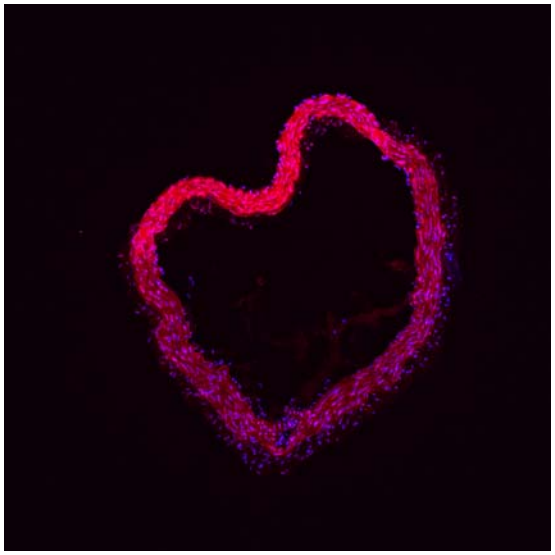
INSTITUT DE CARDIOLOGIE DE MONTRÉAL

Université de Montréal

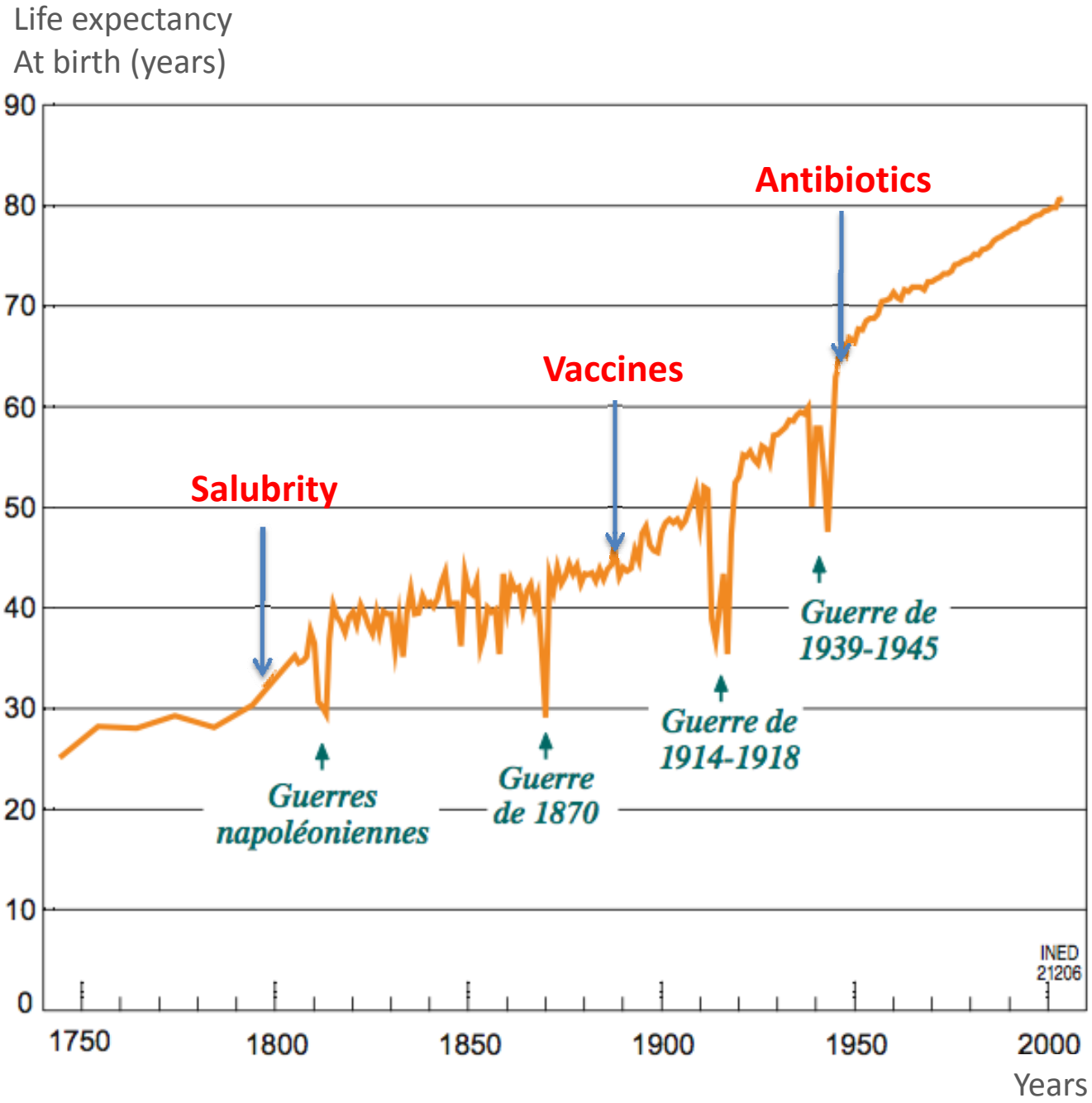


Early environmental changes influence long-term vascular function in mice

Eric Thorin
Department of Surgery

The Dose-Response Society

Life expectancy at birth in France from 1740 to 2005



Centenarians:

France: 1/4000
Japan: 1/5000
Canada: 1/8000

Genetics is too simple to explain aging

What is aging: definition

Aging = Entropy

Degradation of organized matter

No Old Man Ever Forgot Where He Buried His Treasure: Concepts of Cognitive Impairment in Old Age Circa 1700

Daniel Schäfer, PhD, MD

Current concepts on aging

The biochemical basis of aging in general, and of vascular aging in particular, is the progressive failure of maintenance and repair systems due to cumulative molecular damage leading to cell death.

BUT, the structure of the vascular system collapses from “wear and tear”.

Therefore, to limit the consequences needs to target cells that regenerate the matrix.

"It has been said that one is as old as one's arteries. In view of the supreme importance of endothelium in arterial function, I should like to modify… this statement by saying that one is as old as one's endothelium."

R. Altschul 1954

Aging is No Longer an Unsolved Problem in Biology

Ann. N.Y. Acad. Sci. 1067: 1–9 (2006).

ROBIN HOLLIDAY

maintenance and repair systems:

1. the multiple pathways of DNA repair, which are vital for the removal of spontaneous lesions in DNA;
2. the defenses against oxygen-free radicals, which include antioxidants and enzymes;
3. the removal of defective proteins by proteases;
4. protein repair, such as the renaturation of proteins by chaperones, and the enzymic reversal of oxidization of amino acids;
5. the accuracy of synthesis of macromolecules, which depends on proof-reading mechanisms;
6. the immune response against pathogens and parasites;
7. the detoxification of harmful chemicals in the diet by the monooxygenase enzymes coded for by the P450 gene superfamily;
8. wound healing, blood clotting, and the healing of broken bones and torn ligaments;
9. physiological homeostasis, including temperature control;
10. the epigenetic stability of differentiated cells, and the defenses against neoplastic transformation;
11. apoptosis, which is the means of removing unwanted or damaged cells;

Current concepts on aging

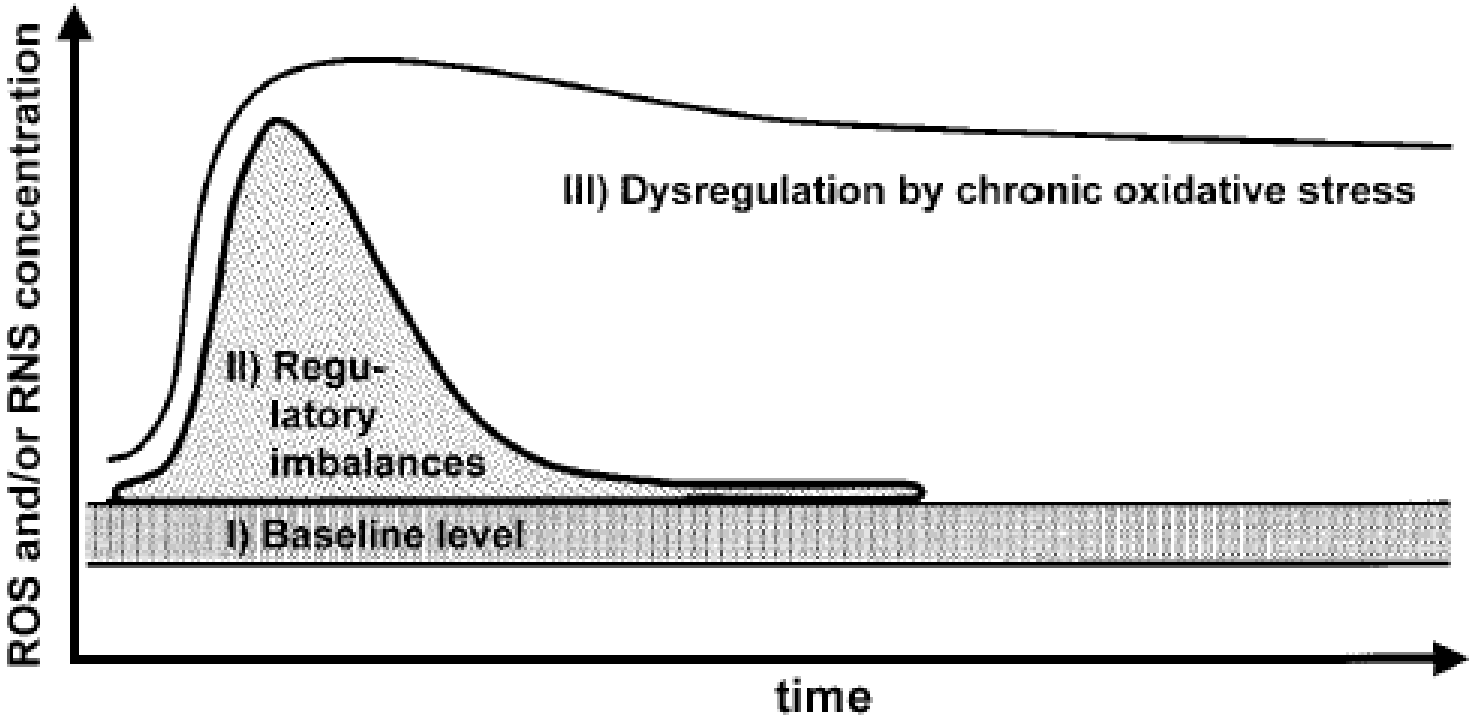
The free radical theory of aging hypothesizes that the accumulation of oxidative damage is a central mediator of the aging process and age-related disorders (D. Harman, 1956).

