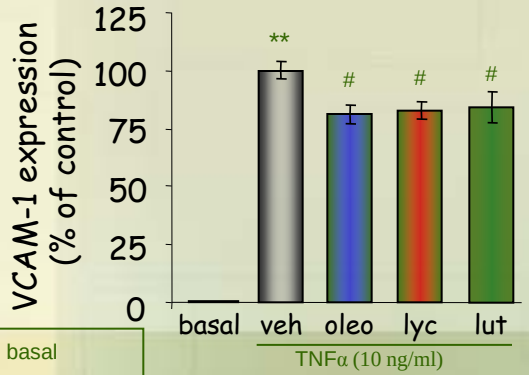
NFkB

2a. Carotenoids and tomato-oleoresin reduce expression of adhesion molecules (ICAM-1 & VCAM-1) in stimulated EC

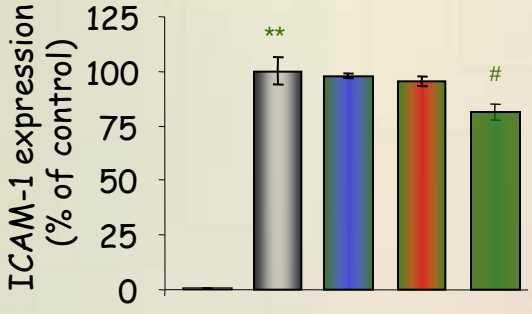
A EA.hy 926 cells

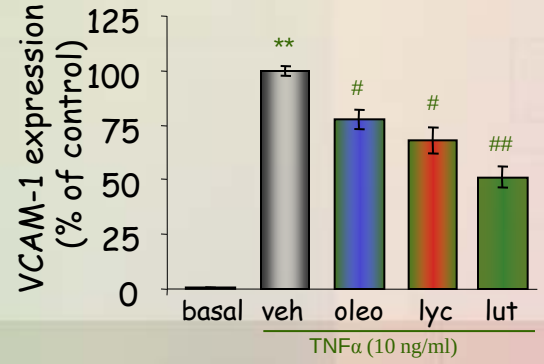






B hUVEC





** p<0.001, * p<0.05 vehicle vs. basal

p<0.001, # p<0.05 treatments vs. vehicle

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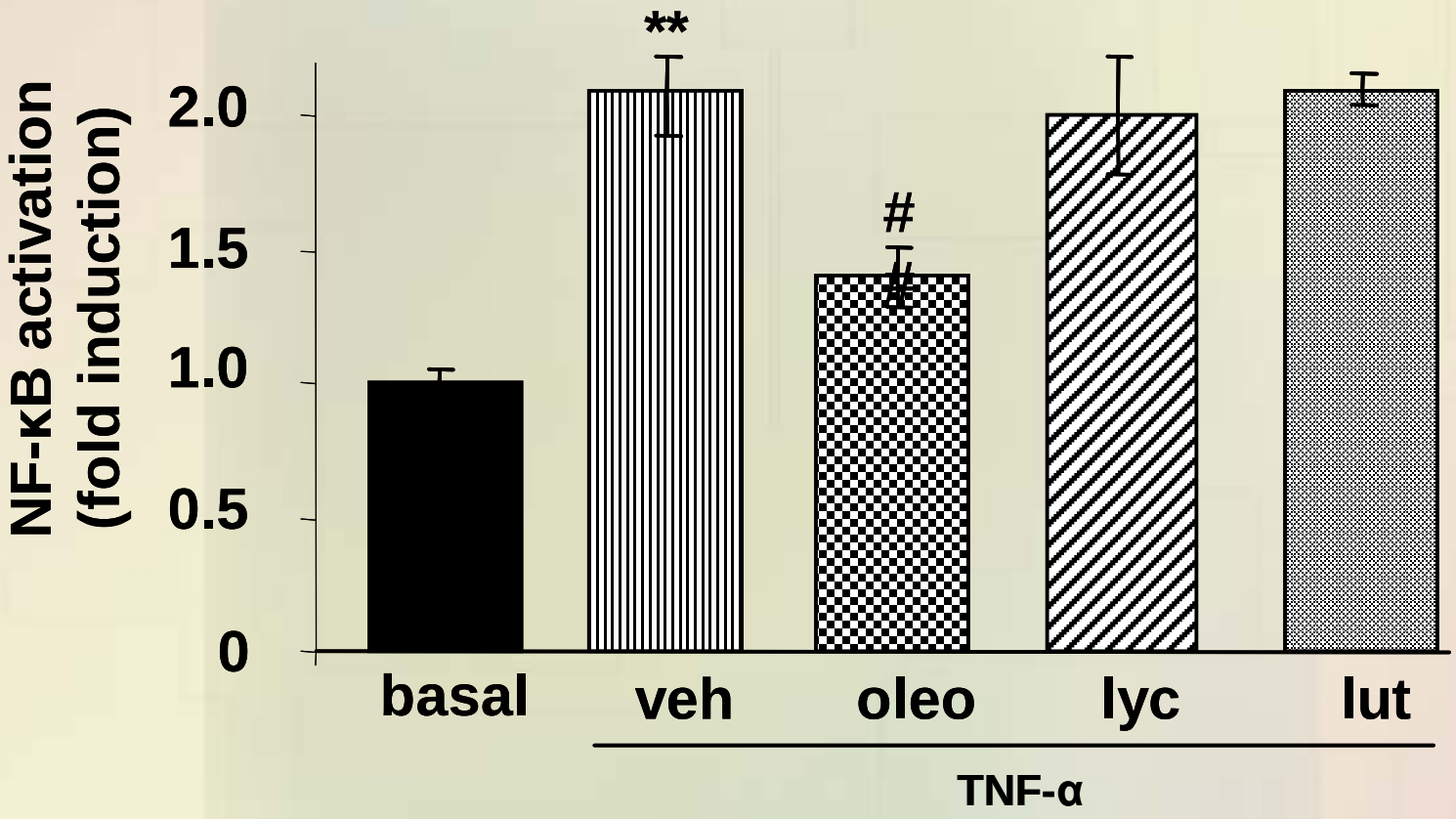
Effects of carotenoids on TNF-α-induced NF-κB activation in endothelial cells



