

**The Endothelial Saga: *Regenerate  
to ...be old***

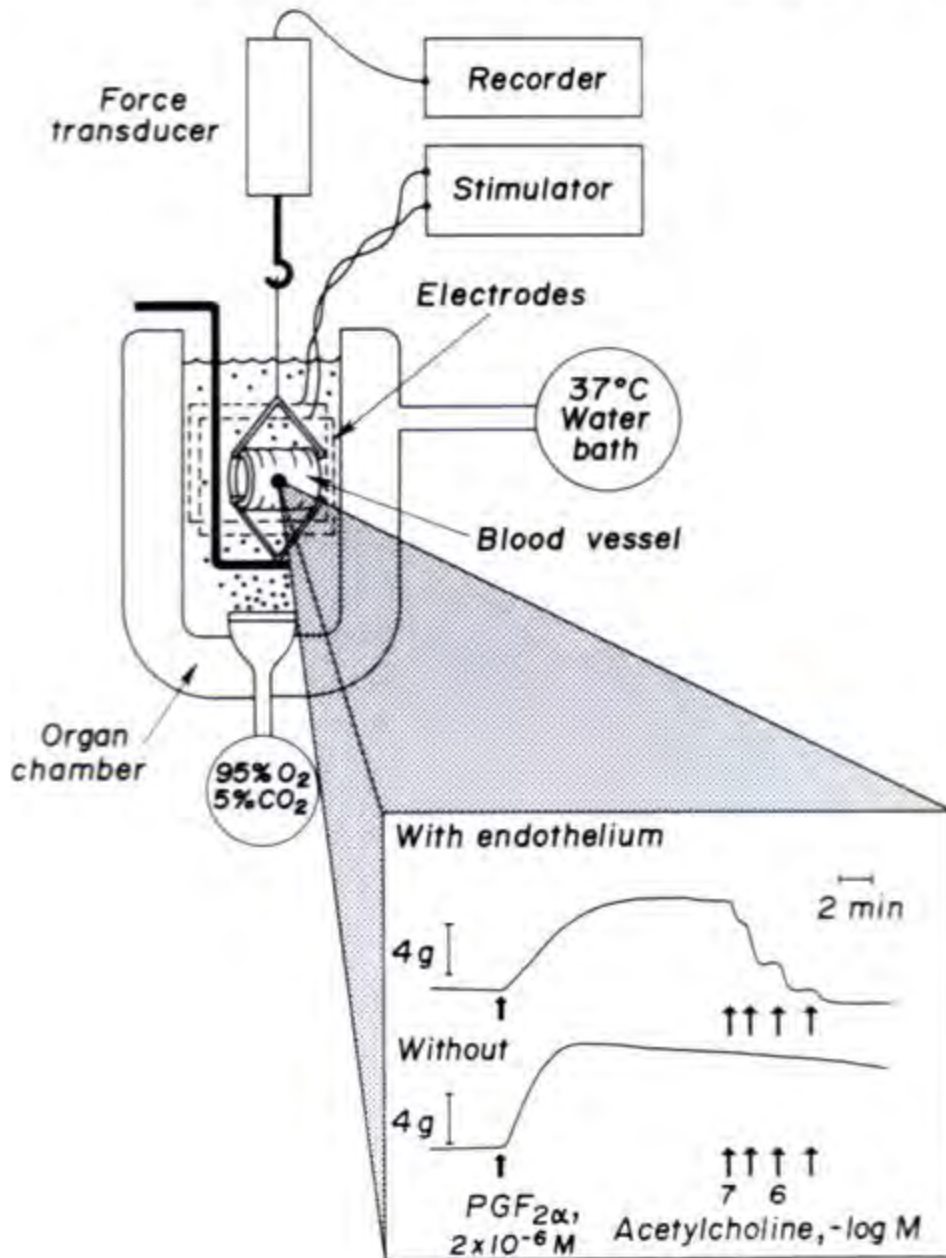
**Paul M. Vanhoutte M.D., Ph.D.**

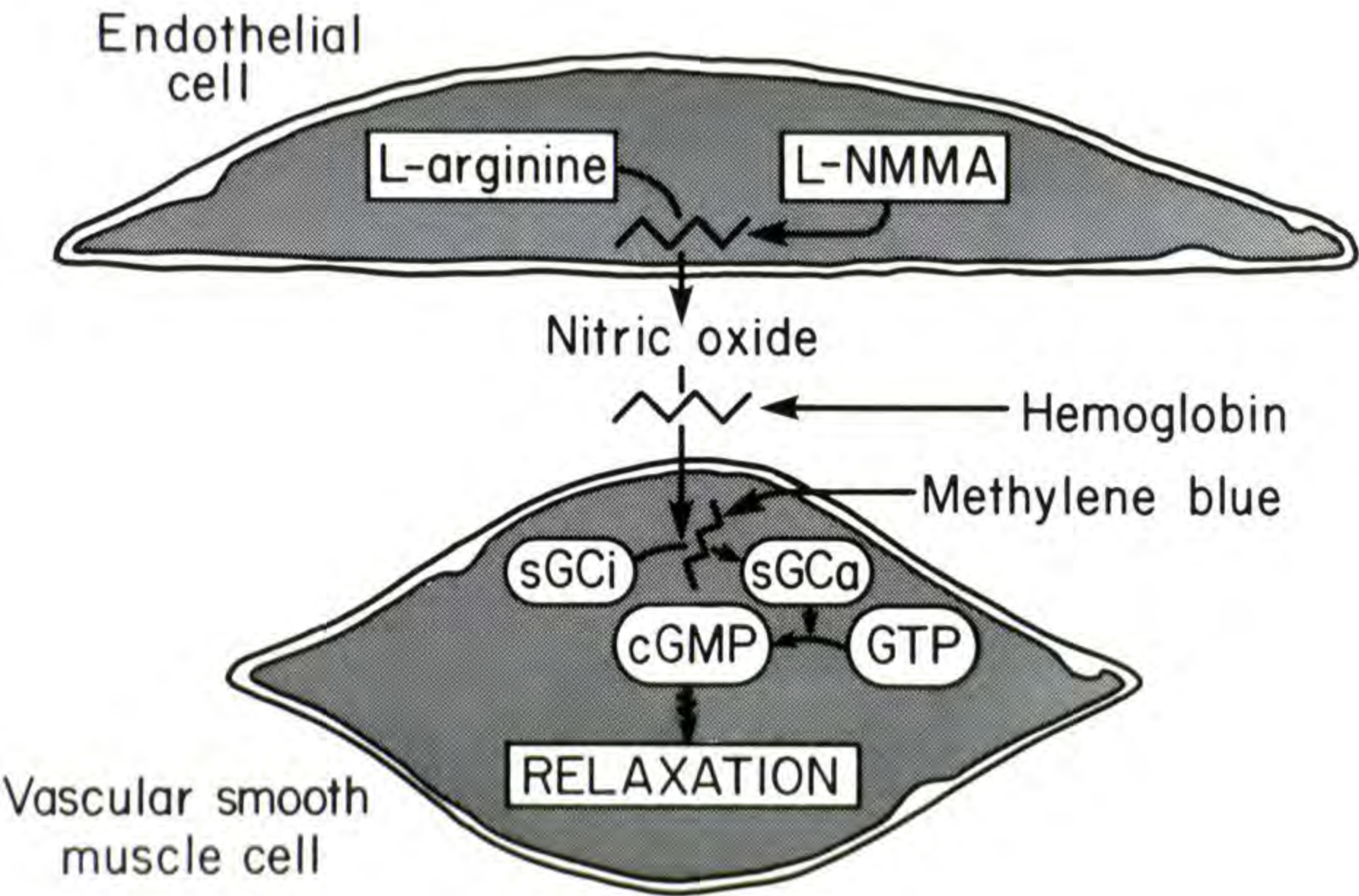
**University of Hong Kong**

**Nice June 12<sup>th</sup> , 2011**



# METHOD FOR STUDYING ISOMETRIC TENSION OF ISOLATED CORONARY ARTERY RINGS WITH OR WITHOUT ENDOTHELIUM





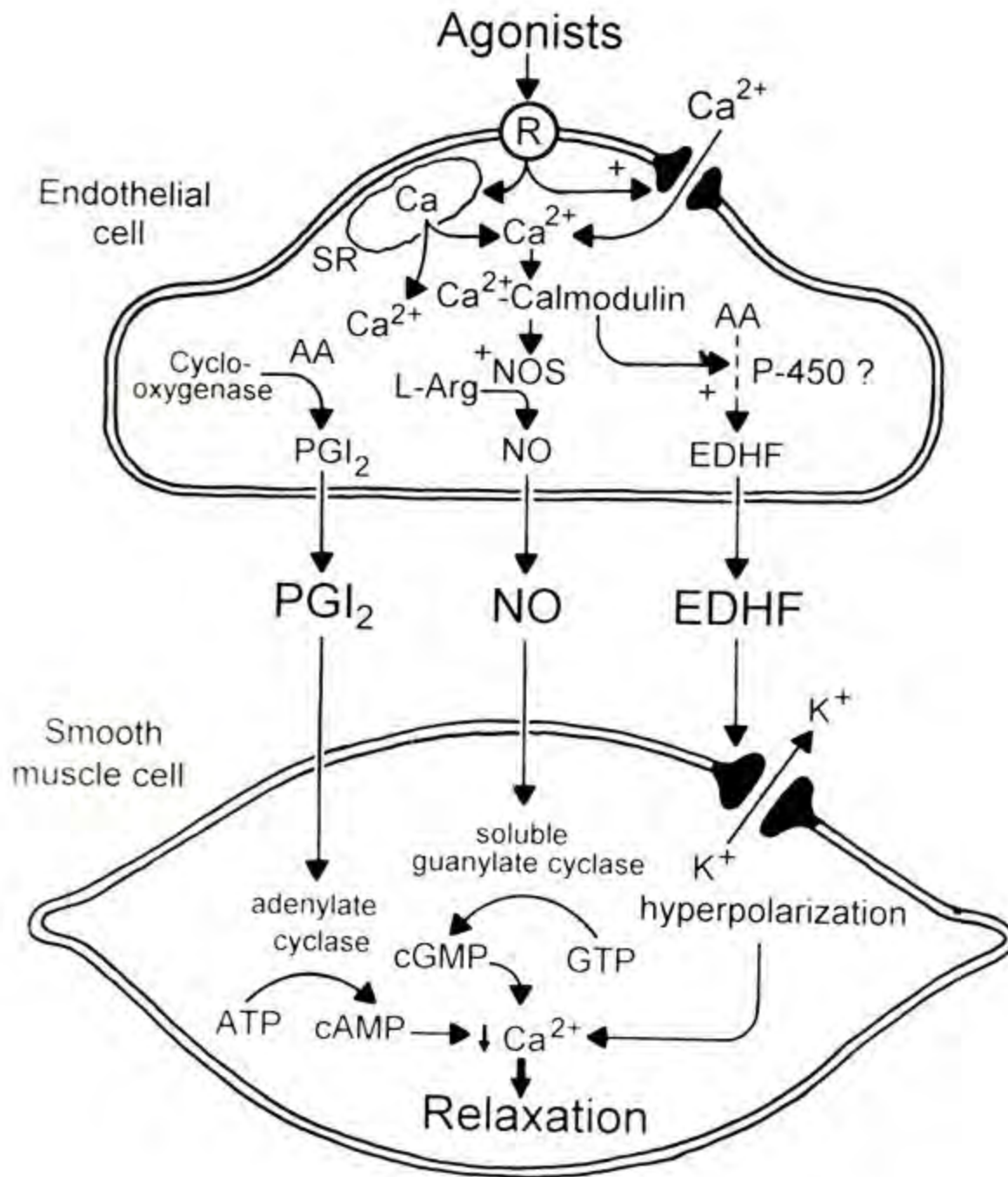
NO

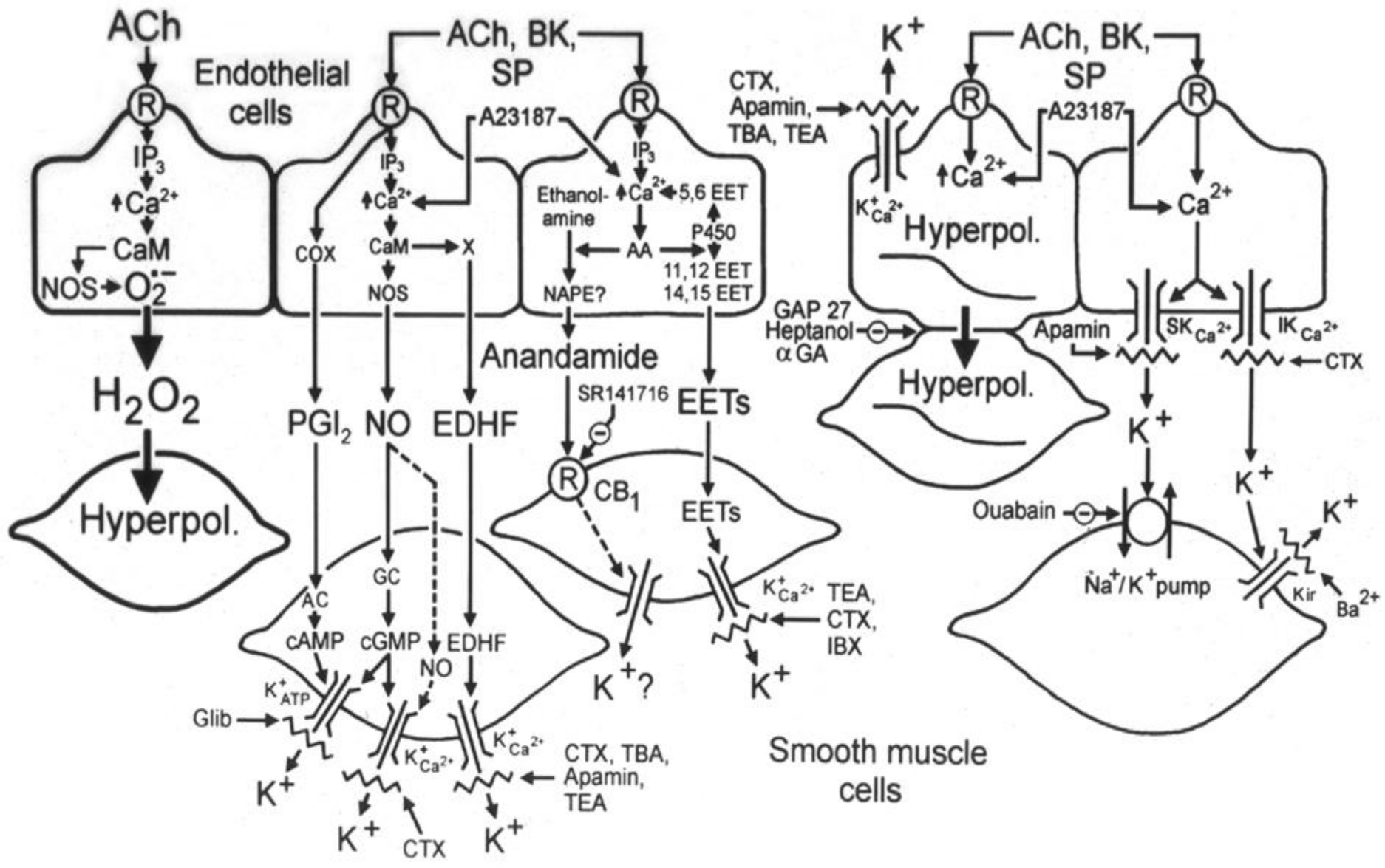


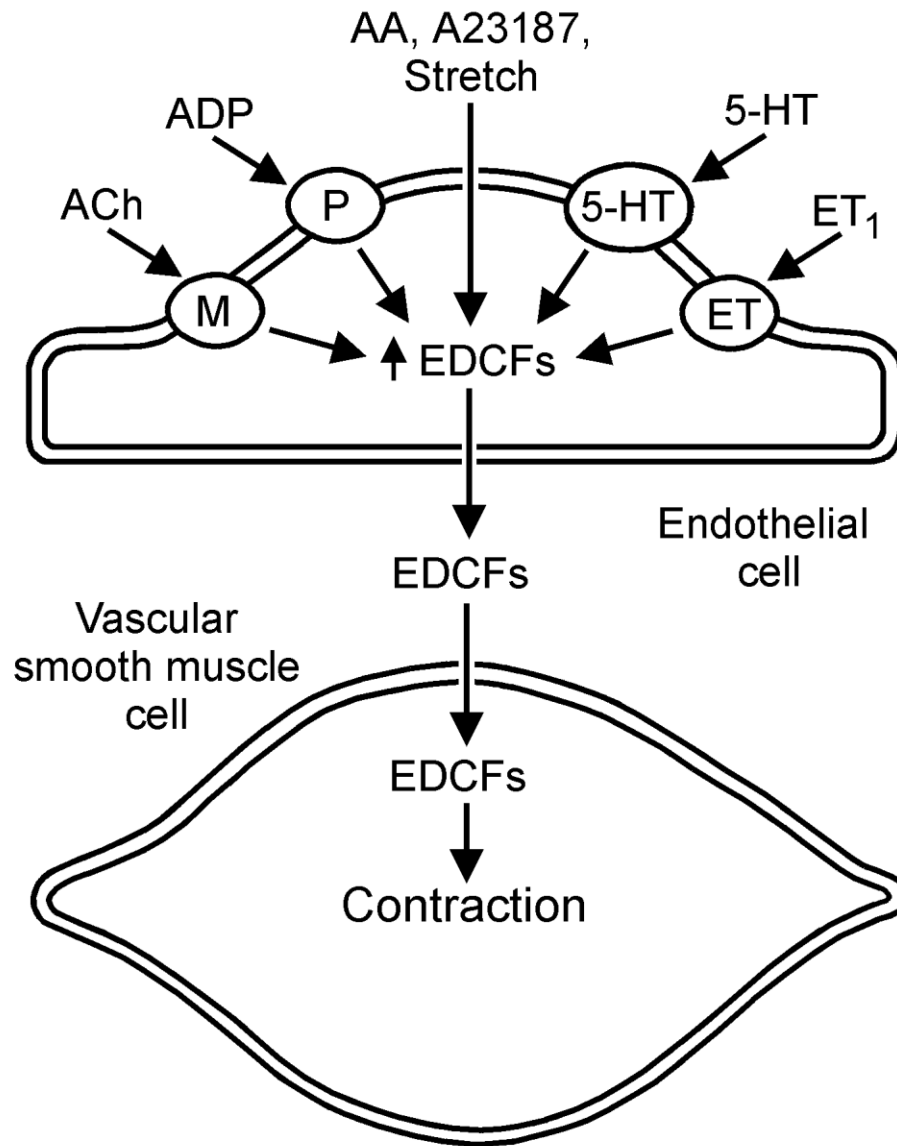