This theory applies to vascular endothelial aging.

Current concepts on aging

ROS also play a crucial role in physiologic cell function, when present at a low physiologic concentration.

Valko M *et al*, Int J Biochem Cell Biol . 2006

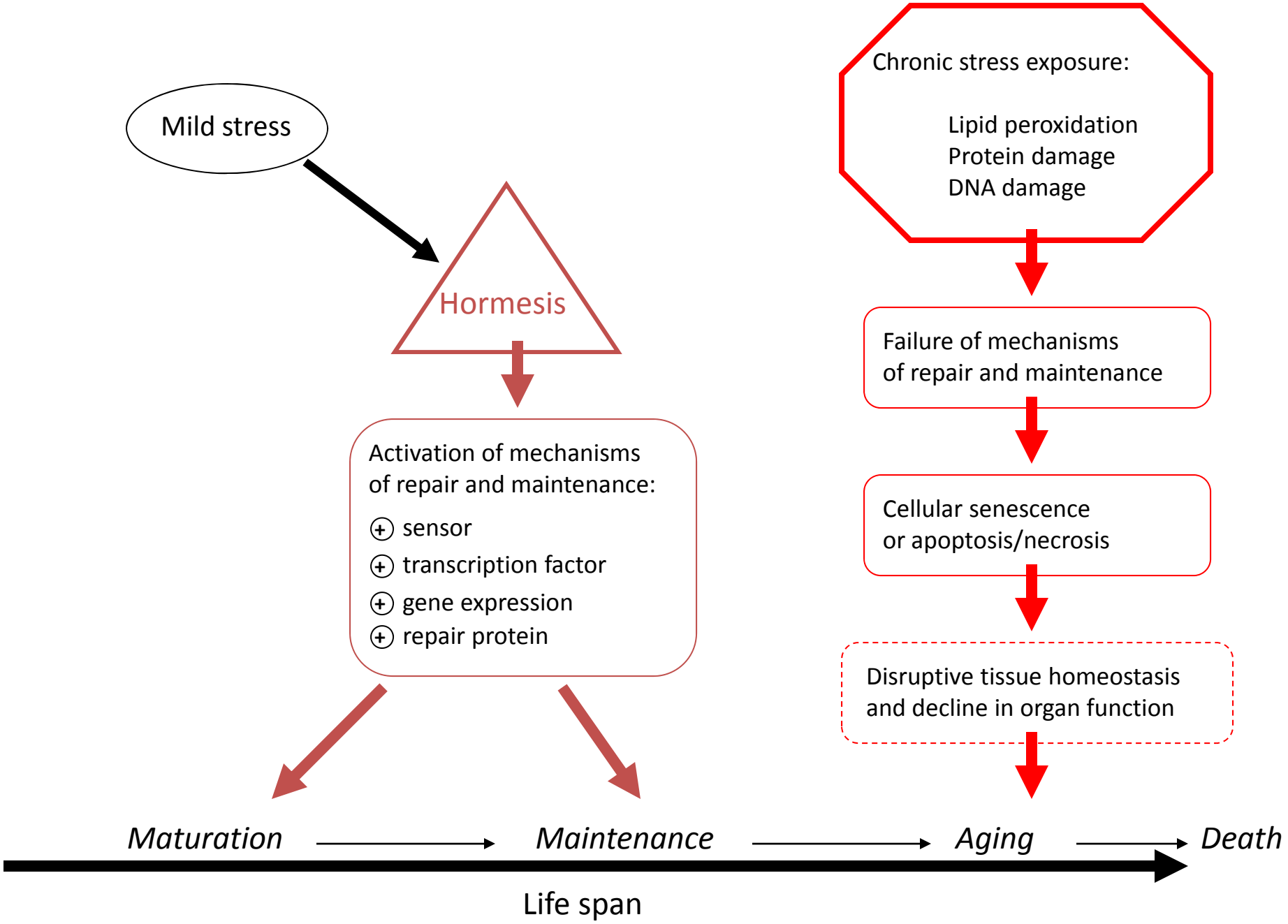


Dröge W, Physiol Rev. 2002

Our hypothesis

Exposure to mild oxidative stress during the maturation phase of the endothelium will activate protective pathways involved in stress resistance.

The exposure of the endothelium to physiologic oxidative stress during its maturation phase determines vascular longevity.


Mild stress
Hormesis
Activation of mechanisms
of repair and maintenance:
⊕ sensor
⊕ transcription factor
⊕ gene expression
⊕ repair protein
Chronic stress exposure:
Lipid peroxidation
Protein damage
DNA damage
Failure of mechanisms
of repair and maintenance
Cellular senescence
or apoptosis/necrosis
Disruptive tissue homeostasis
and decline in organ function
Maturation
Maintenance
Aging
Death
Life span

There are controversies to the free radical theory of aging because of the lack of understanding of the mechanisms of aging:

under oxidative stress or not?

The overexpression of major antioxidant enzymes does not extend the lifespan of mice

Aging Cell (2009) **8**, pp73–75

Viviana I. Pérez,[1,2] Holly Van Remmen,[1,2,3,4] Alex Bokov,[1,3] Charles J. Epstein,[5,6] Jan Vijg[7] and Arlan Richardson[1,2,4]

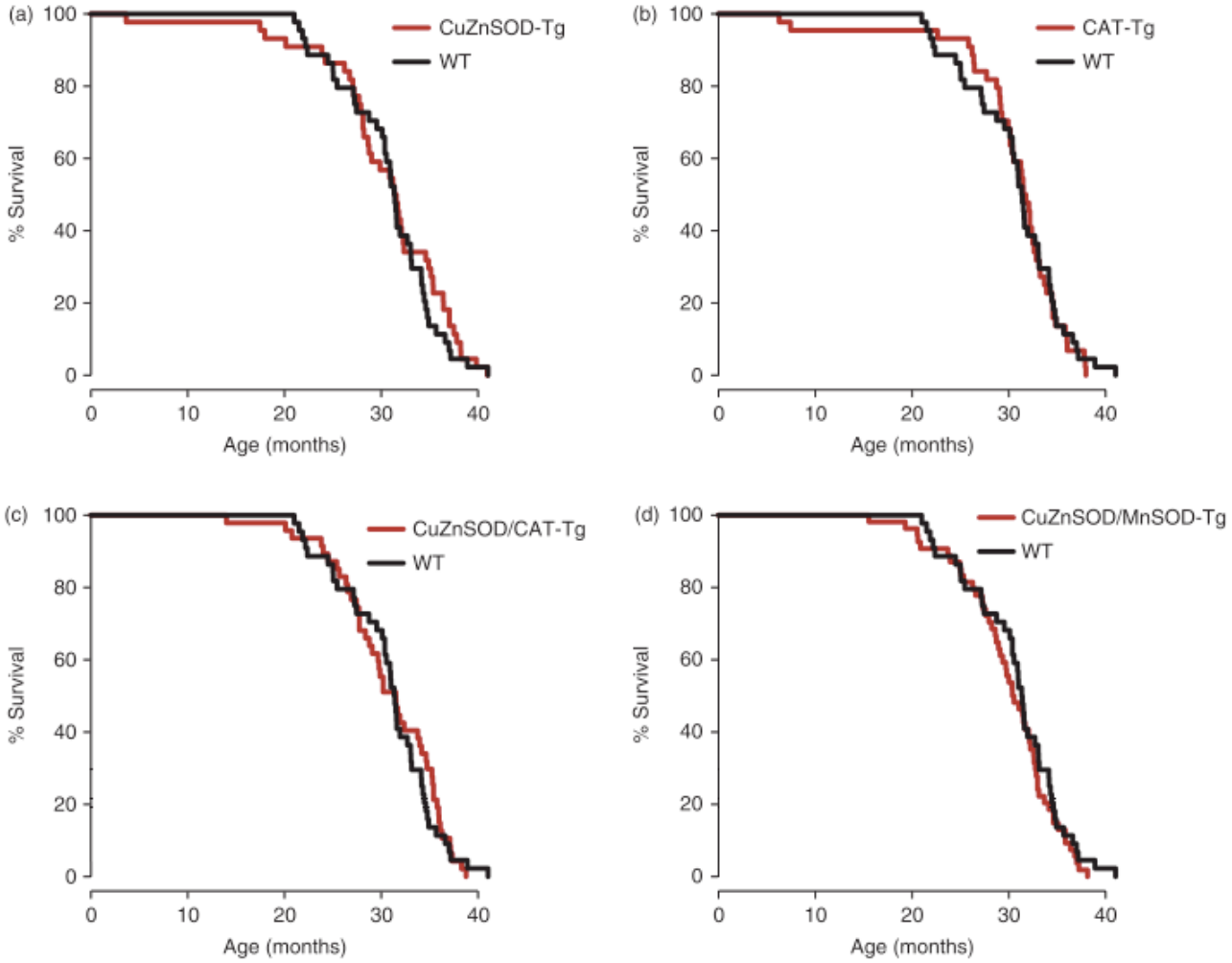


Fig. 1 Lifespans of male transgenic mice overexpressing various antioxidant enzymes. Survival studies were conducted as described by Ran *et al.* (2007), and all mice were on the C57BL/6J background. The mice were housed four animals per cage starting at 2 months of age and fed a commercial mouse chow (Teklad Diet LM485) *ad libitum*. The survival of the WT cohort is presented in each of the graphs. The number of animals and the survival data are given in Table 1.