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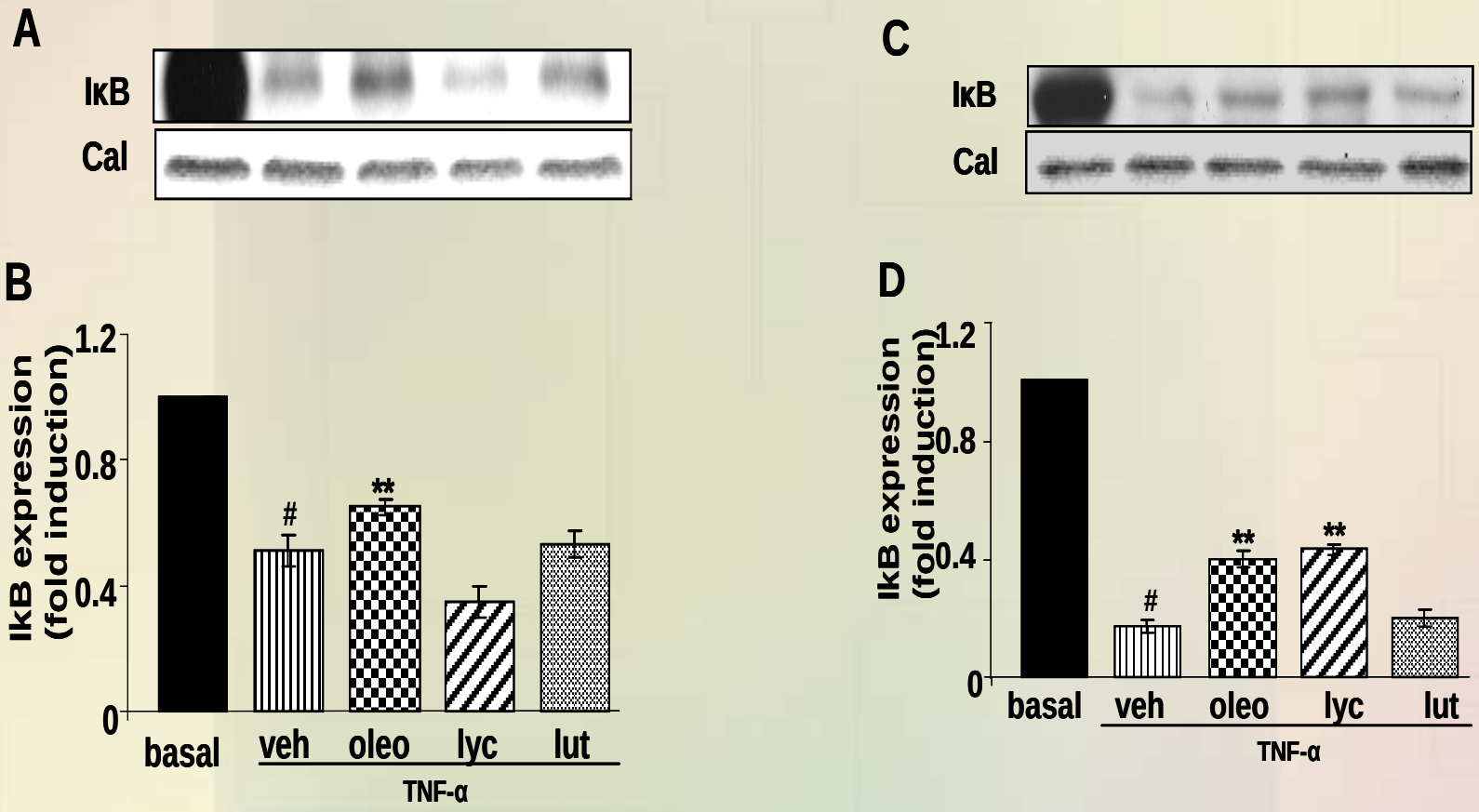
To further investigate whether carotenoids act through regulation of NF-κB pathway, the effects of the carotenoids on dislocation of NF-kB-complex components (IkB, p65 and p50) were tested. Thirty minutes following exposure to TNF-α dramatic reduction in cytoplasm IkB was detected in both cell types