**Is NO the only answer ?**

**... « THE RESULTS INDICATE THAT  
FUNCTIONAL COUPLING BETWEEN  
ENDOTHELIAL CELLS AND THE ARTERIAL  
MEDIA INVOLVES AT LEAST THREE  
DIFFERENT PATHWAYS, TWO OF WHICH  
DEPEND ON THE METABOLISM OF THE  
FATTY ACID ».**

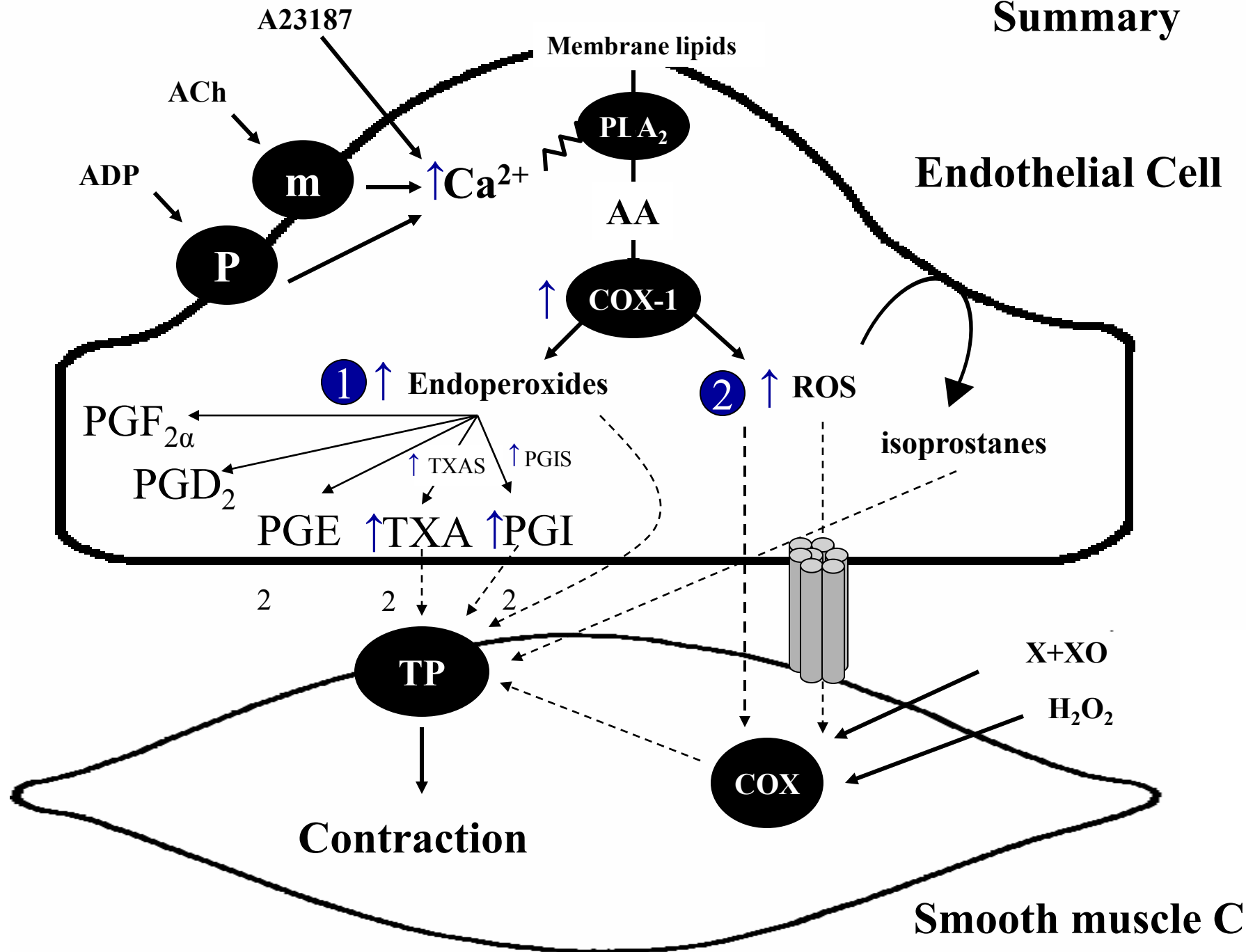
**J.G. De Mey, M. Claeys and P.M. Vanhoutte  
JPET, 222 : 166, 1982**







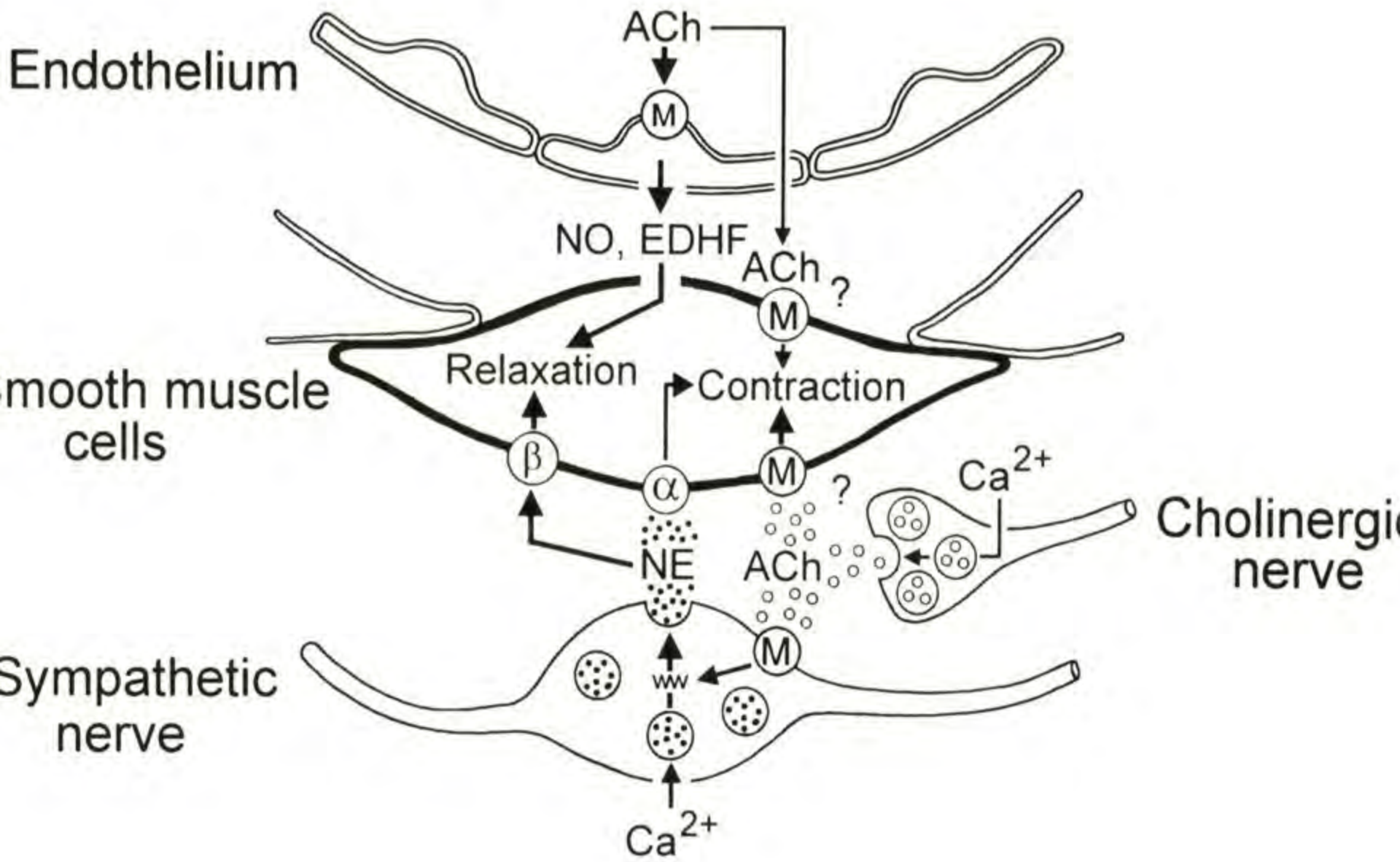
# Summary



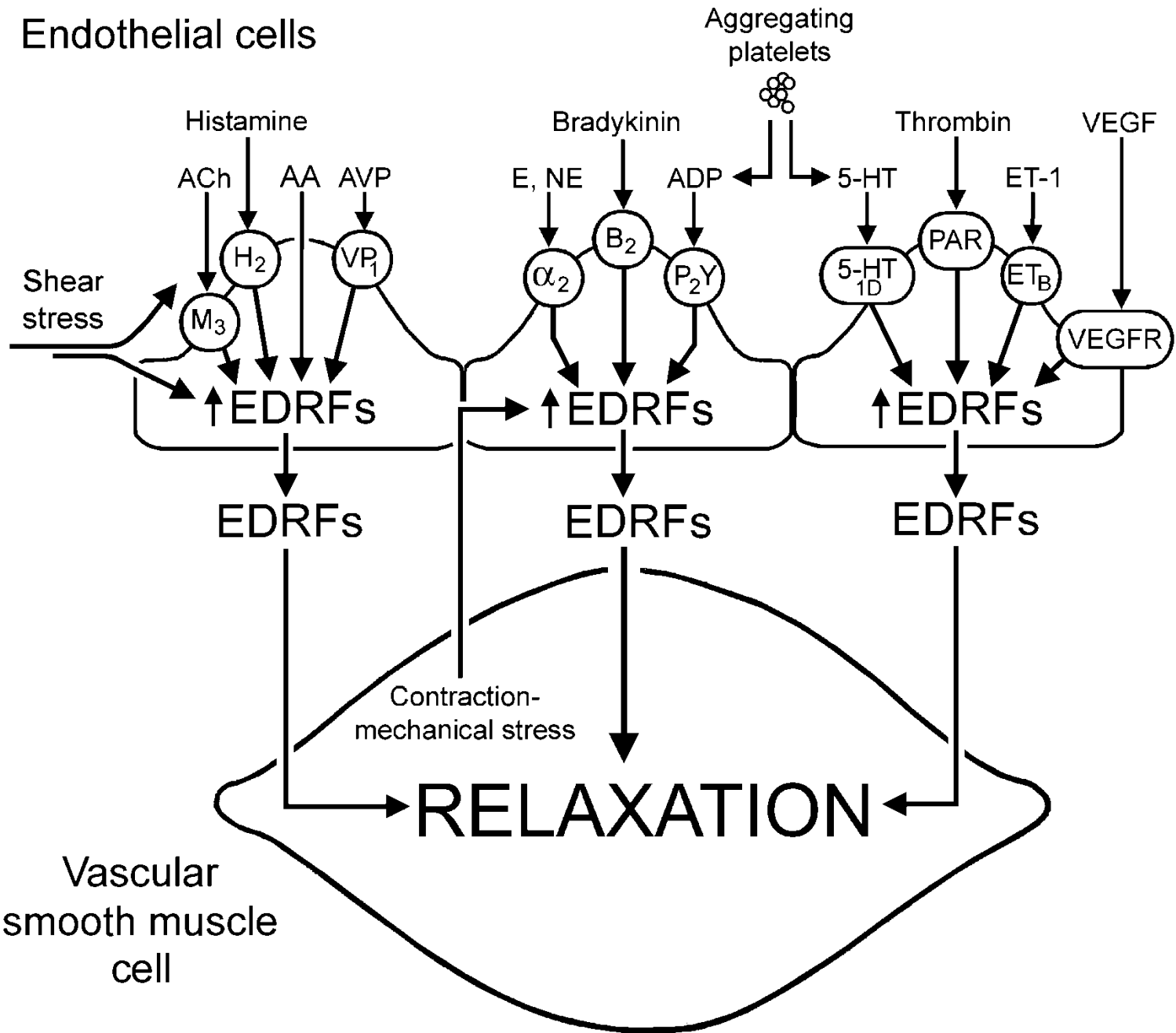
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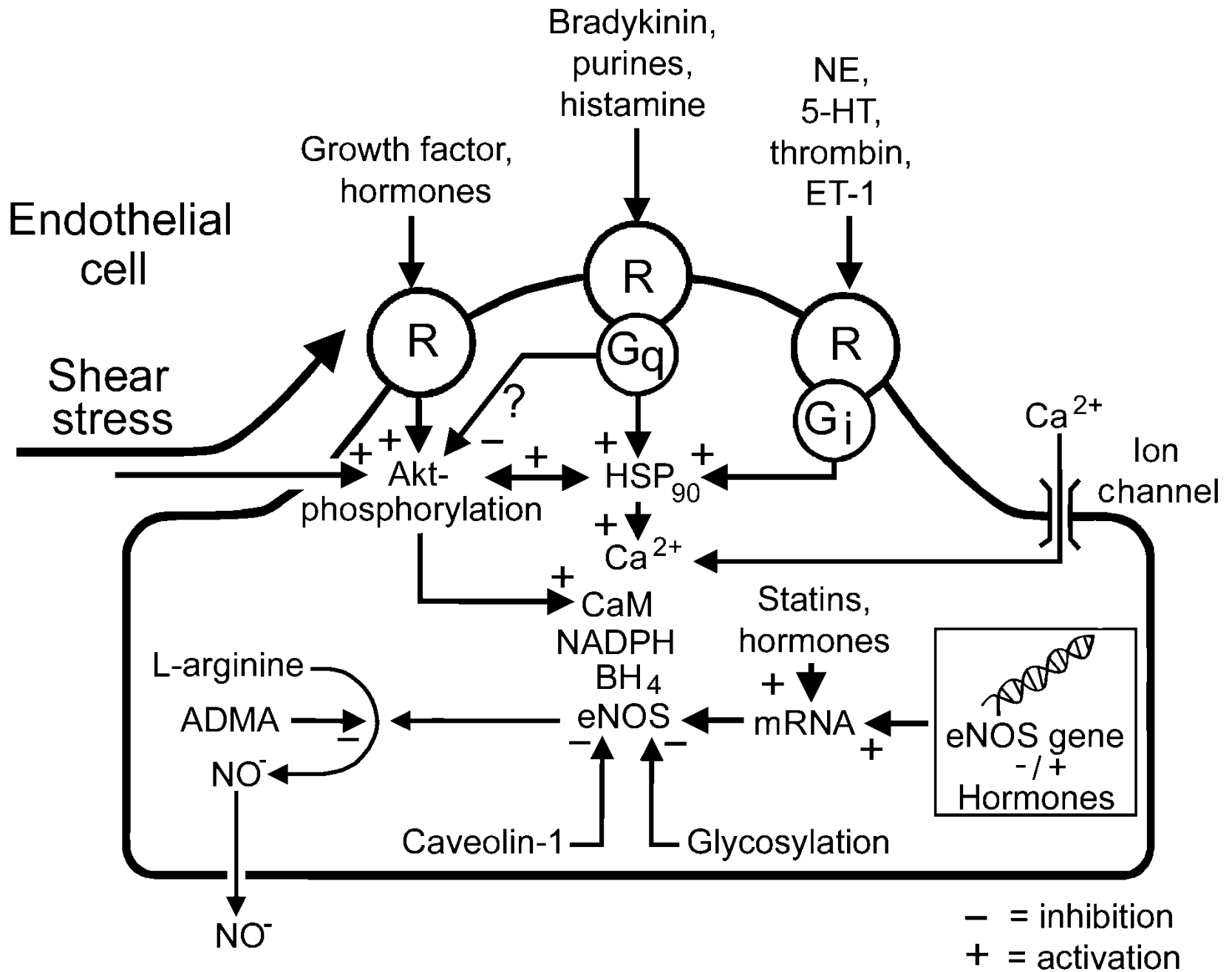