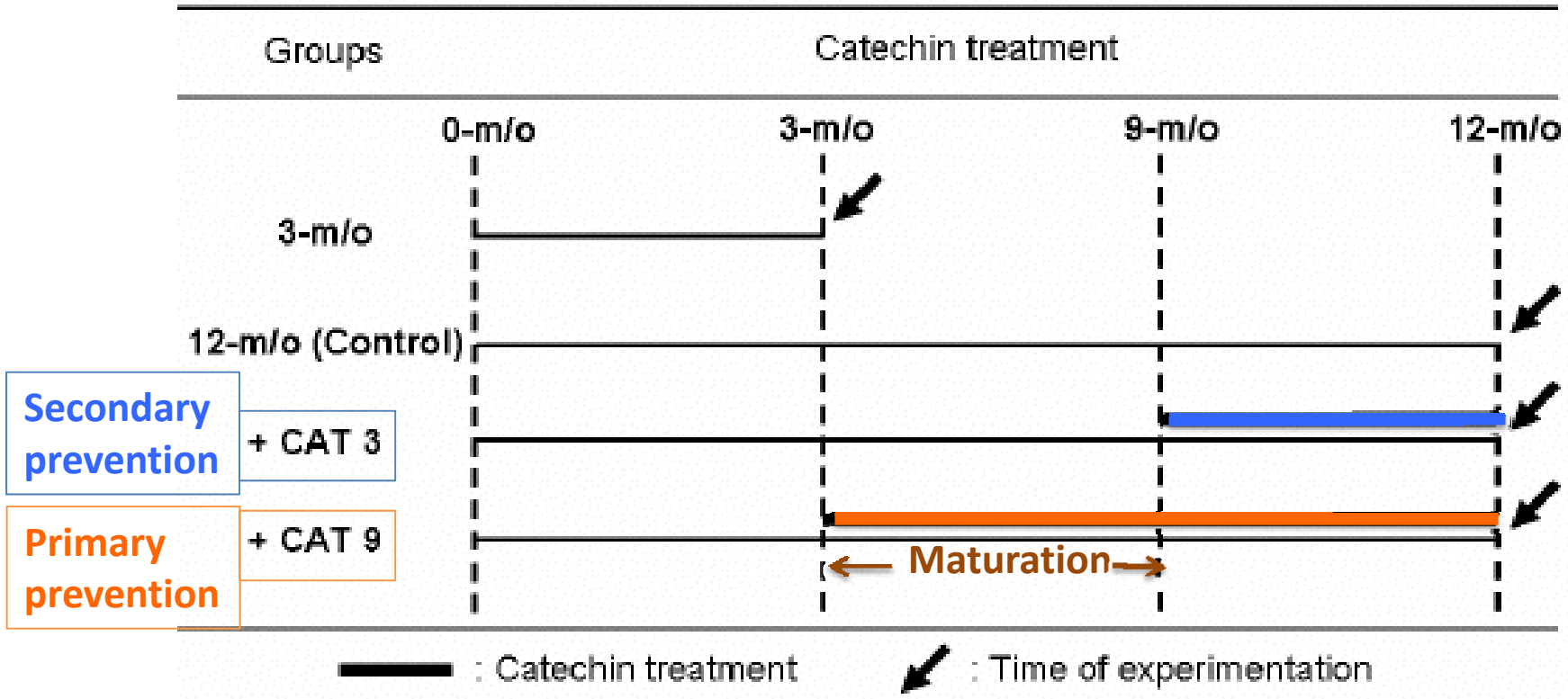
Clinical trials with antioxidants have failed to demonstrate efficacy at limiting the progression of age-related diseases:

intervention too late? too short? with the wrong drug?

Some clinical trials:

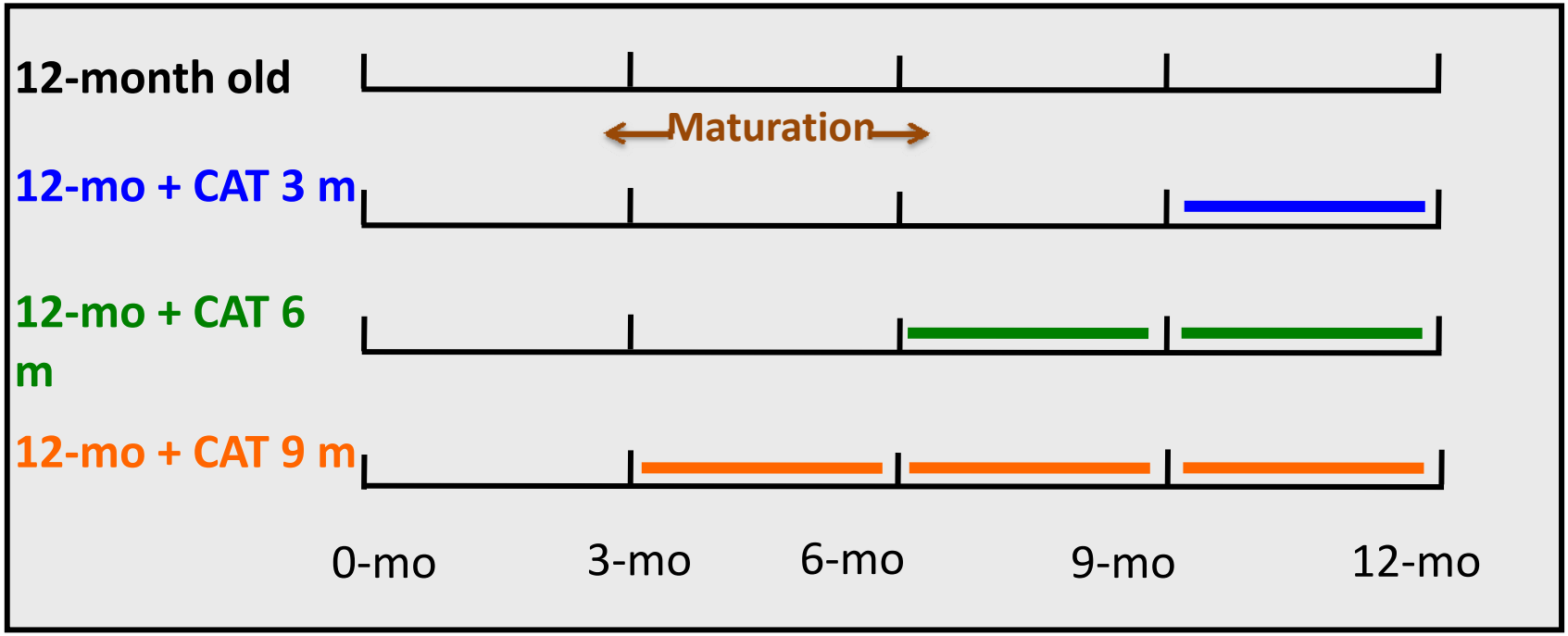
Trial	Study type	Intervention	Outcome
Yusuf *et al* (HOPE study) 2000	9,541 W&M >55y with CVD. F/up >4.5y	Vit E 400 IU/d ± ACEI	No significant effect on all cause mortality or major cardiovasc events
ATBC Cancer prev 1994	29,133 Finnish M smokers 50-69, no history, F/up >8y	Vit E 50 mg/d, ±β-carotene 20 mg/d	idem
Primary prevention project 2001	4,495 Italian W&M, 1 CVRF, >64y, F/up 3.6y	Synthetic α-tocopherol 300 IU/d ±aspirin 100 mg/d	idem
Physician Health Study 1996	22,071 M US physicians, 40-84y, F/up 12y	β-carotene 50 mg alternate day, aspirin 325 mg/d	idem
Skin cancer prevention 1996	1,188 US M & 532W, <85, F/up 8.2y	β-carotene 50 mg/d	idem
Womens Health Study 1999	39,876 US W <45y, no history, F/up 2.1	β-carotene 50 mg alternate day, aspirin 100 mg/d, VitE 600 IU/d	idem

To test our hypothesis that *Exposure to mild oxidative stress during the maturation phase of the endothelium will activate protective pathways involved in stress resistance*