Reduction in cytoplasm IkB



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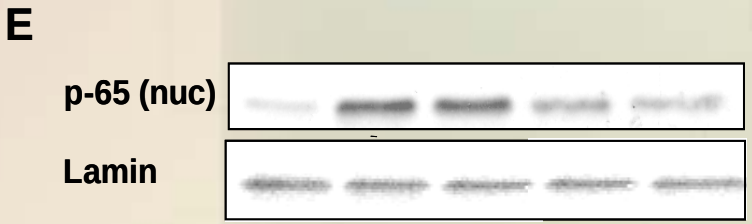
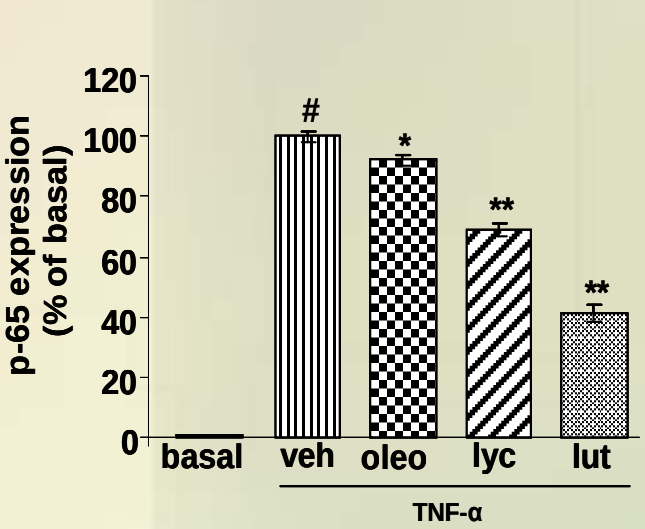
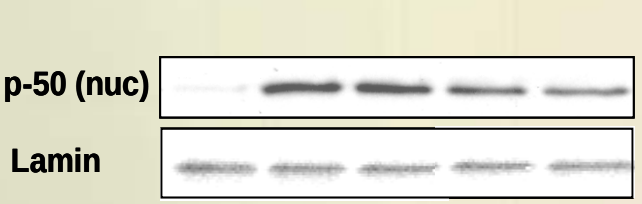
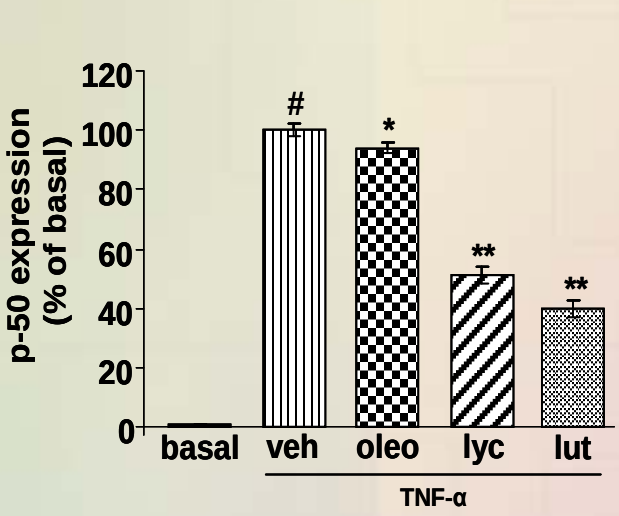
Reduction in the production of the subunits P65 and P50









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Conclusions

(*in-vitro models*)

- Carotenoids attenuate the adhesion of neutrophils to stimulated endothelial cells.
- Carotenoids reduce expression of adhesion molecule ICAM-1
- Carotenoids inhibit activation of transcription factor NFkB

Carotenoids inhibit vasoconstrictive and pro-inflammatory effects and improve endothelial function


Thank you