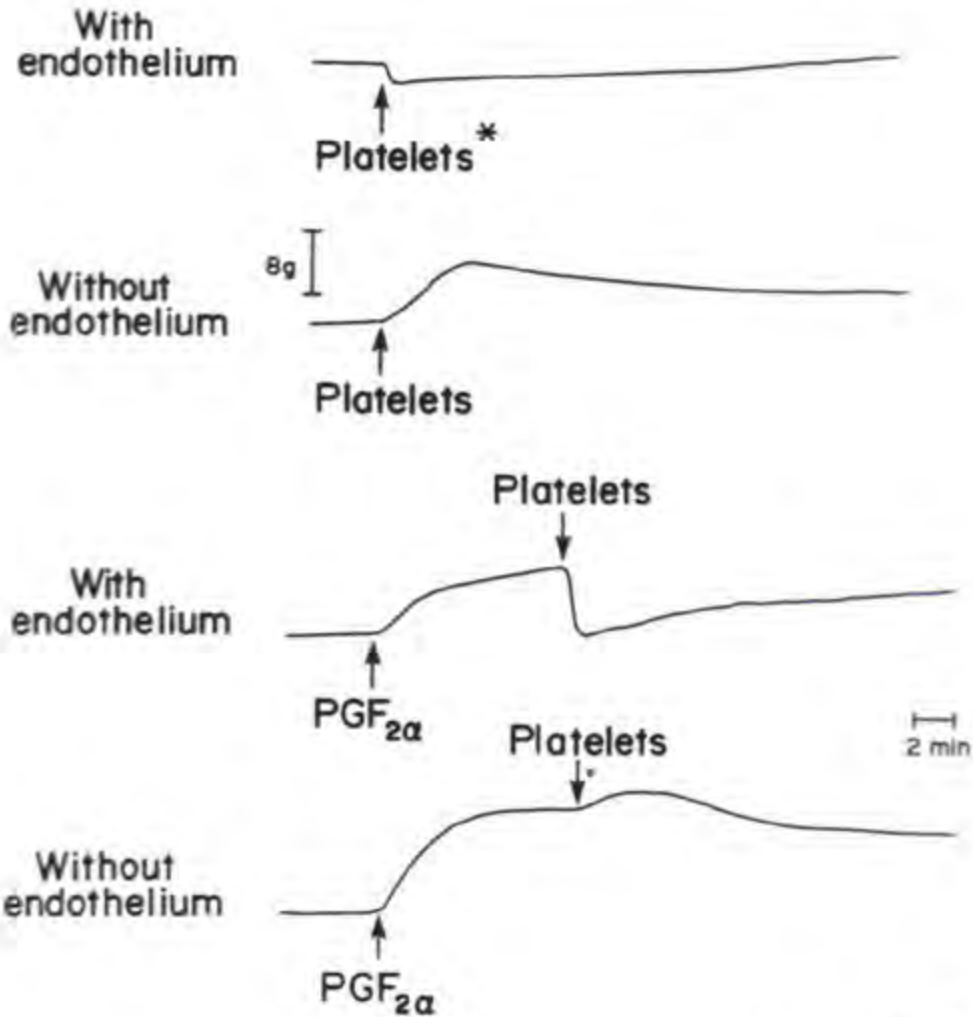
# Endothelial cells





# ENDOTHELIUM-DEPENDENT EFFECT OF AGGREGATING HUMAN PLATELETS ON CANINE CORONARY ARTERY

Subject PMV  
9/28/84

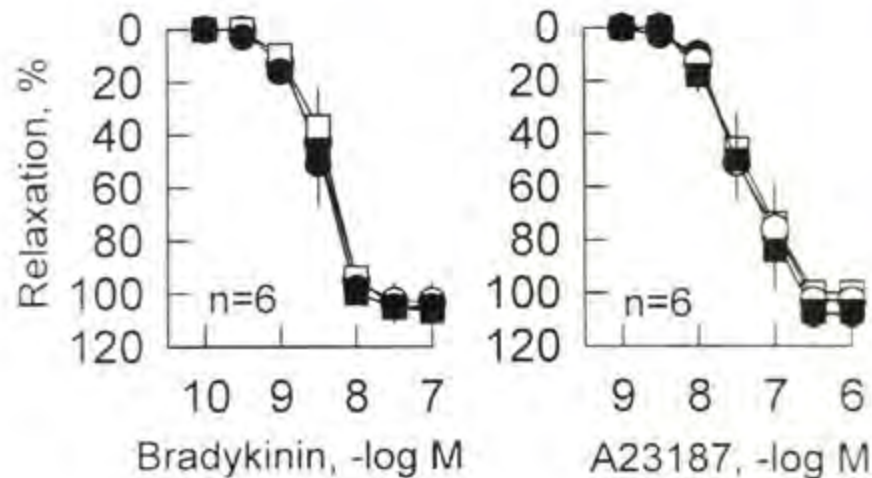
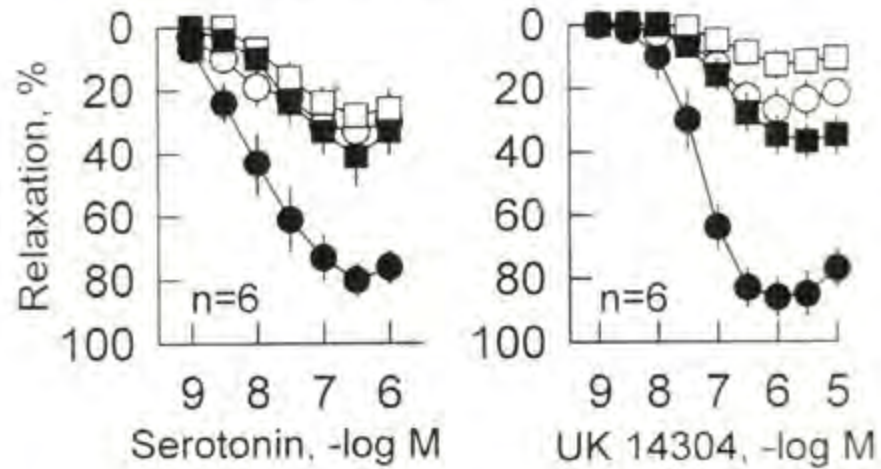


\* 70,000/mm<sup>3</sup>

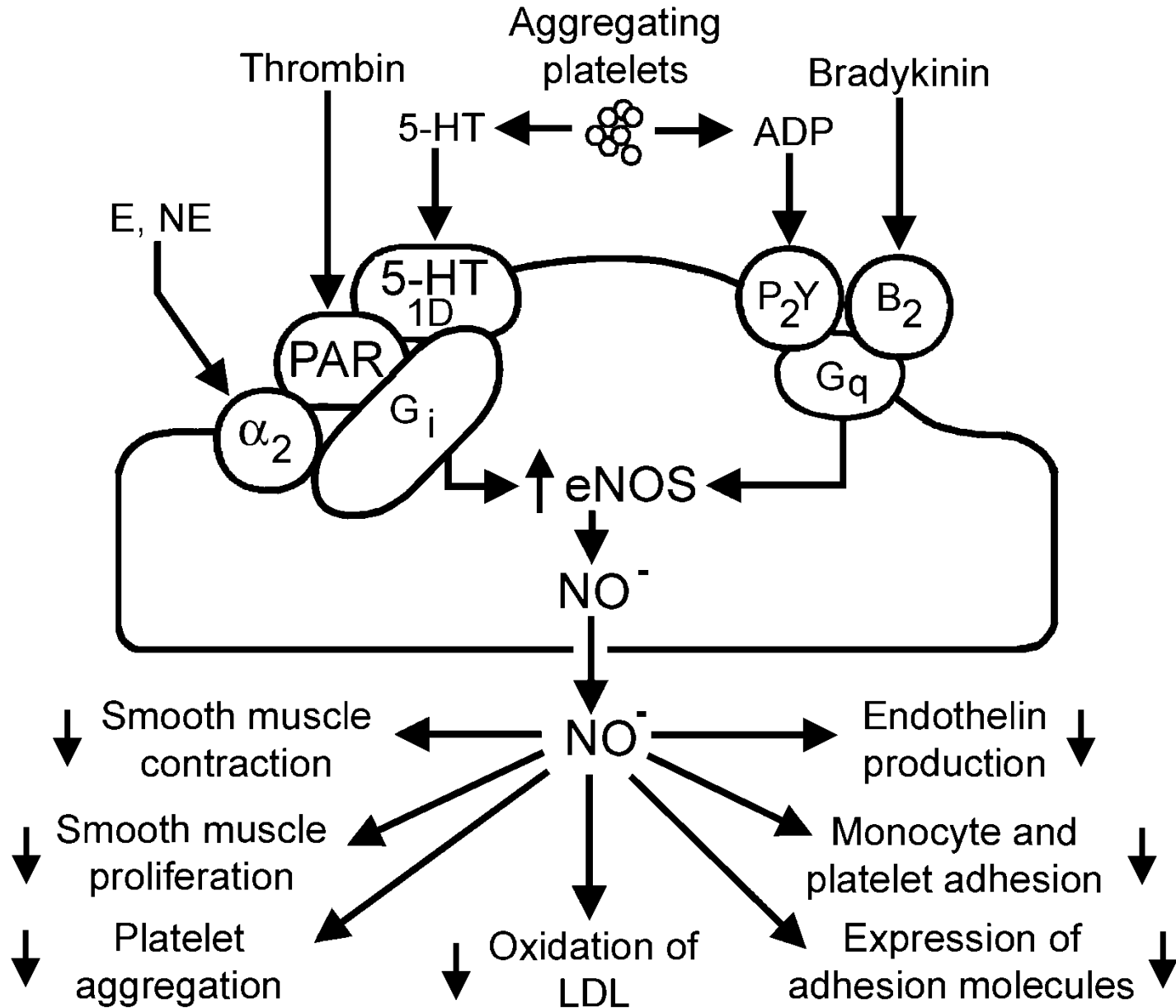
# ENDOTHELIUM-DEPENDENT RELAXATIONS IN PORCINE CORONARY ARTERIES

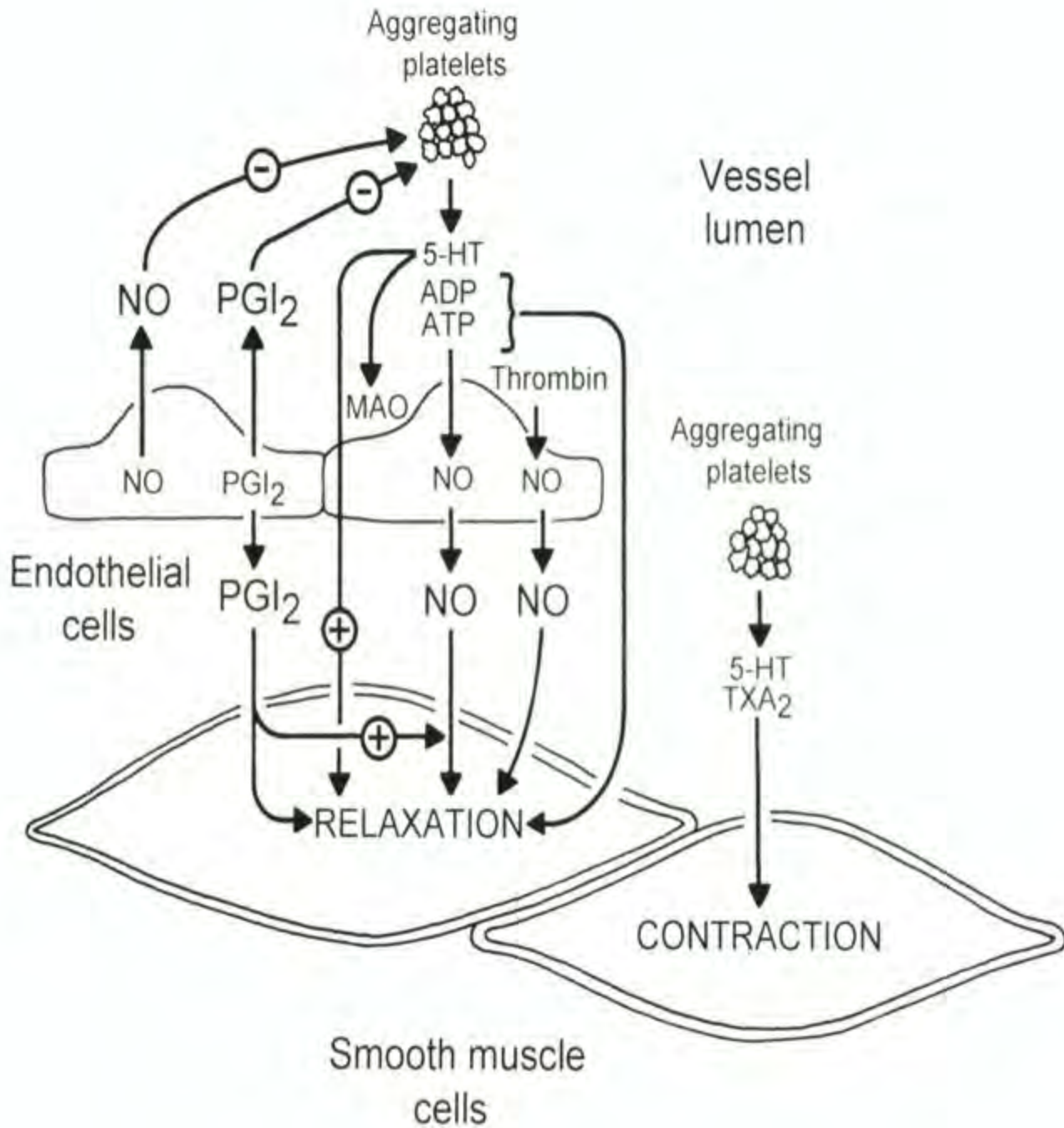
( in the presence of indomethacin,  $10^{-5}$  M )