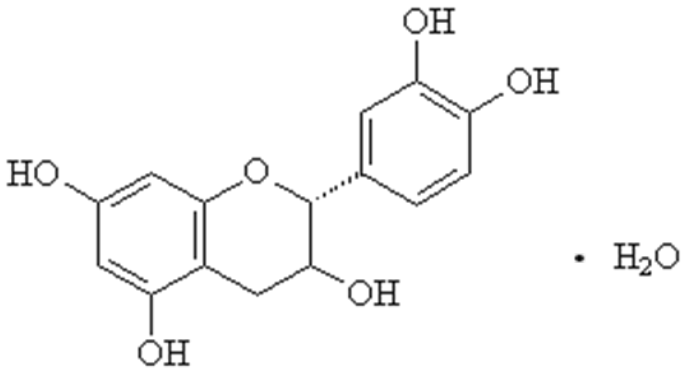
Treatment with catechin, a polyphenol during maturation or after maturation in control mice

Treatments with catechin for the last 6 or 9 months gave similar results: the maturation phase occurs from 3 to 6 months of age



Chronic treatment with catechin (30 mg/kg/day)

(+) Catechin hydrate



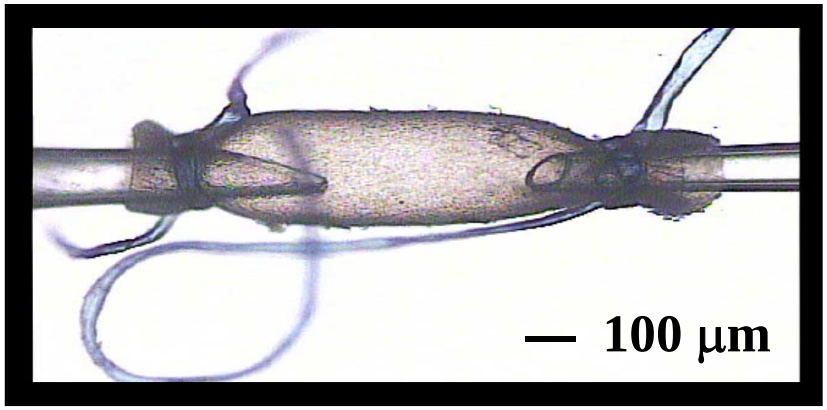
- Catechins are polyphenols and belong to the flavonoid family.
- Catechins are antioxidants that also display large cardioprotective properties, including anti-inflammatory effects (Suzuki *et al.*, Mediators Inflamm, 2009).
- Catechins are found in green tea, in fruits and in vegetables.

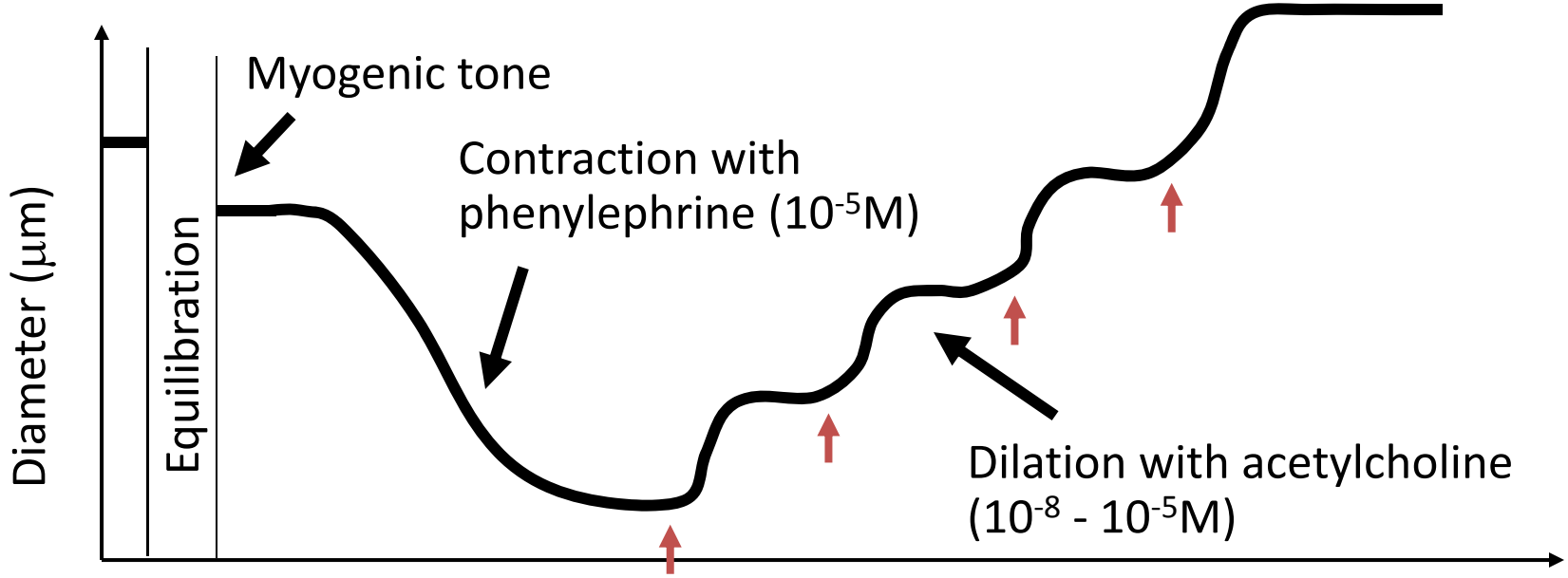
Mice were treated with catechin 30 mg/kg/day.
In humans, 50 mg of catechin ≅ one cup (200 ml) of black tea + 20 g of dark chocolate
≅ 2 large apples

In humans, catechin intake (25-500 mg) from tea or from other sources has been shown to be inversely correlated with ischemic heart disease mortality and to reduce the risk of stroke.

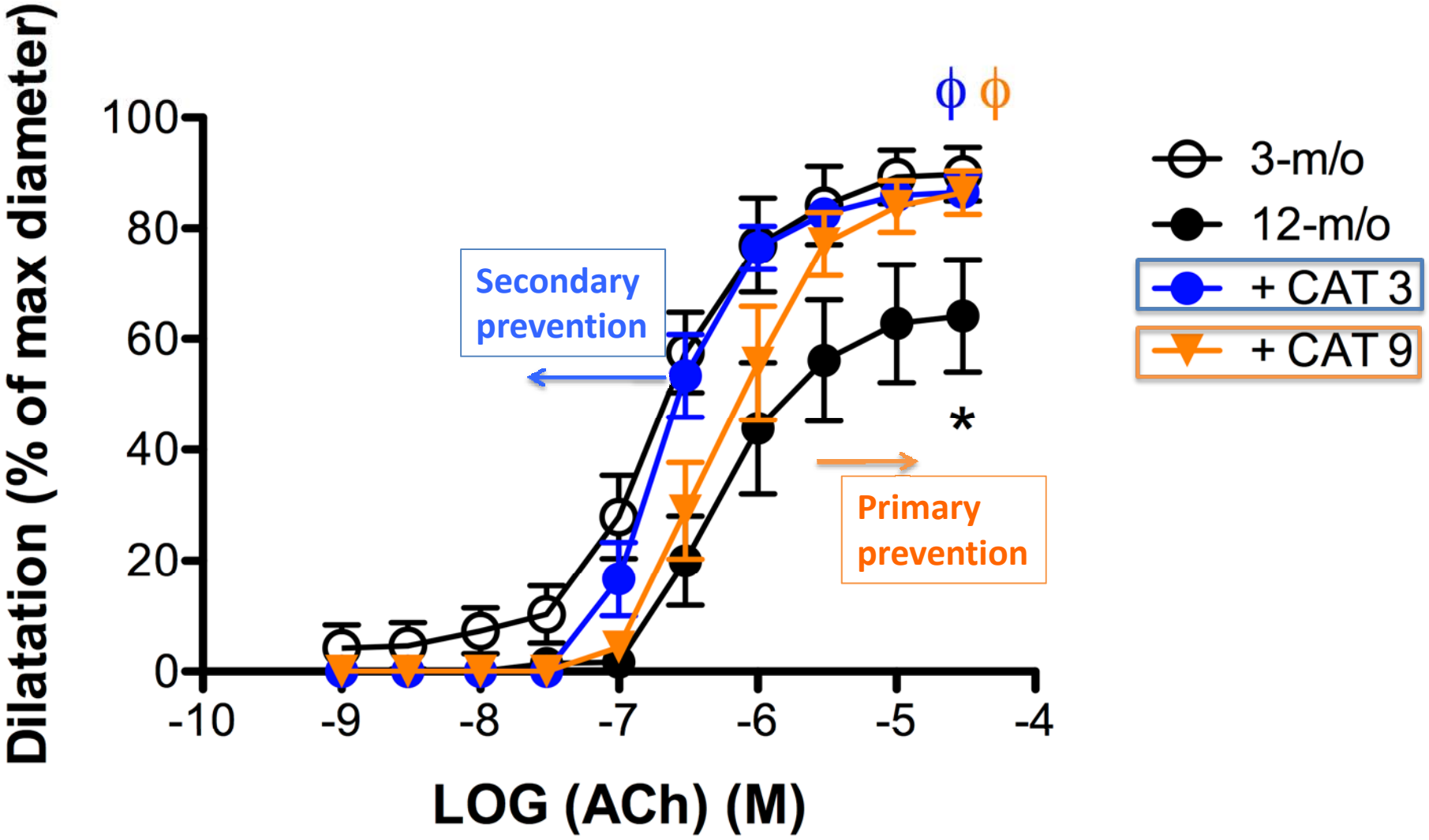
Arab and Liebeskind, *Arch Biochem Biophys*, 2010, 501: 31-36
Arts et al., *Am J Clin Nutr*, 2001, 74: 227-232
Keli et al., *Arch Intern Med*, 1996, 156: 637-642

Pressurized renal mouse artery



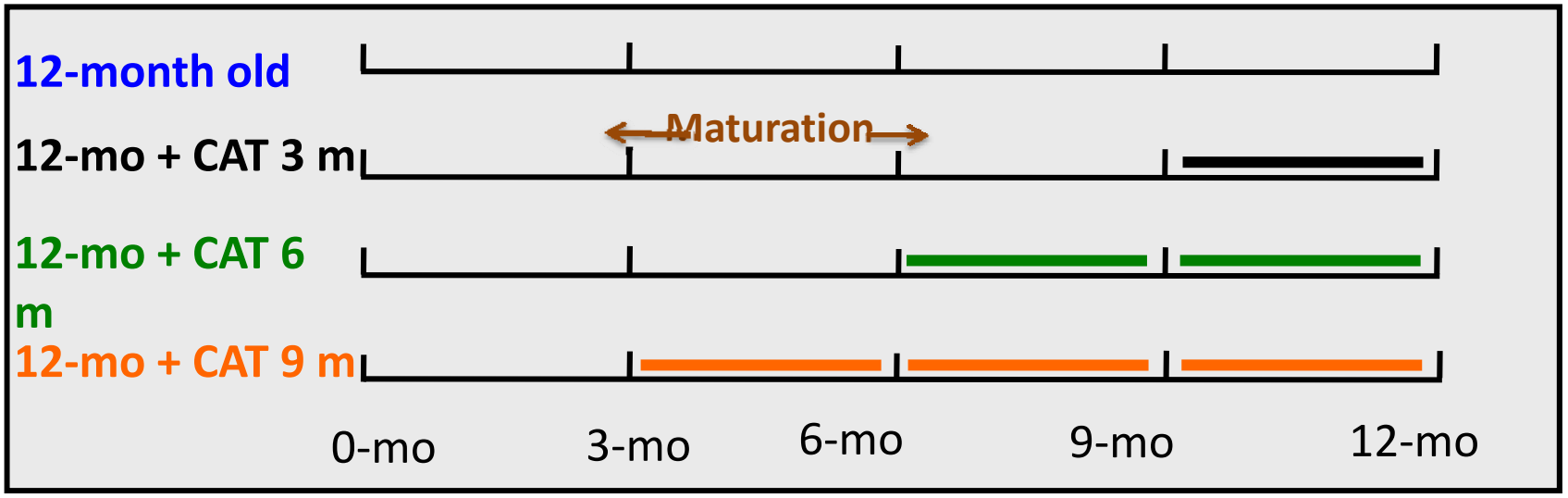


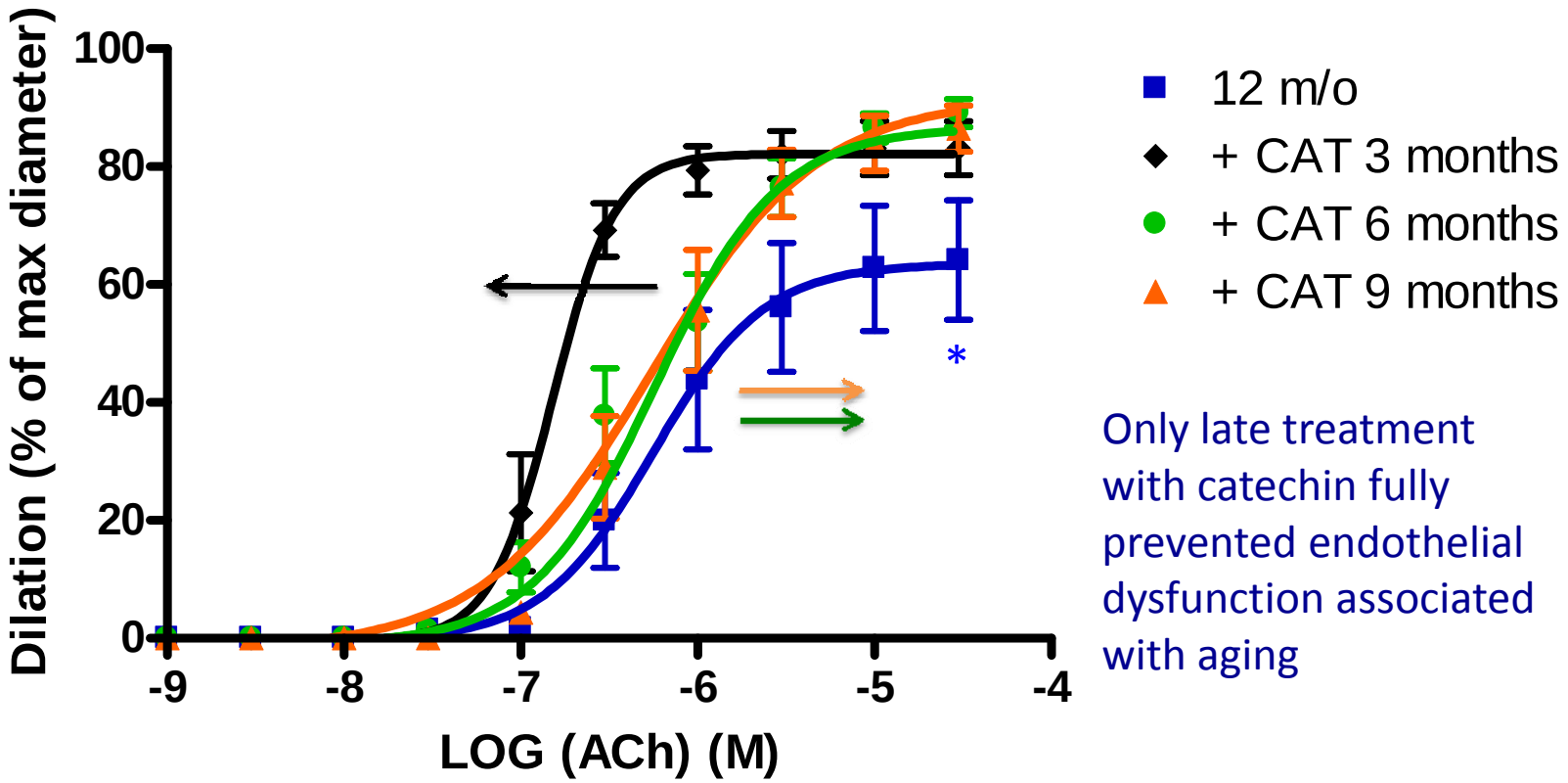
Only late treatment with catechin fully prevented endothelial dysfunction associated with aging



Only late treatment with catechin fully prevented endothelial dysfunction associated with aging

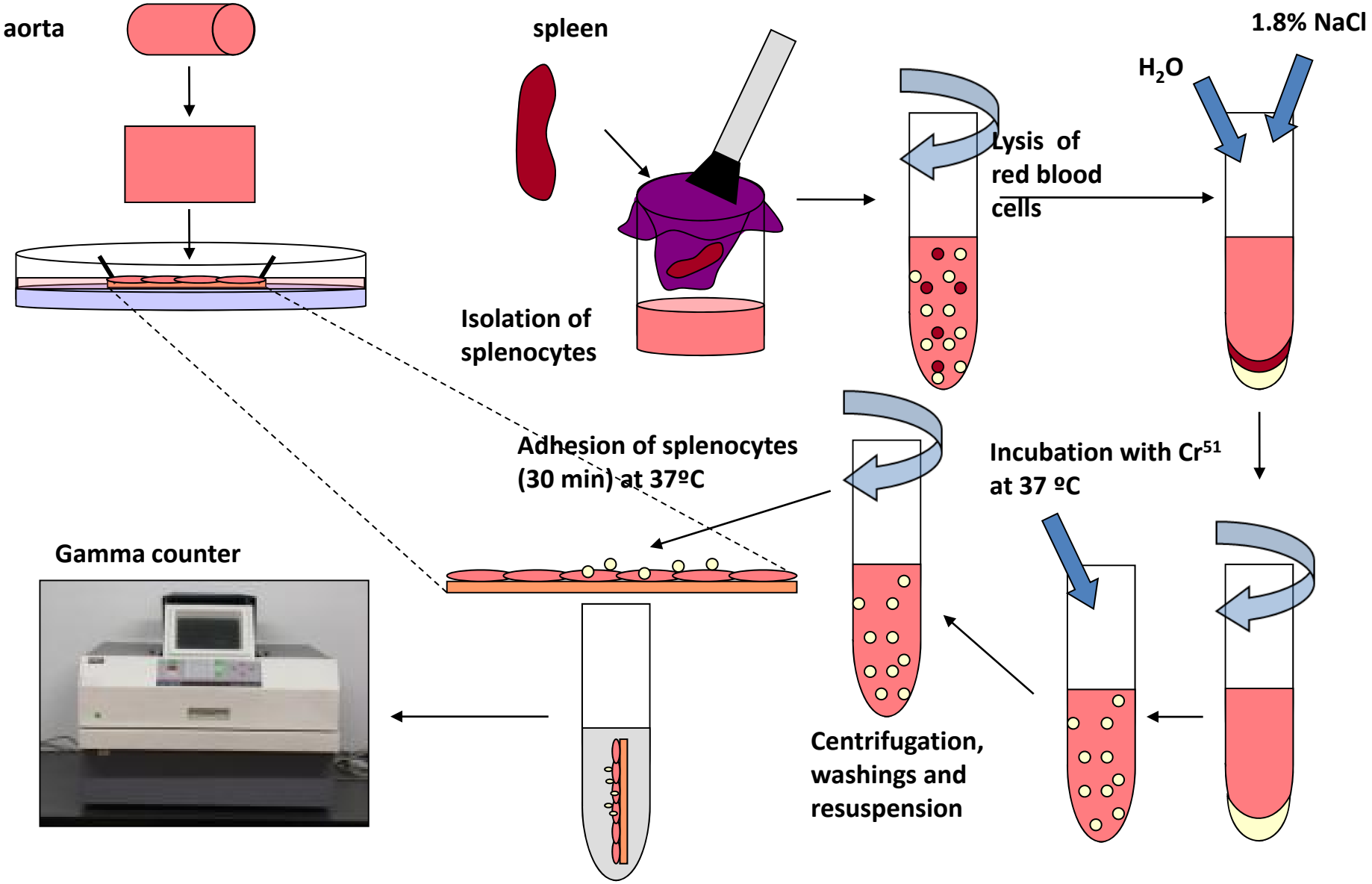
	Sensitivity (pD_2)	Efficacy (E_{max}, %)
3-mo	6.7±0.1	90±5 (7)
12-mo	6.3±0.1 *	64±10 (7) *
Secondary prevention: 12-mo + CAT 3	6.6±01 Φ	87±3 (11) Φ
Primary prevention: 12-mo + CAT 9	6.2±0.1	86±4 (8) Φ