- control, - PTX      ■ denuded, - PTX
- control, +PTX      □ denuded, +PTX



# Native Endothelial Cell

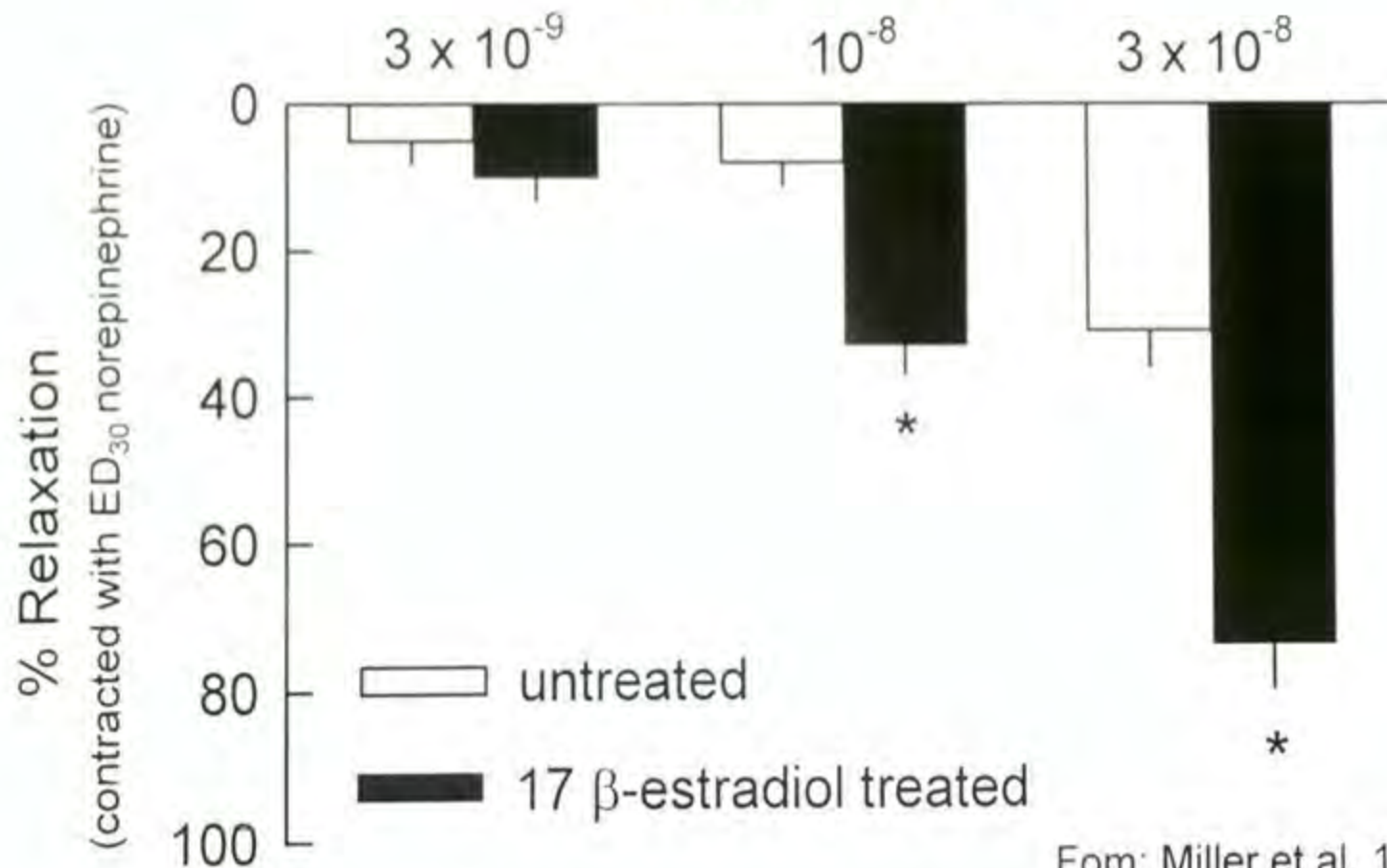




# 17 $\beta$ -ESTRADIOL AND ENDOTHELIUM-DEPENDENT RELAXATION

(rabbit femoral arteries)

Acetylcholine, M

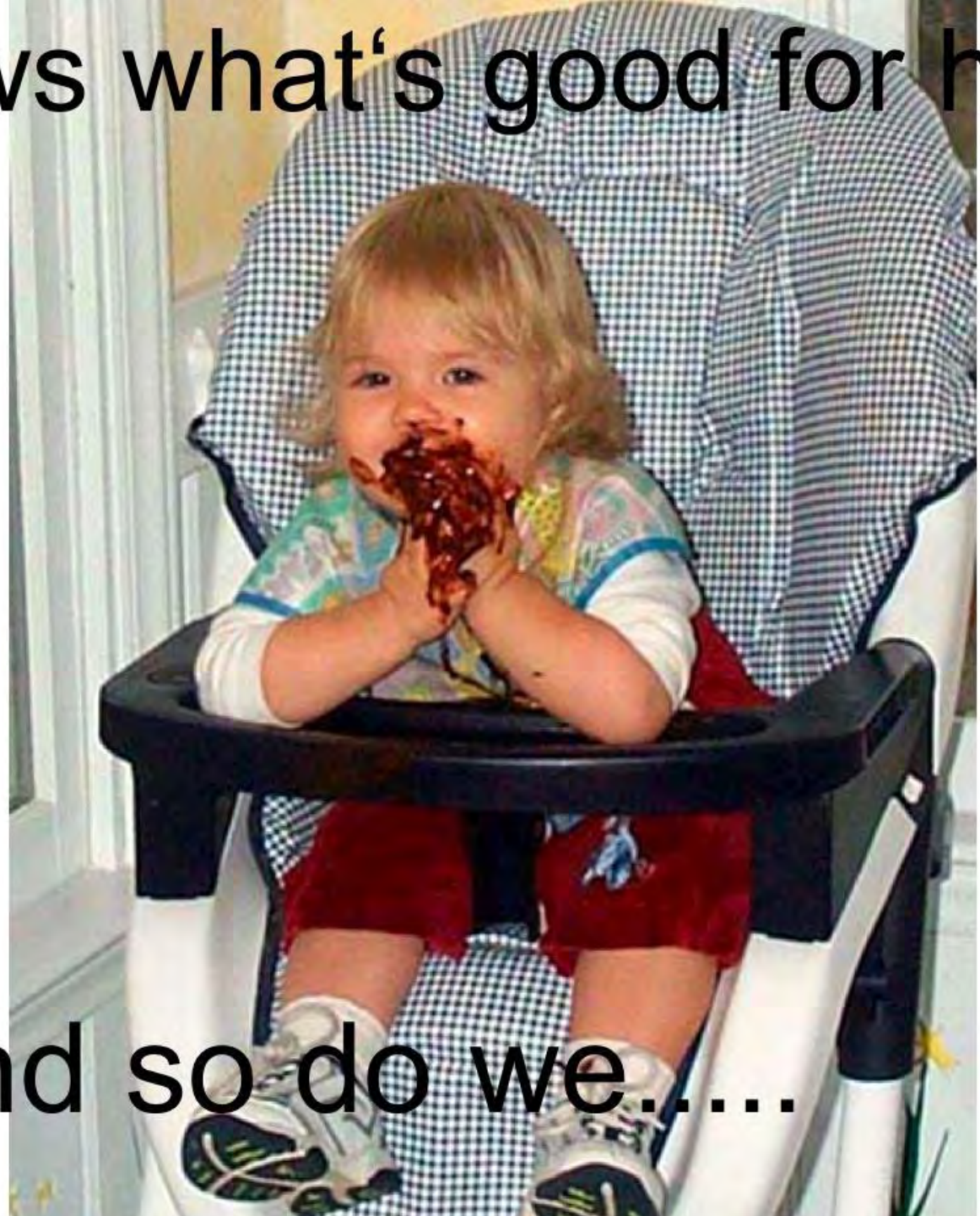


From: Miller et al, 1988

# NON SEQUITUR

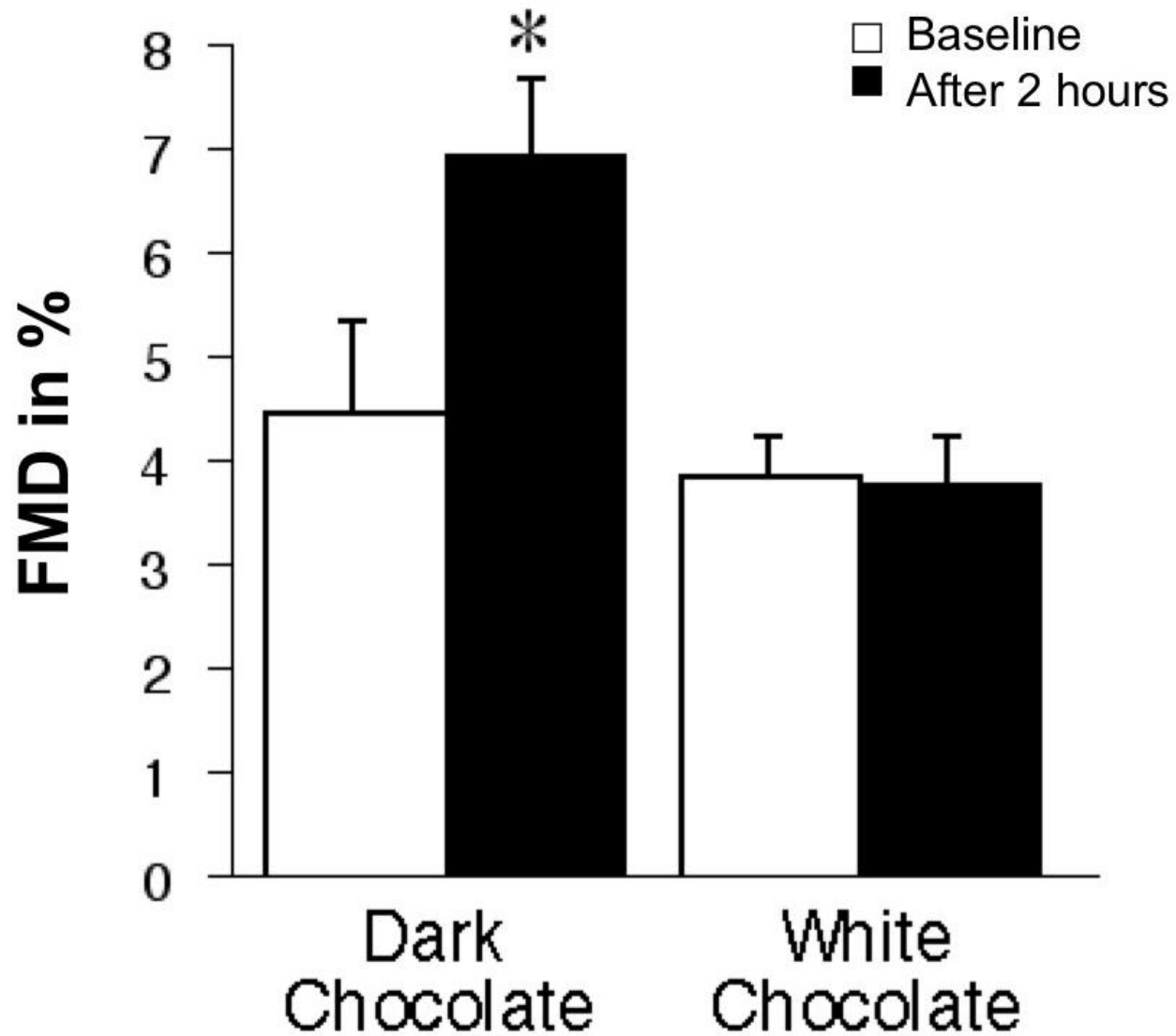


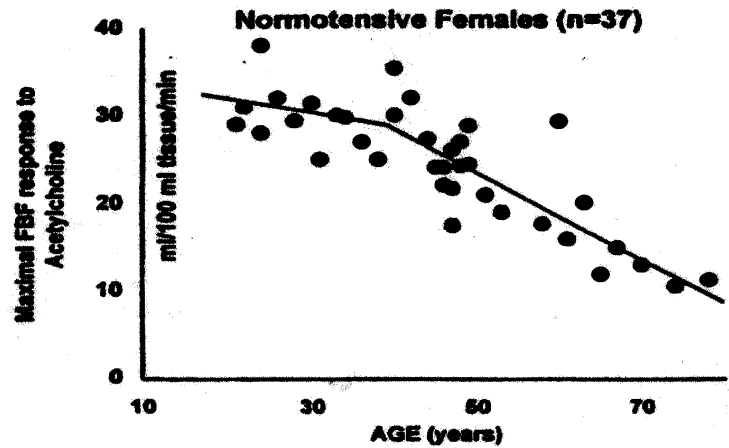
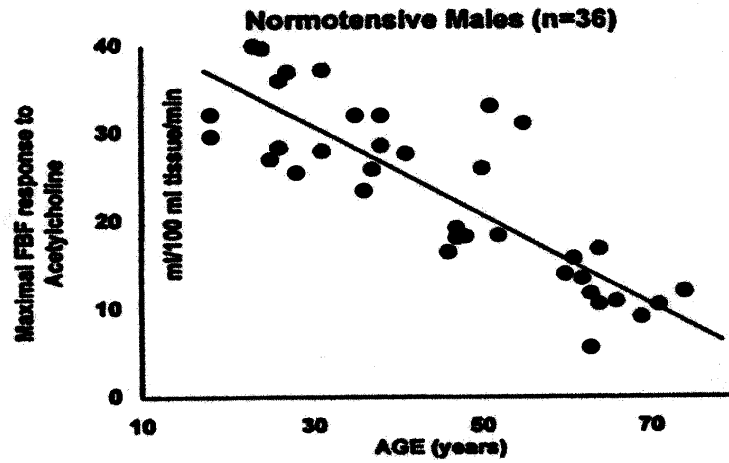
He knows what's good for him



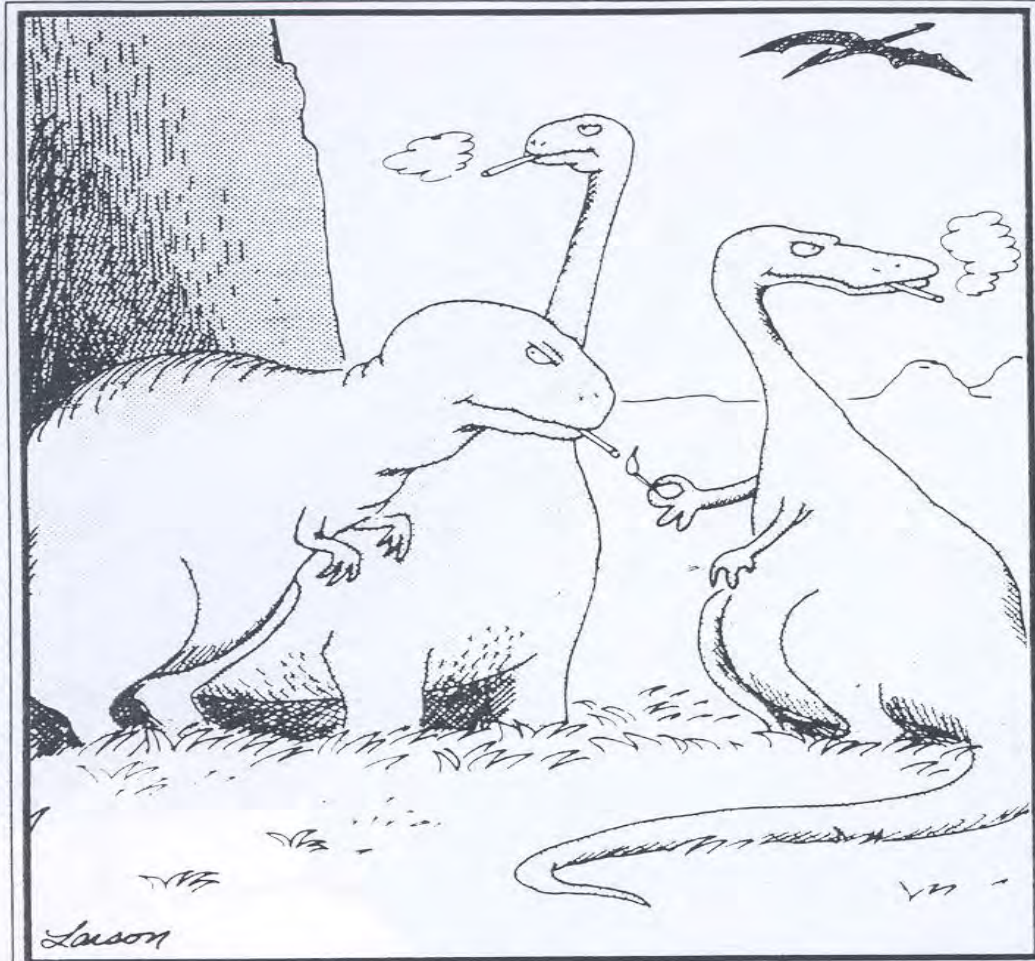
And so do we.....