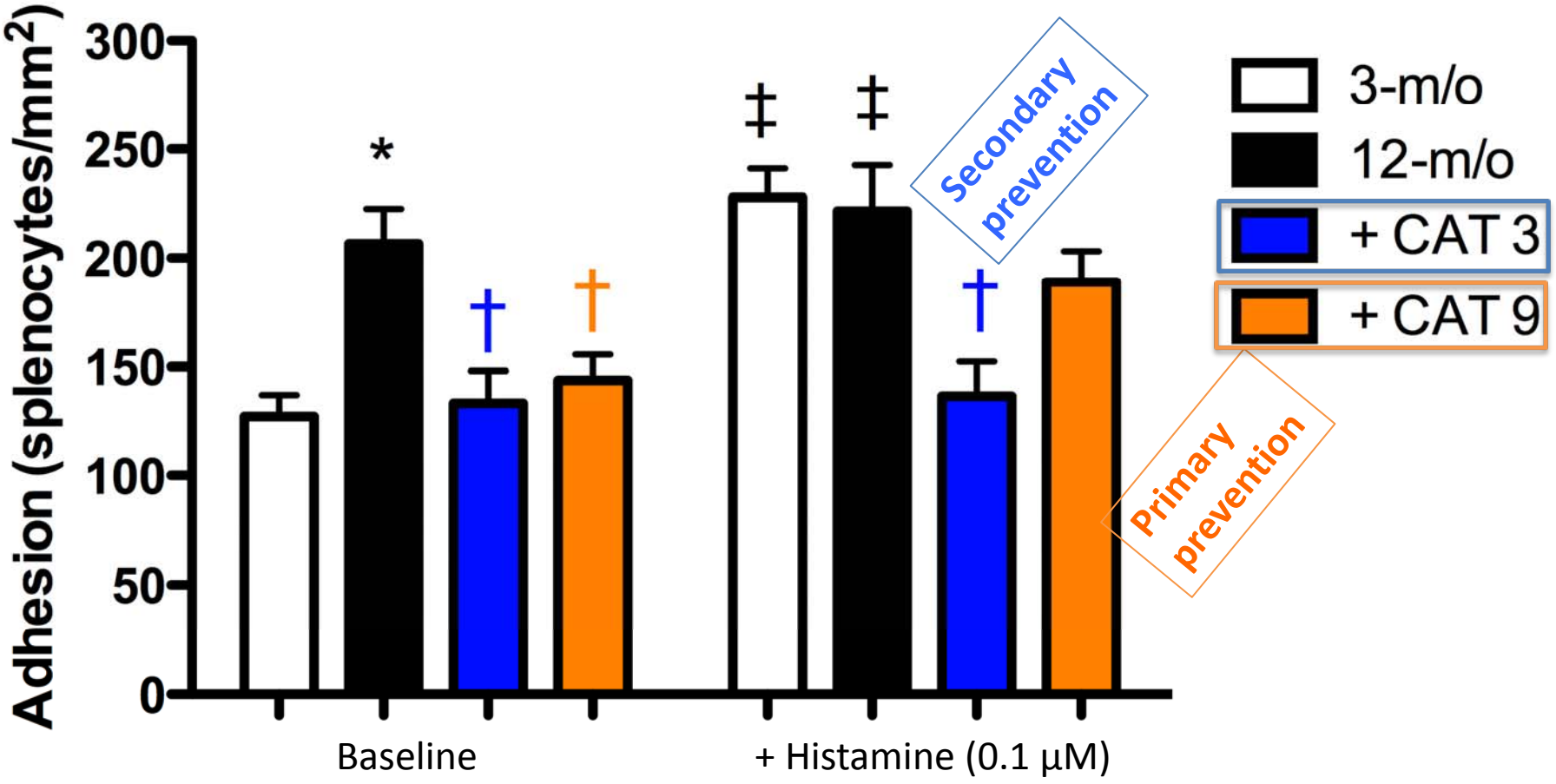


Only late treatment with catechin fully prevented endothelial dysfunction associated with aging

Adhesion of lymphocytes on native endothelium

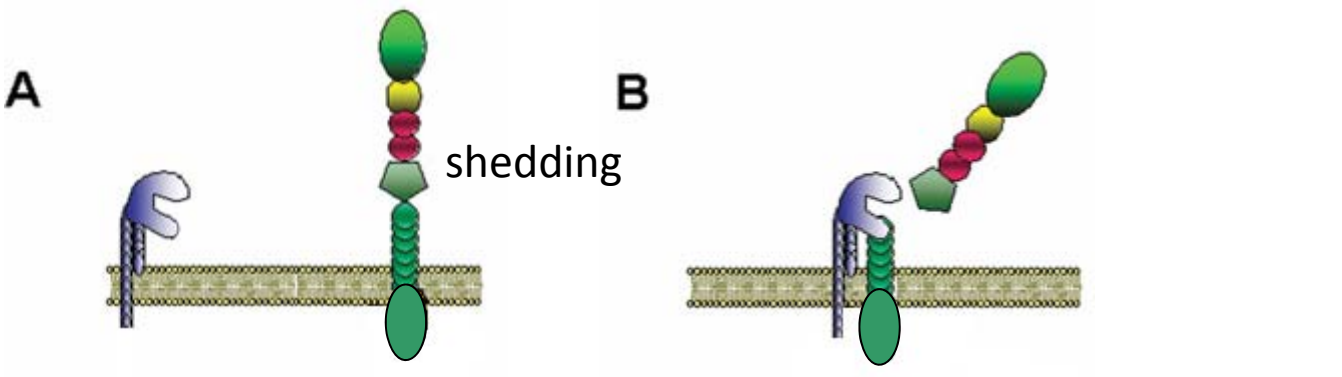


Only late treatment with catechin fully prevented splenocytes adhesion onto the native endothelium