NN





From Taddei et al 1996

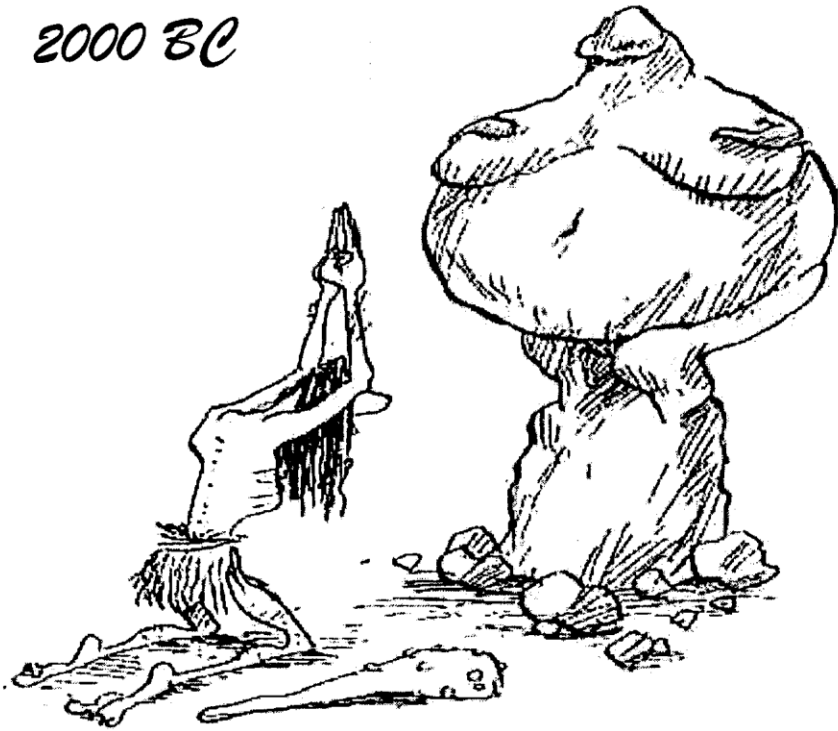


The real reason dinosaurs became extinct

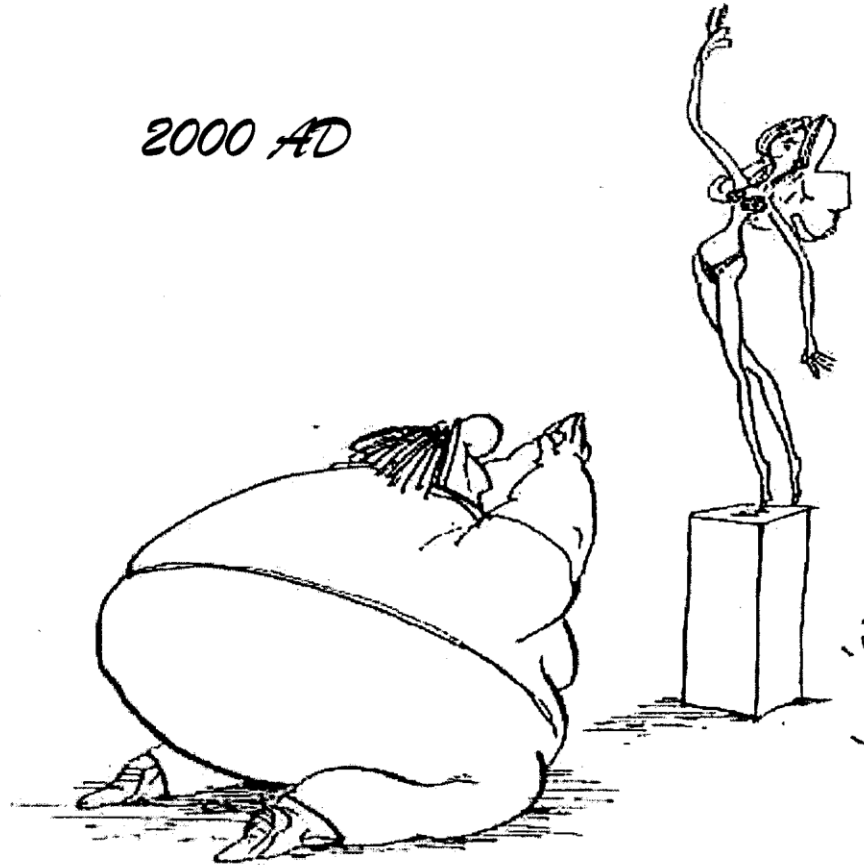




2000 BC



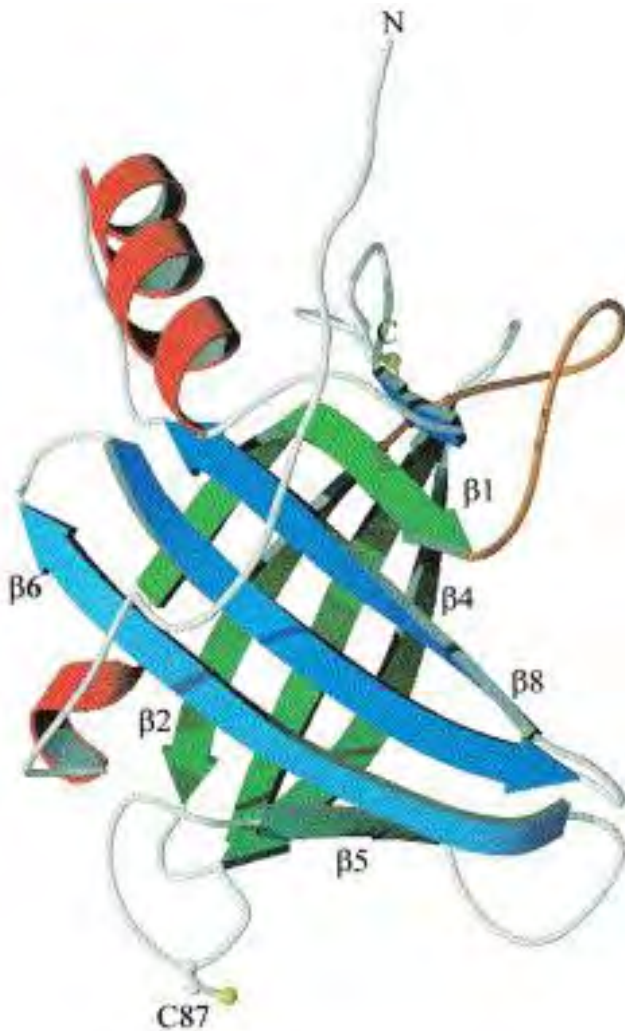
2000 AD



F. J. J. J.

EVOLUTION...

# Lipocalin-2



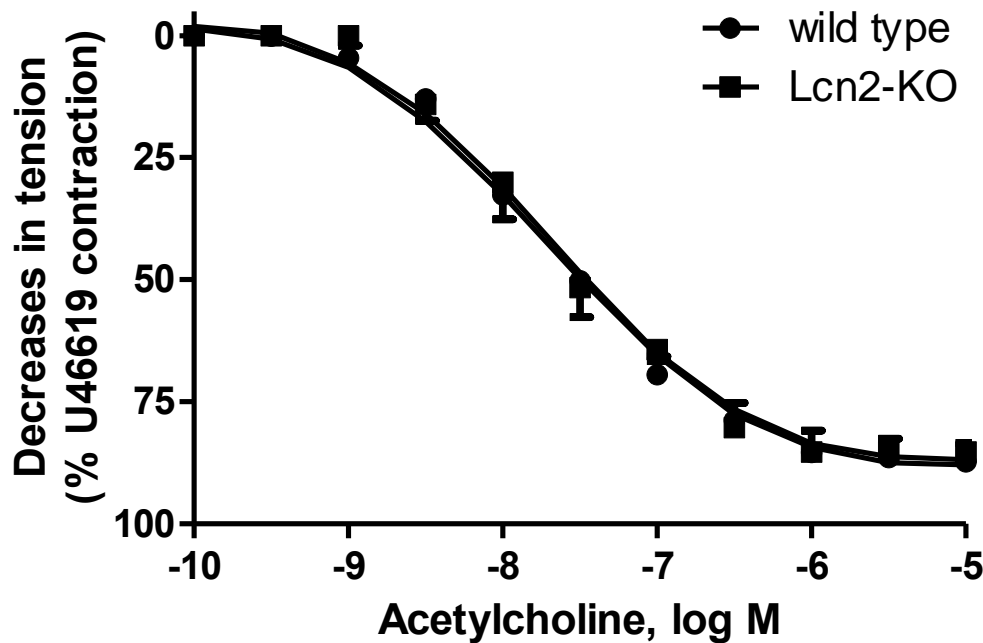
- 25kDa pro-inflammatory glycoprotein
- ↑ in obese mice and humans
- ↓ by rosiglitazone
- In humans, lipocalin-2 is positively associated with several parameters of the metabolic syndrome [adiposity, hypertriglyceridemia, hyperglycemia, insulin resistance index], and the levels of the inflammatory marker, C-reactive protein. [Wang *et al.* (2007) *Clin. Chem* 53: 34-41]

Cole *et al.* (1999) *J Mol Biol* 289(1):139-57

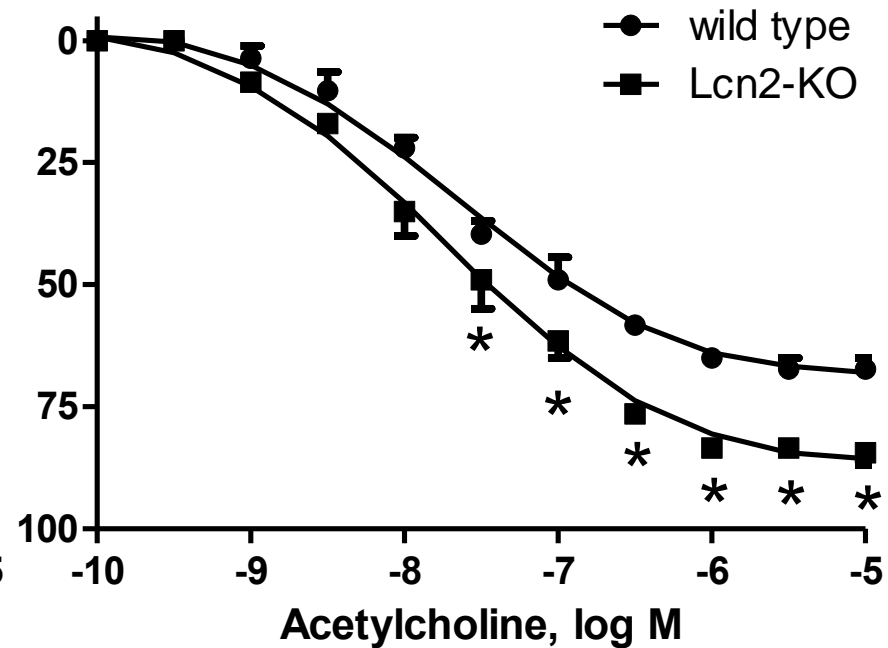
# Relaxations to acetylcholine in mouse aortae

[with endothelium; 12-13 weeks]