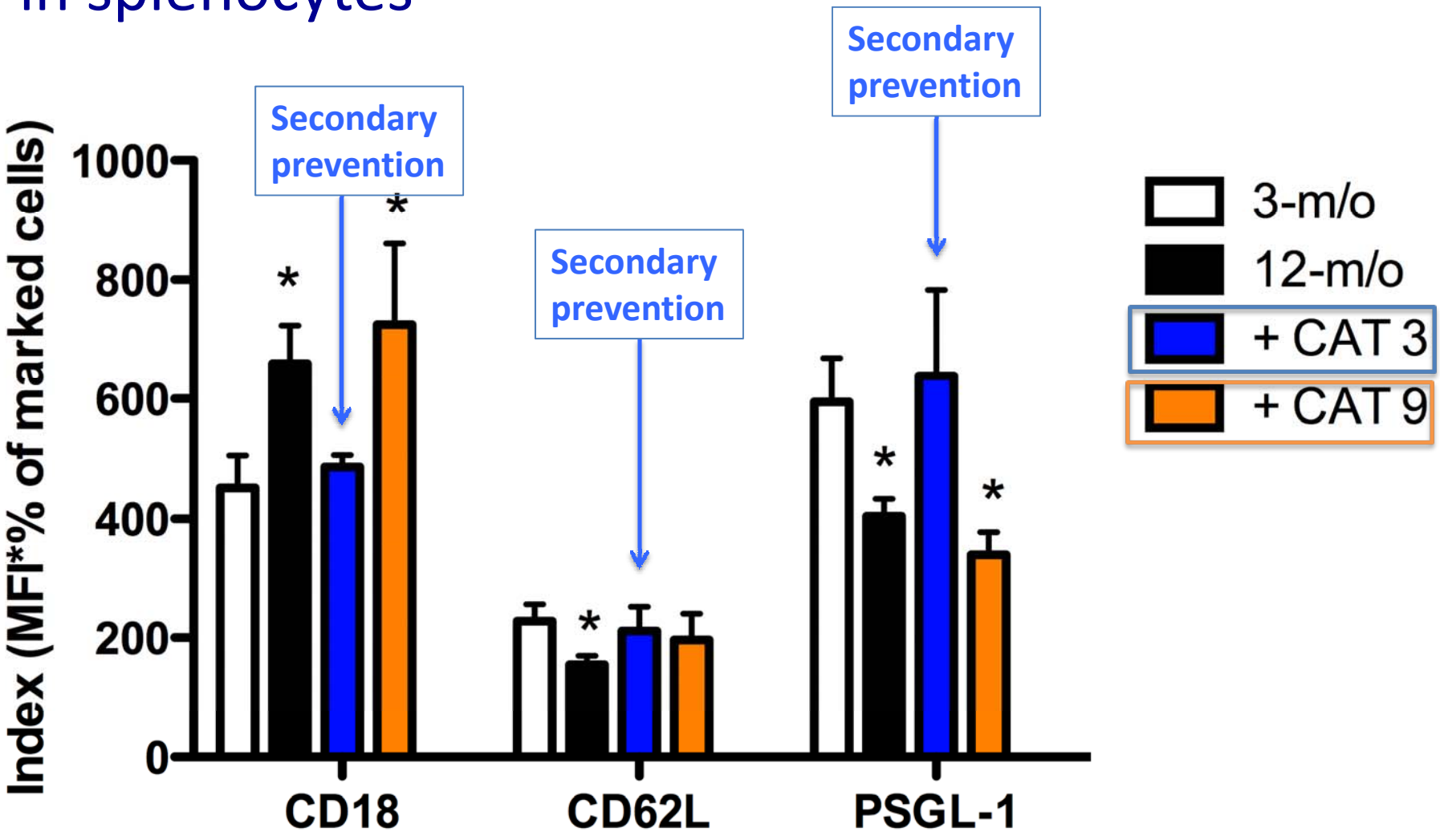


Adhesion molecules

- CD18, CD62L and PSGL-1 expression was studied by FACS
  - An increase in CD18 (β-integrin) promotes adhesion
  - An increase in CD62L (L-selectin) and in PSGL-1 (P-selectin) shedding activates the lymphocytes and promotes adhesion

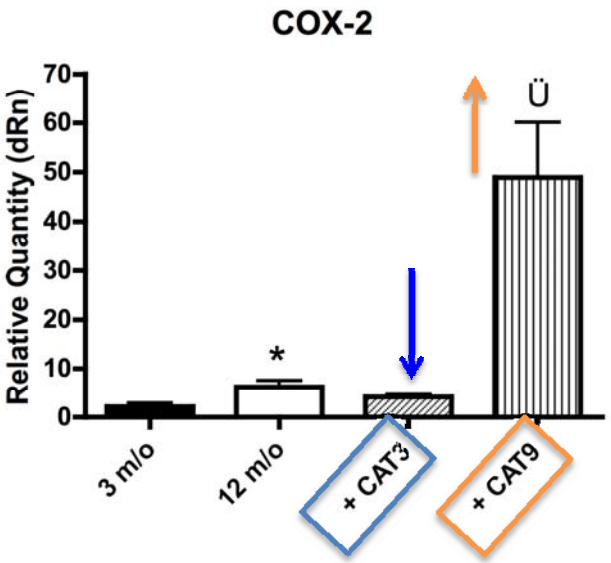


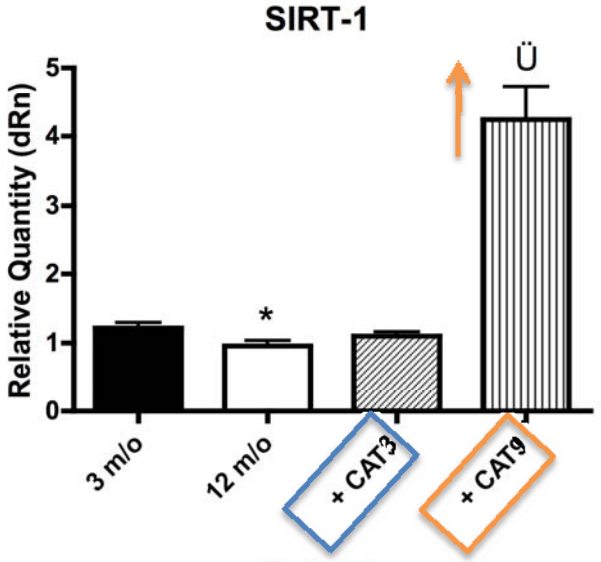
Only late treatment with catechin fully prevented alteration in the expression of adhesion molecules in splenocytes



Early treatment with catechin leads to deleterious aortic gene expression

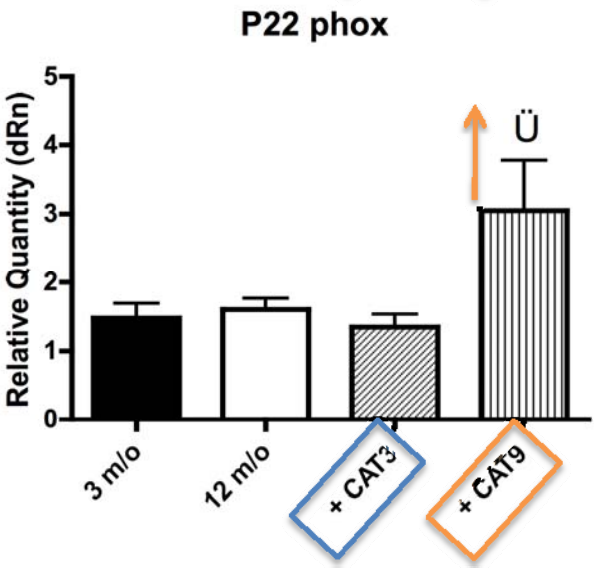
Increase in inflammation

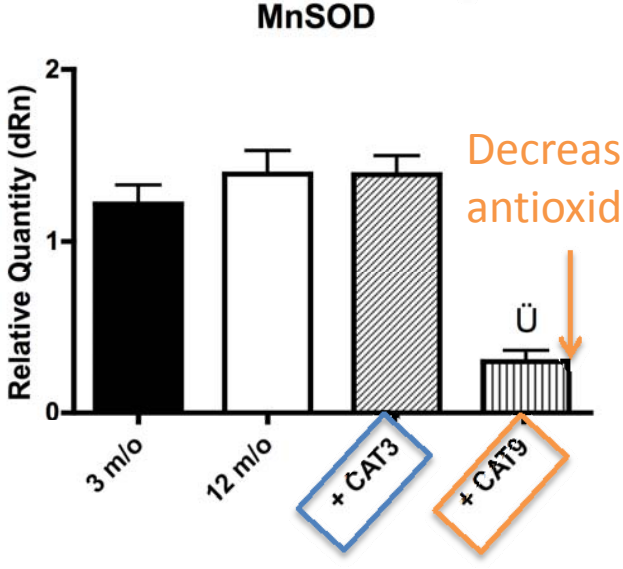




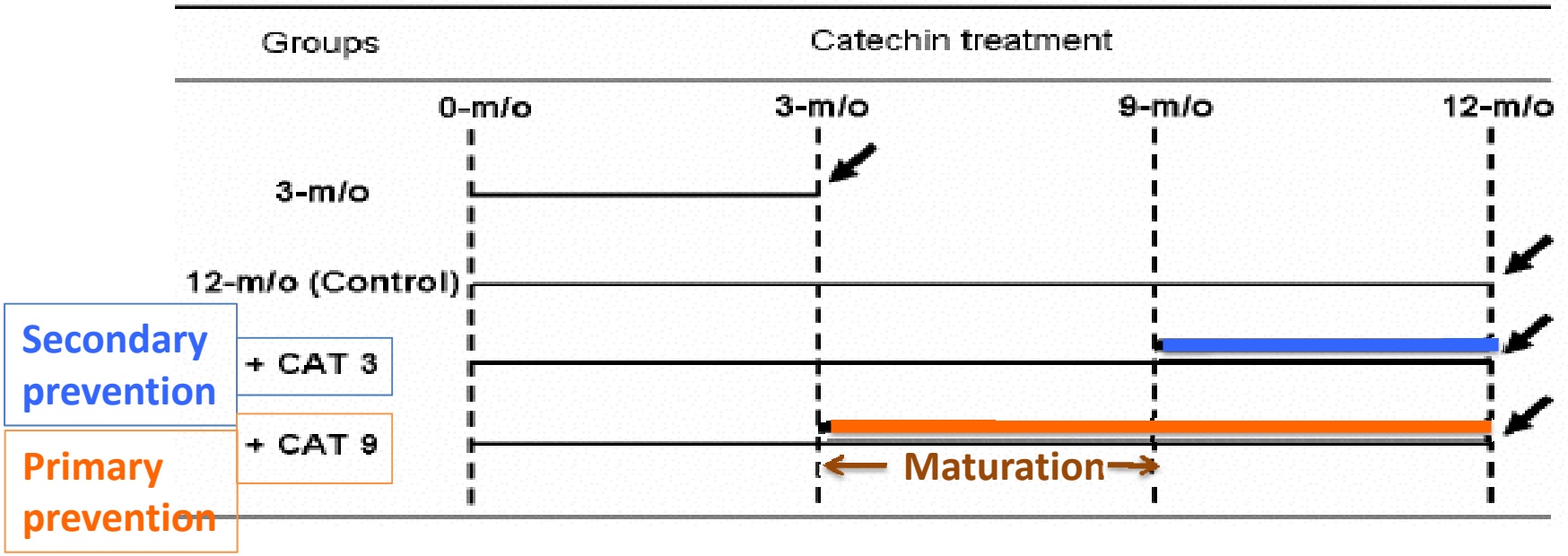
?