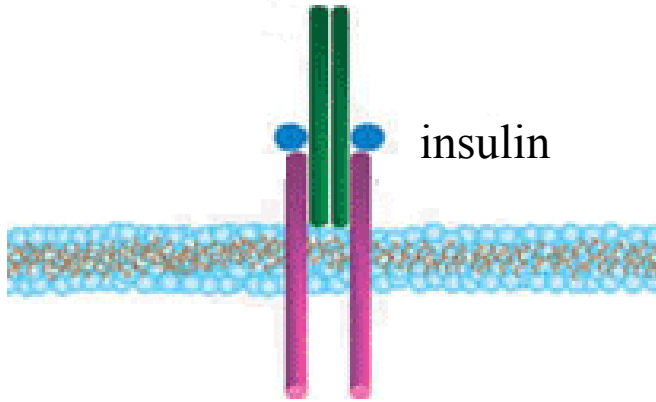
## Control diet



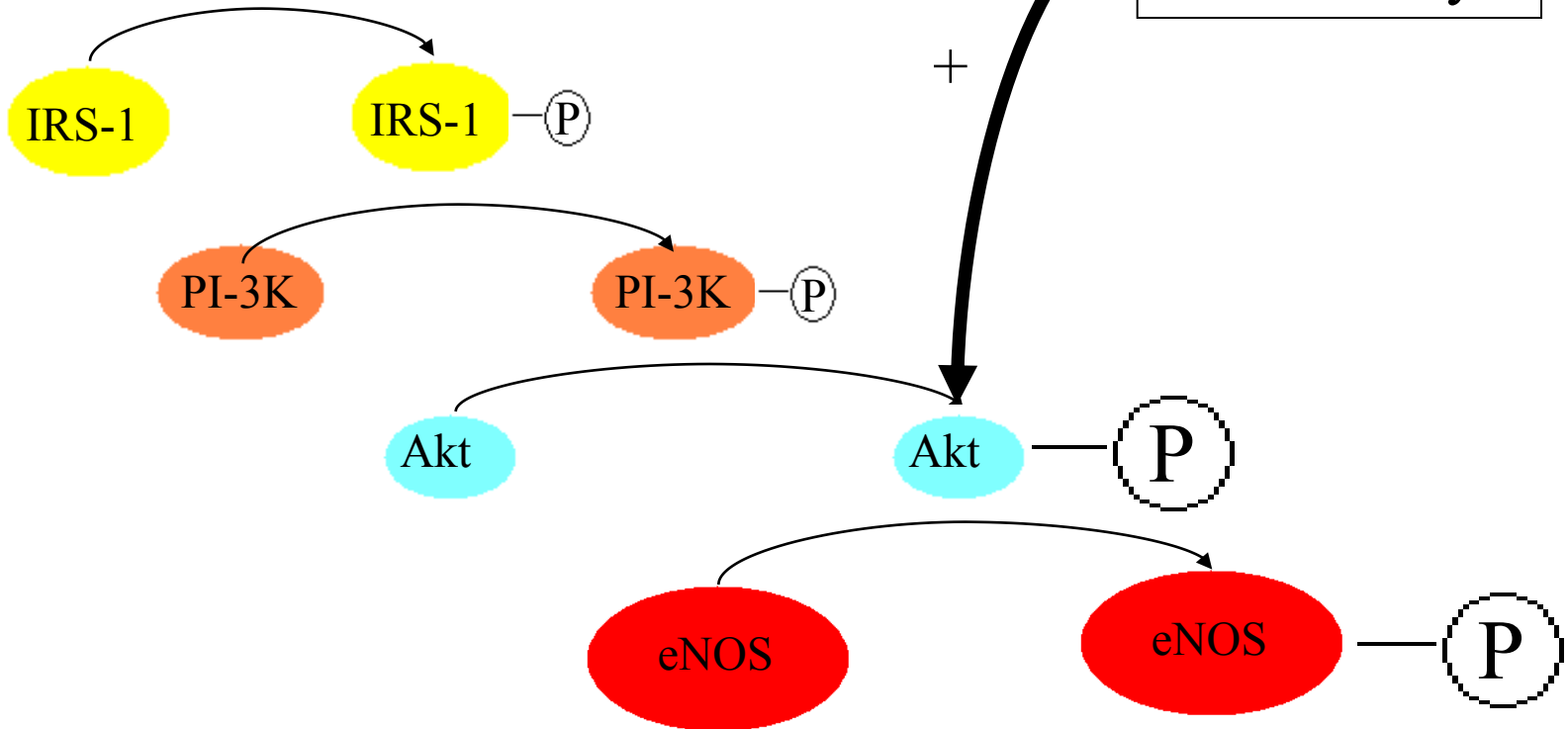
## High fat diet



Insulin receptor

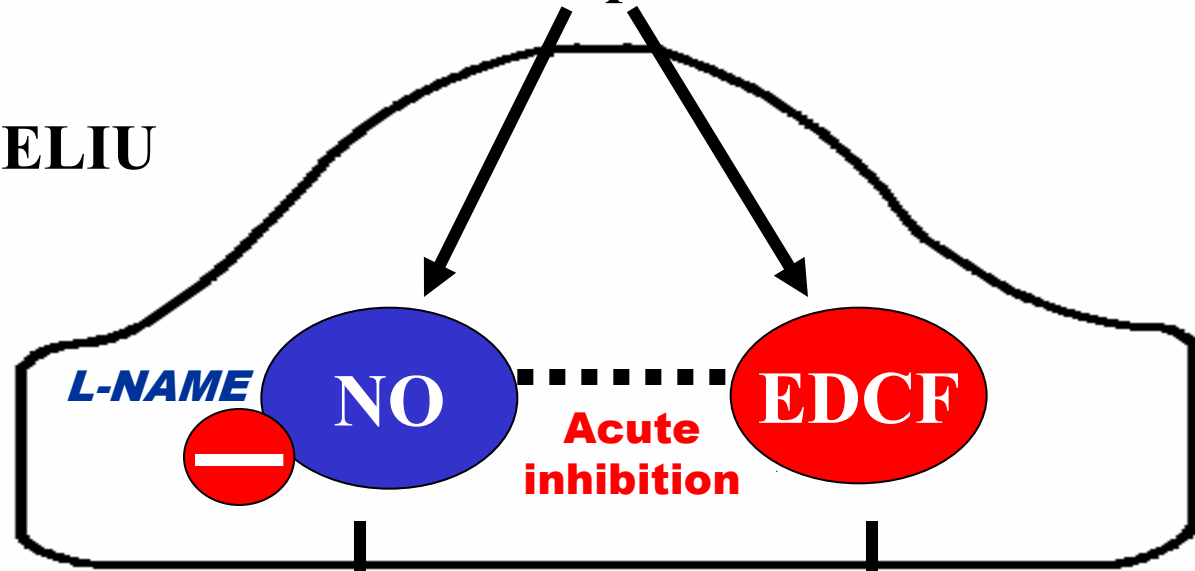


insulin

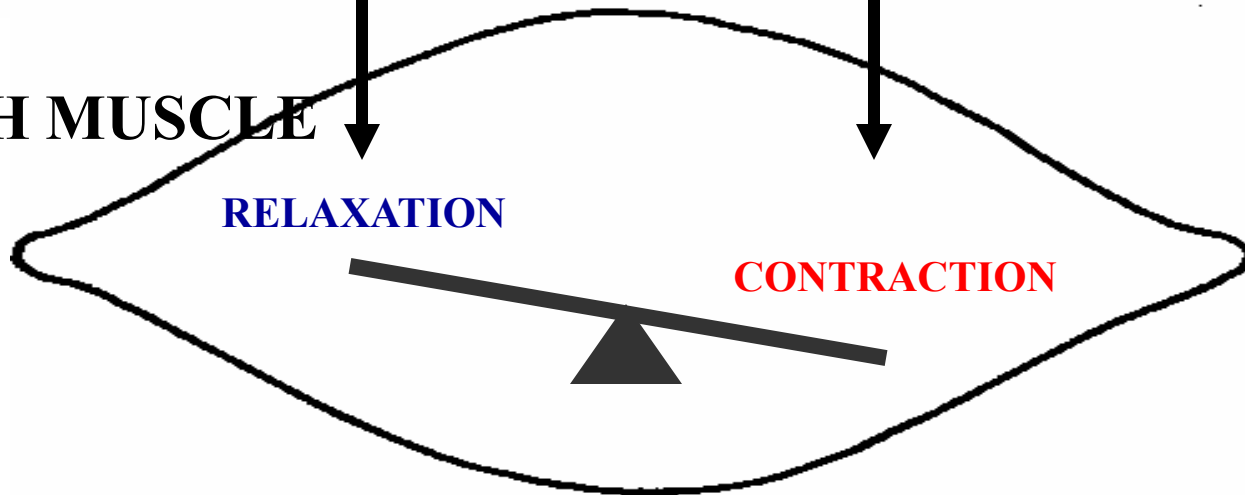


**ACETYLCHOLINE or  
Calcium Ionophore A23187**

**ENDOTHELIUM**



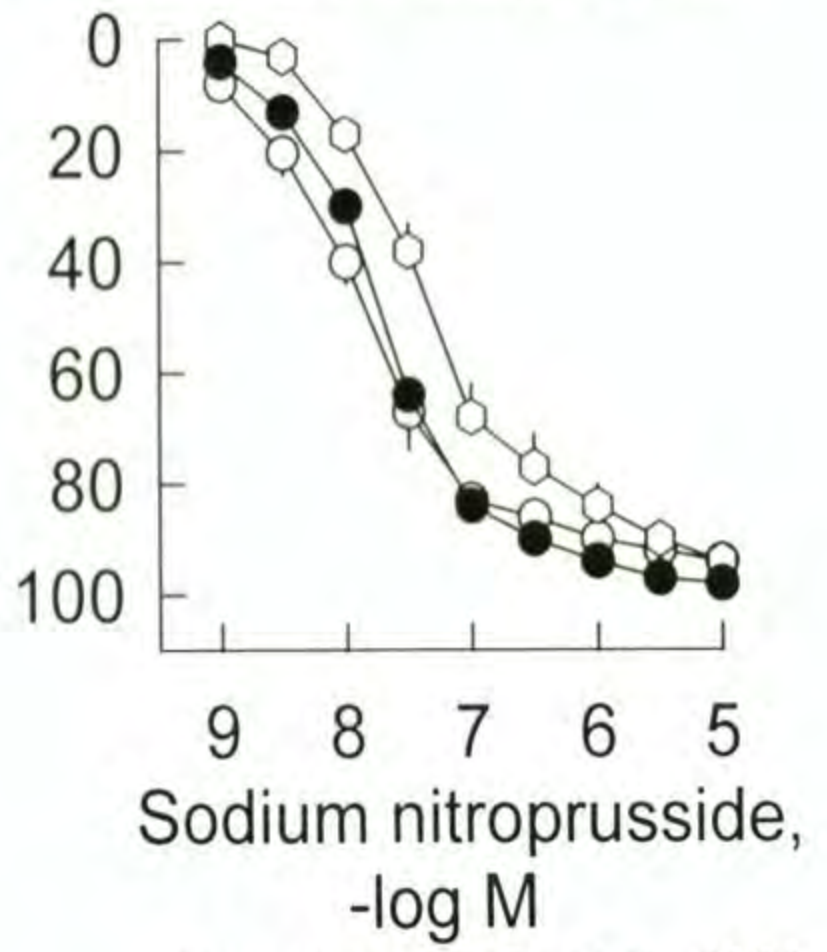
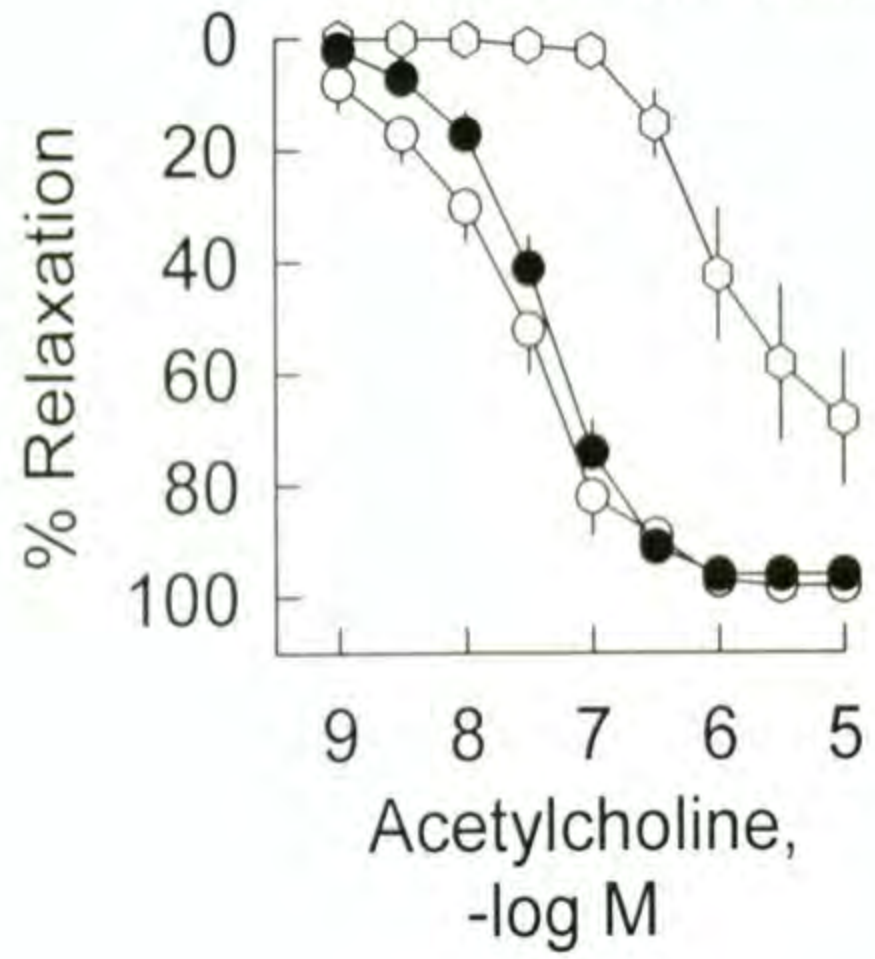
**SMOOTH MUSCLE**



# RELAXATIONS OF RAT MESENTERIC ARTERIES

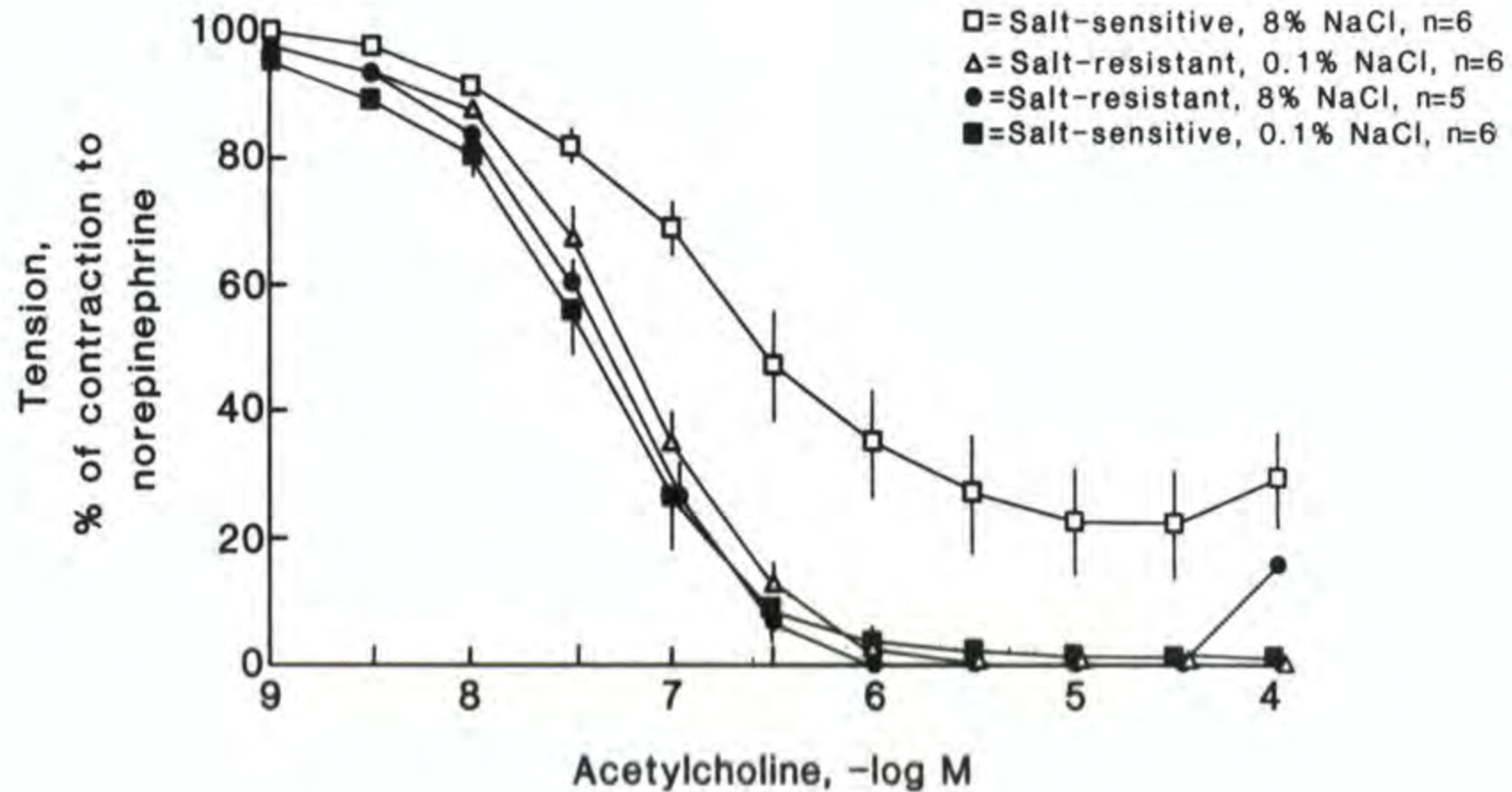
○ control, n=13-17  
● diabetics + insulin, n=8-13

◇ diabetics, untreated, n=16-22



From Taylor P.D. et al., 1994

# ACETYLCHOLINE AND DAHL RAT AORTAS ( Endothelium intact )



A:



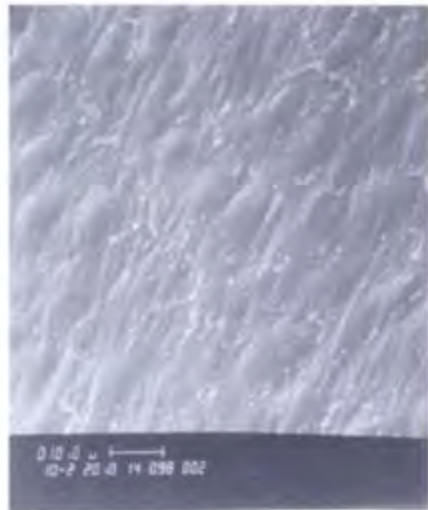
Control

B:



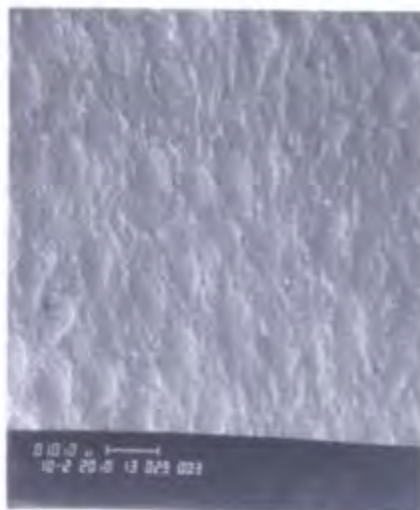
Immediately after  
denudation

C:



8 days after  
denudation

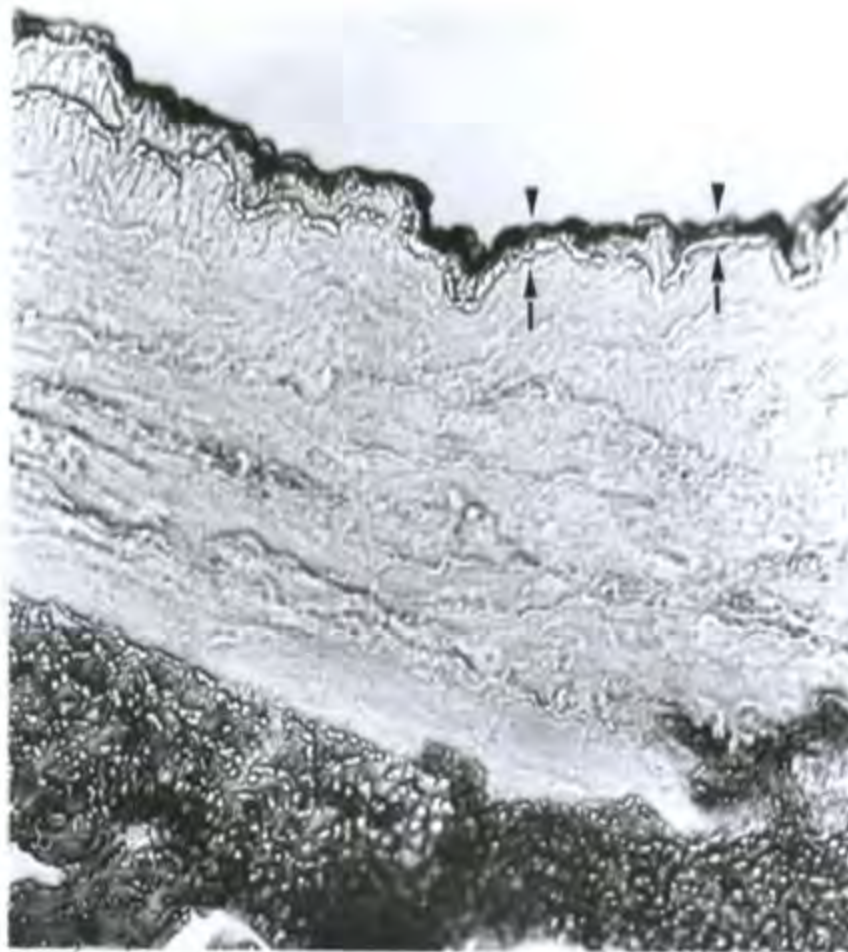
D:



4 weeks after  
denudation

# ENDOTHELIAL NOS (TYPE III) IMMUNOSTAINING IN PORCINE CORONARY ARTERIES

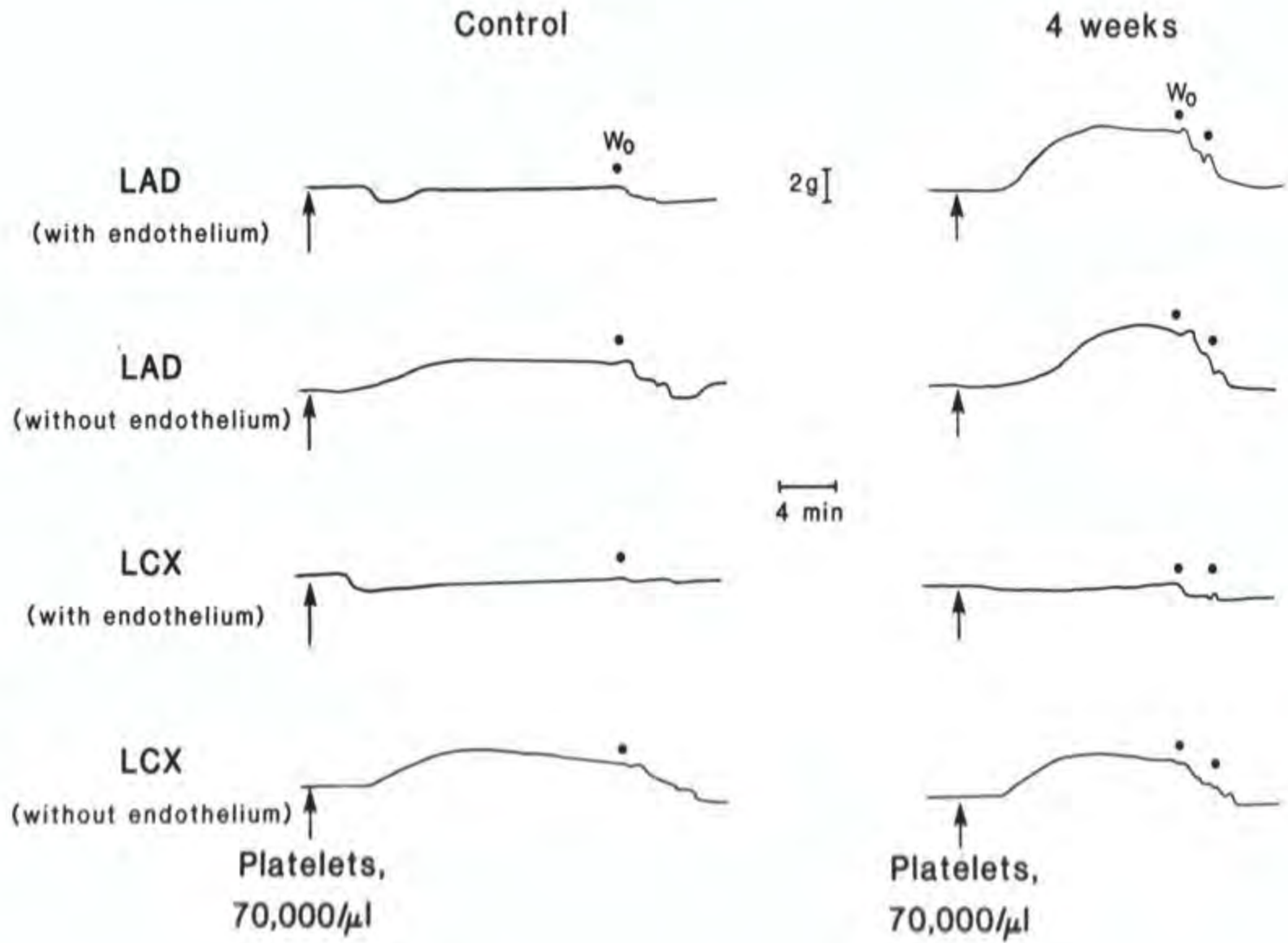
LCX  
(control)



LAD  
(regenerated endothelium)

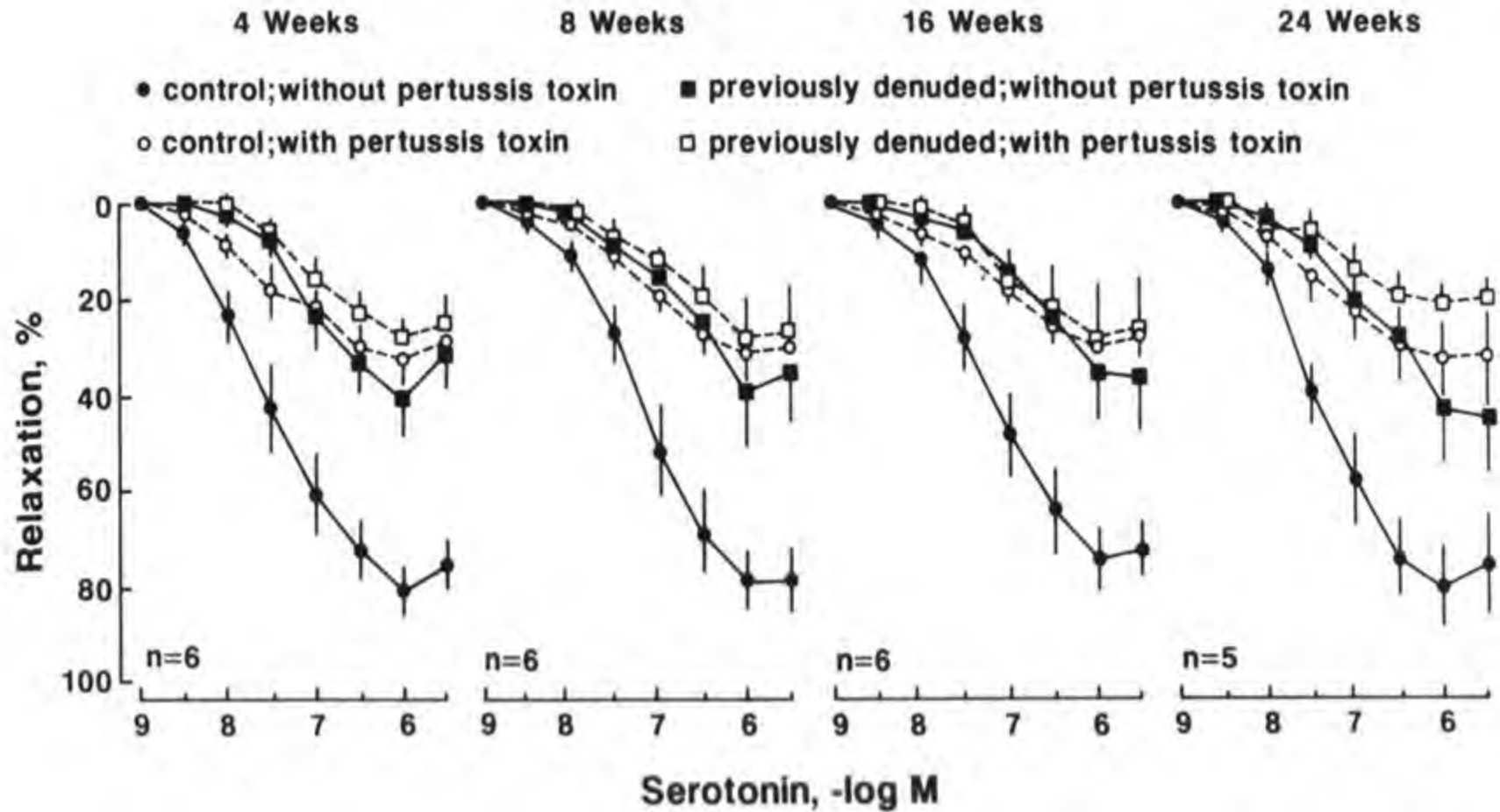


# PLATELETS AND REGENERATED ENDOTHELIUM IN ISOLATED PORCINE CORONARY ARTERIES



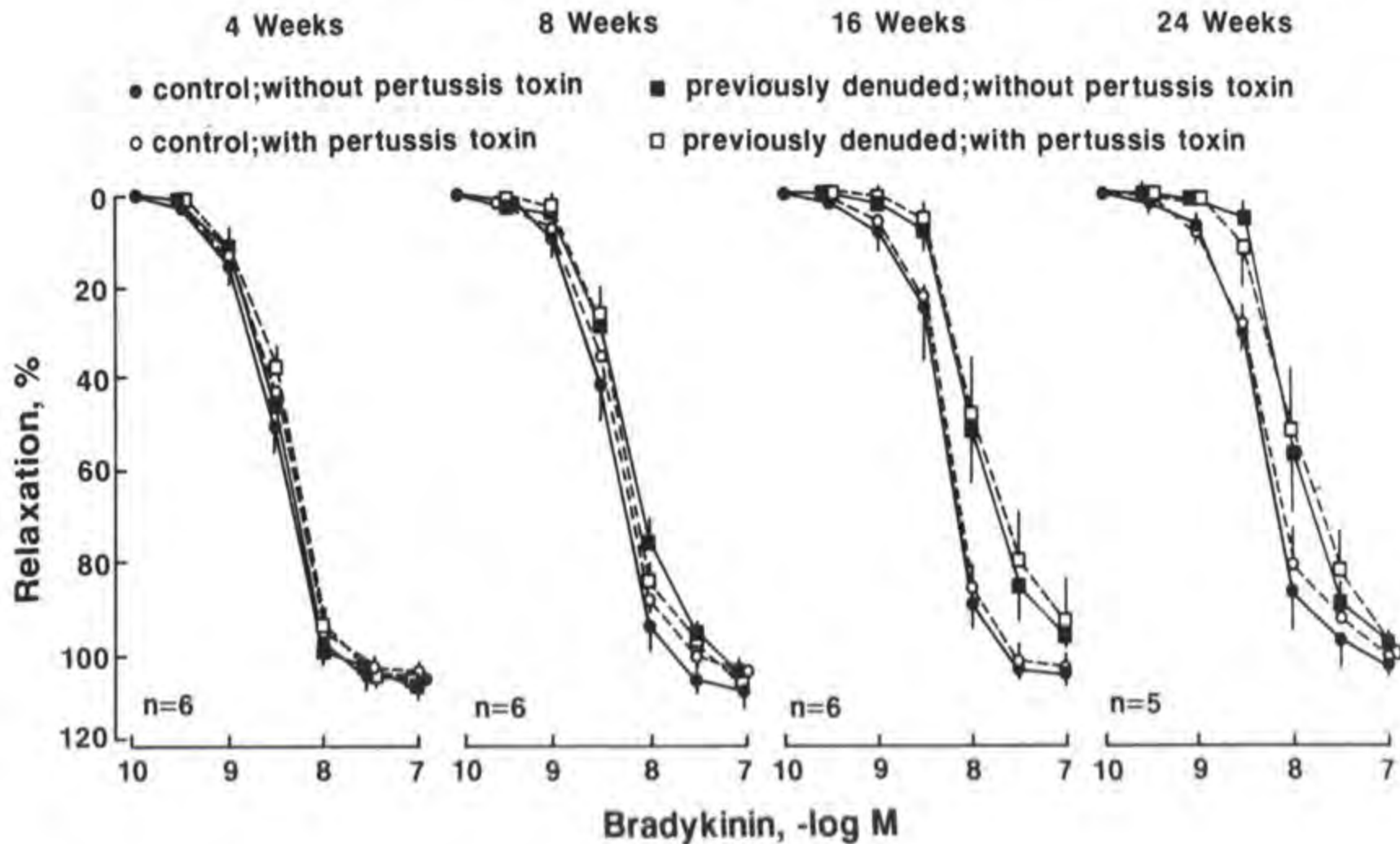
# SEROTONIN AND PORCINE CORONARY ARTERIES

(with endothelium; in the presence of indomethacin and ketanserin)