Increase in ROS production





Decrease in antioxidant defences



SUMMARY

Only late secondary prevention with catechin:

- fully prevented endothelial dysfunction associated with aging,
- fully prevented splenocytes adhesion onto the native endothelium,
- fully prevented alteration in the expression of adhesion molecules in splenocytes,
- did not lead to a gene expression deleterious to the vascular function

Conclusion

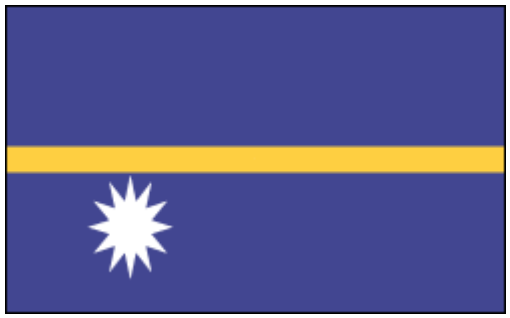
- The contribution of the free radicals during the vascular endothelial maturation phase seems essential to the establishment of protective pathways involved in stress resistance

  - When antioxidant treatment starts before maturation (primary prevention): protective pathways are not activated. Catechin is not able to fully prevent the vascular dysfunctions associated with aging.

  - When antioxidant treatment starts after maturation (secondary prevention): protective pathways are activated by an adequate exposure to free radicals during the maturation phase. Catechin is therefore able to prevent or delay the effects of aging.

Is this an hormetic response?

The South Pacific Example



- Nauru, South Pacific Island, population 12,000
- Second richest country in the world per capita
- Due to “Coca-colonisation”, the diabetes prevalence increased from 0% to 45% <u>in 5 years</u>
- Verge of bankruptcy

The South Pacific Example

From a healthy life-style

healthy diet, rich in fruits and vegetables, fish
physical exercise
no stress
community

Rapid changes

To a modern life-style

Western diet
sendentarity
stress, competition

No previous exposure to “stress”
No mechanisms of adaptation
No hormesis ?

Question

Would the inhabitants of Amherst react better to the abrupt change in life-style experienced by the people from Nauru?

Aging *definition:*

When your broad mind and narrow waist start to change place

Acknowledgements

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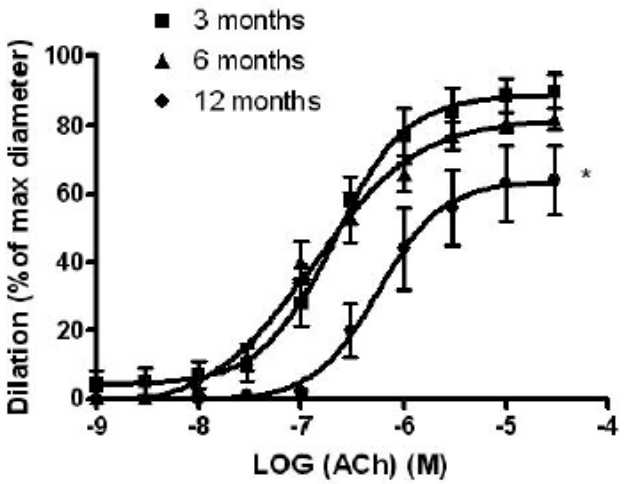
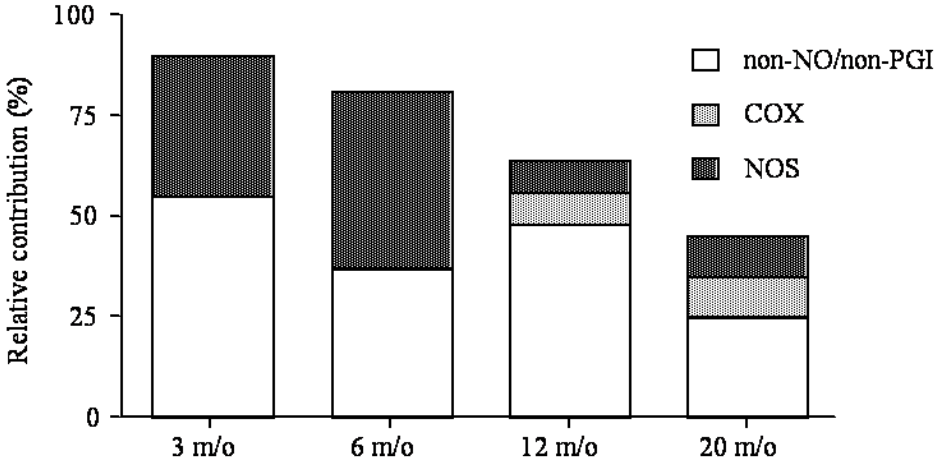
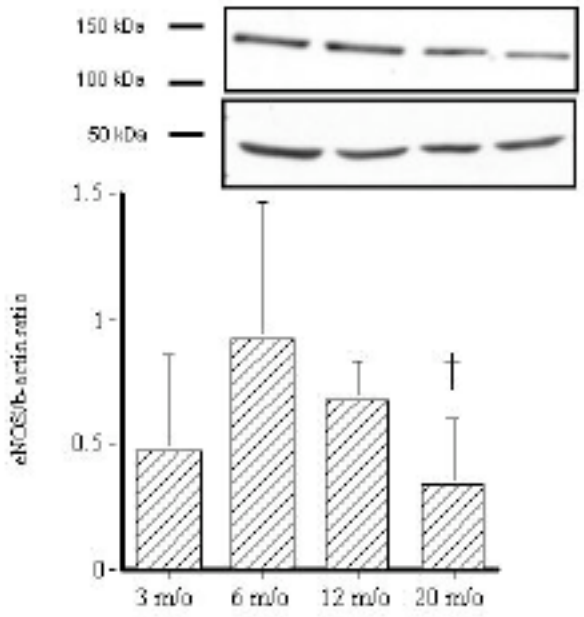
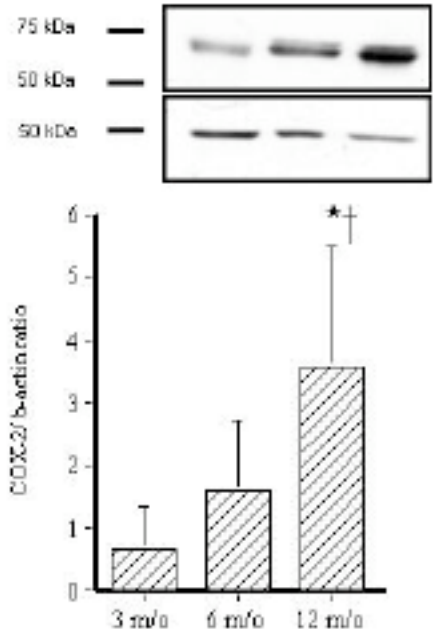
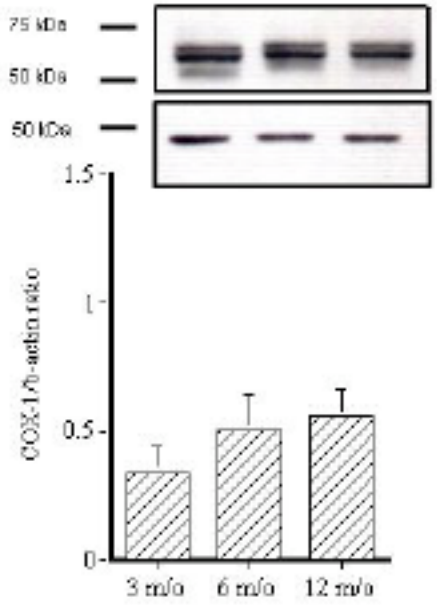

3 months
6 months
12 months
Dilation (% of max diameter)
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
LOG (ACh) (M)
*


Relative contribution (%)
100
75
50
25
0
non-NO/non-PGI
COX
NOS
3 m/o
6 m/o
12 m/o
20 m/o


150 kDa
100 kDa
50 kDa
eNOS/b-actin ratio
1.5
1
0.5
0
†
3 m/o
6 m/o
12 m/o
20 m/o


75 kDa
50 kDa
50 kDa
COX-2/ b-actin ratio
6
5
4
3
2
1
0
*†
3 m/o
6 m/o
12 m/o


75 kDa
50 kDa
50 kDa
COX-1/b-actin ratio
1.5
1
0.5
0
3 m/o
6 m/o
12 m/o