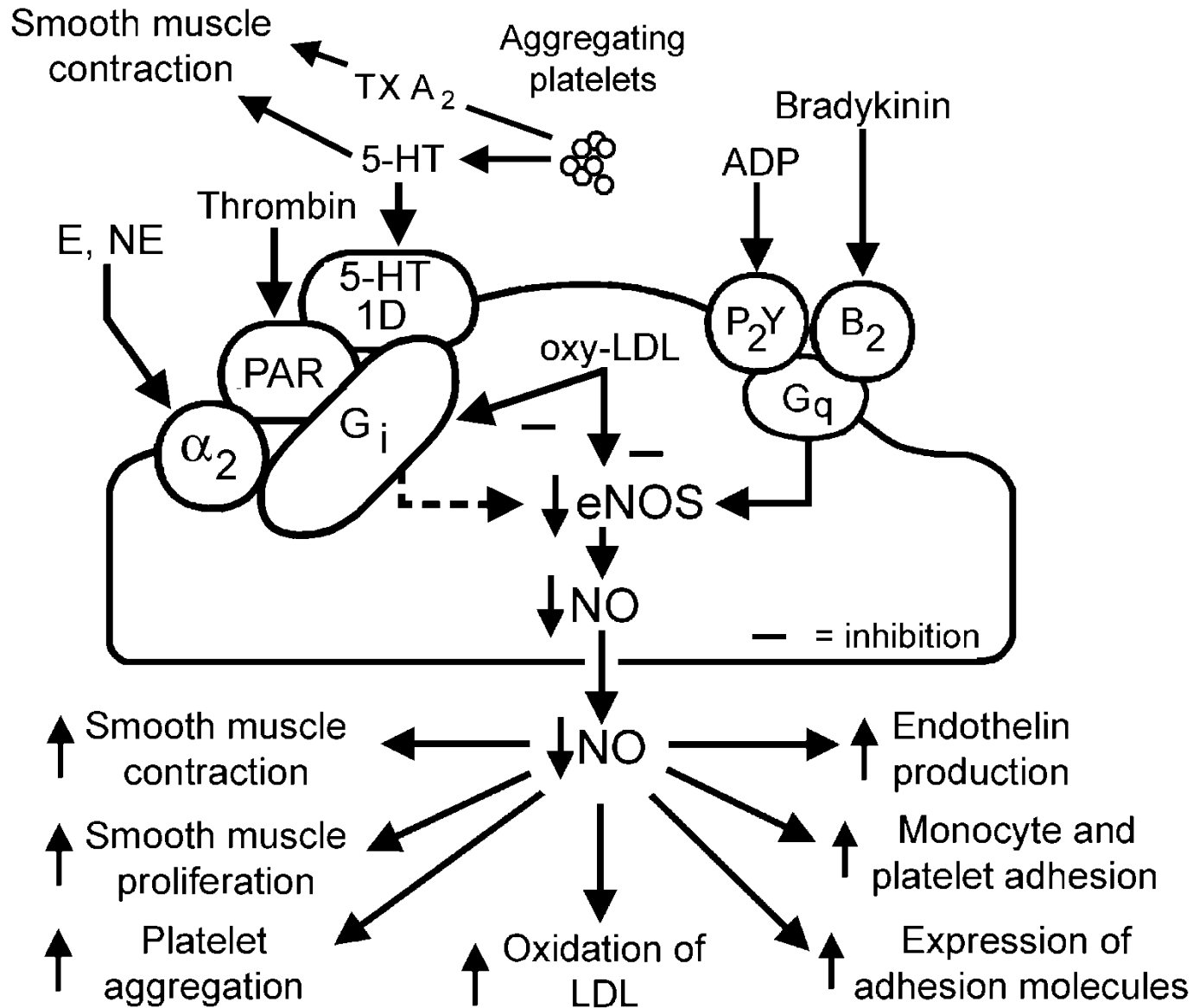


# BRADYKININ AND PORCINE CORONARY ARTERIES

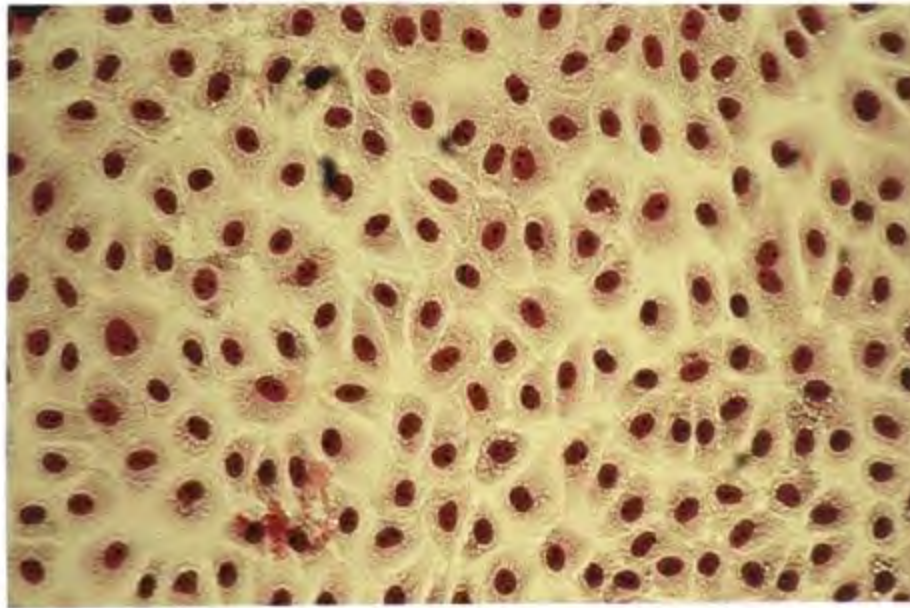
(with endothelium; in the presence of indomethacin)



# Regenerated Endothelial Cell



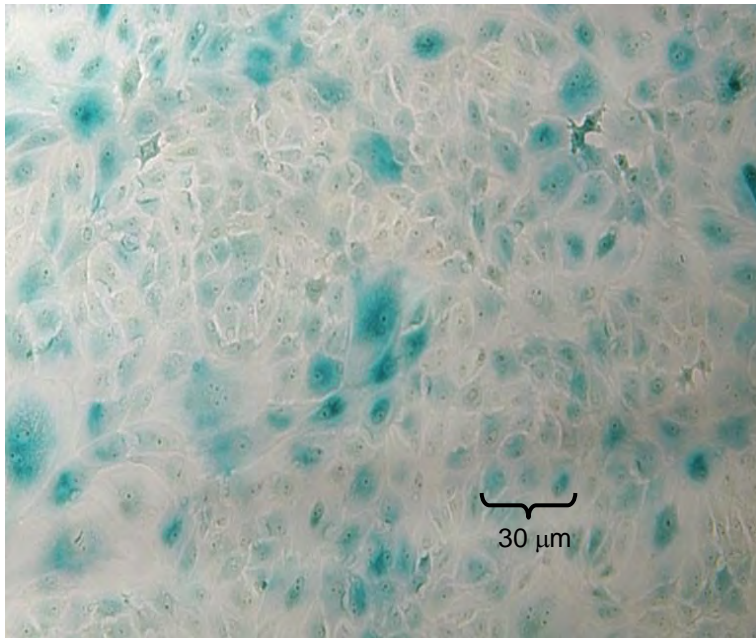
**Control**



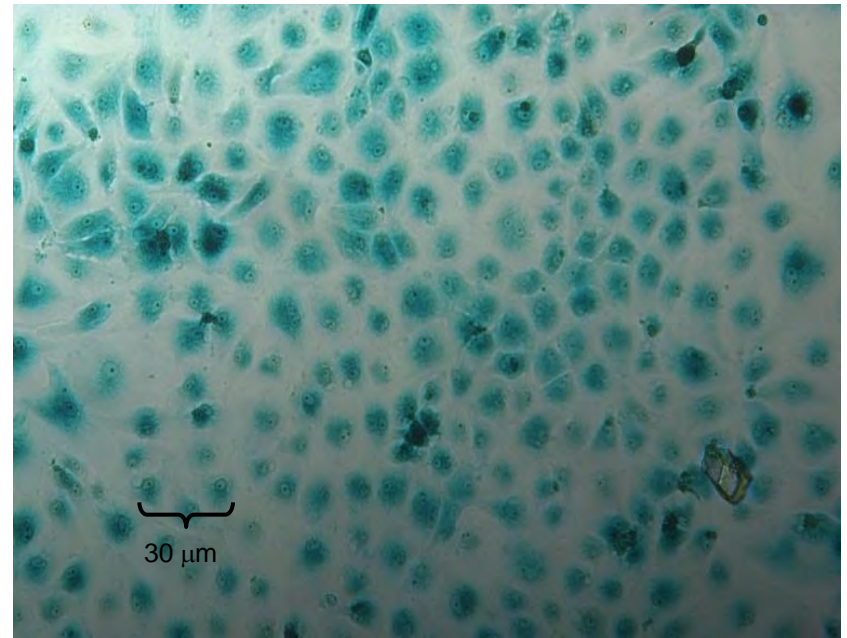
**Regenerated**



# Elevated cytoplasmic detection of $\beta$ -galactosidase in regenerated endothelial cells (P1)



Native cells

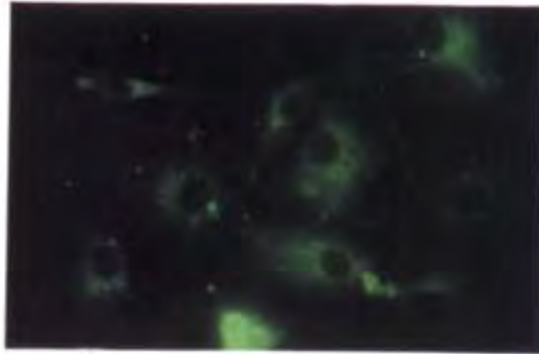


Regenerated cells

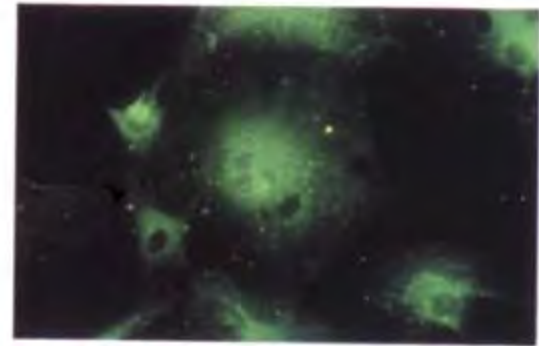
**Regenerate to be old...**

**G1 $\alpha$  family IMMUNOSTAINING IN ENDOTHELIAL CELLS  
FROM PORCINE CORONARY ARTERIES**

Native



Regenerated  
28 days

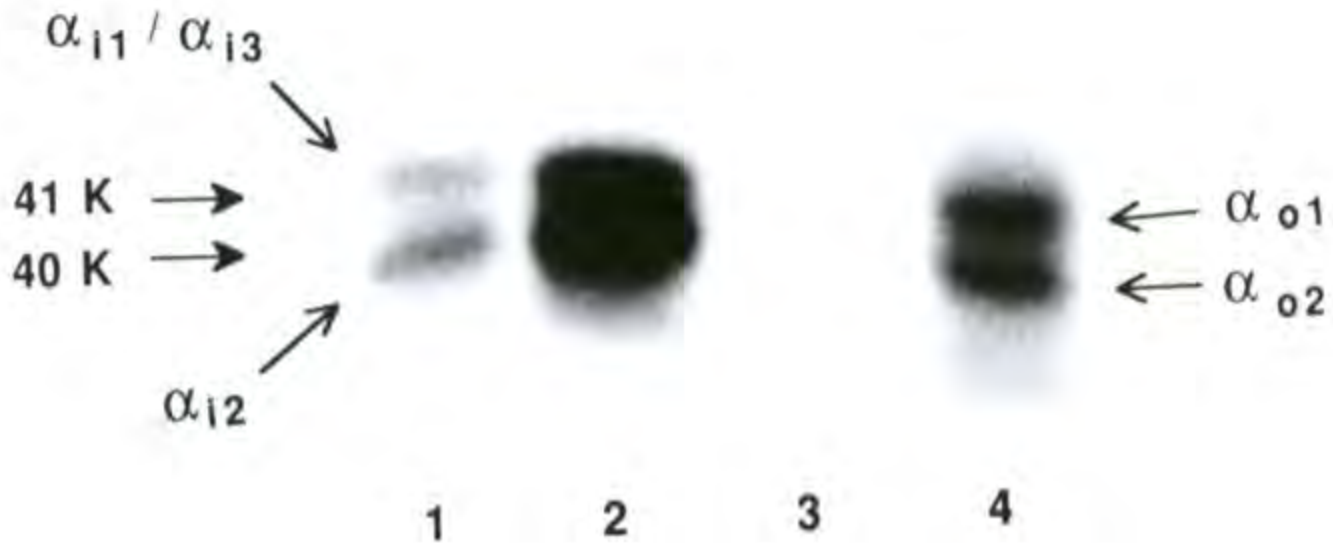


Control



# Gi PROTEINS IN PORCINE MEMBRANE FRACTIONS

(ADP-ribosylation with pertussis toxin)

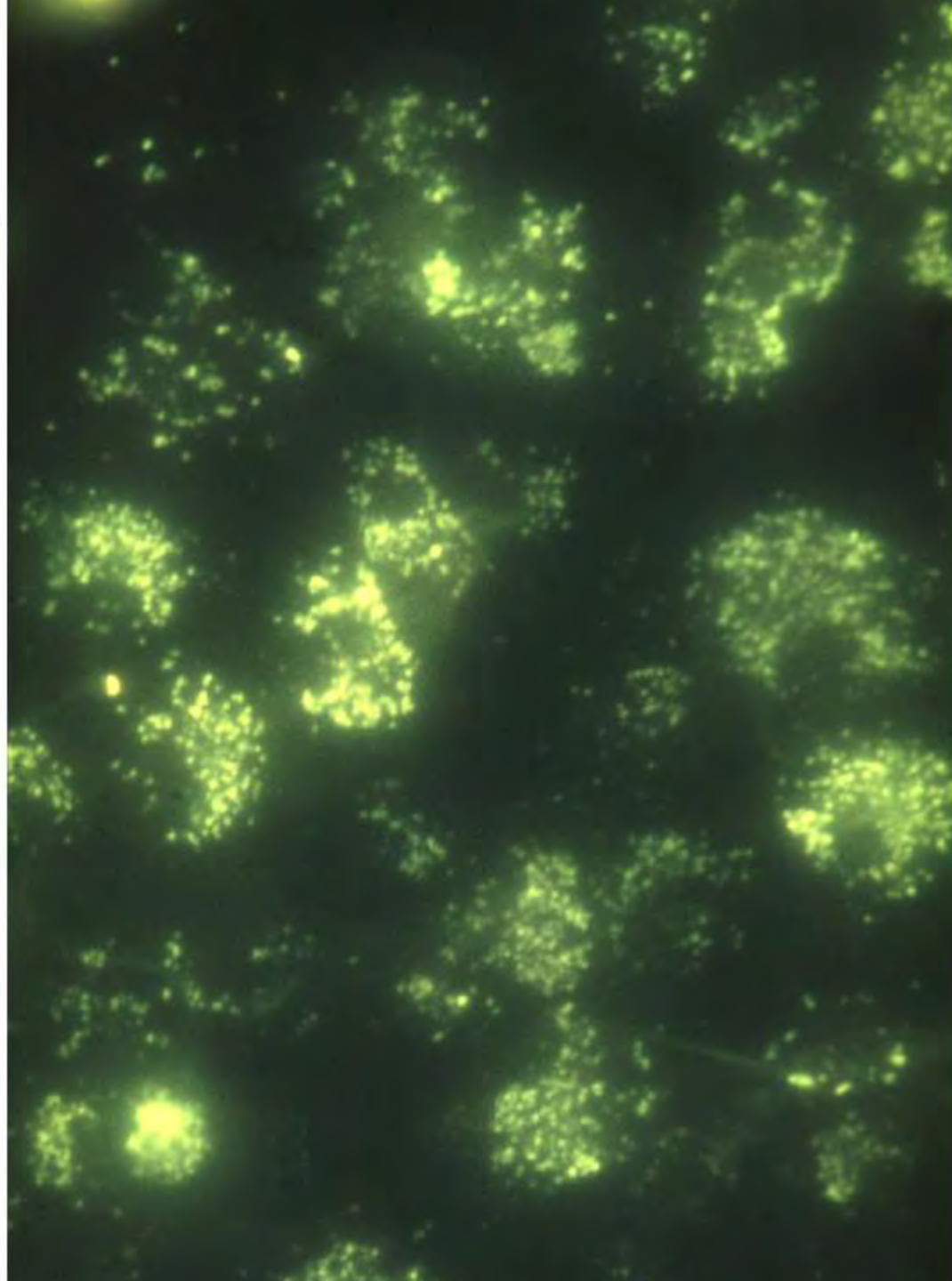


1: regenerated endothelial cells (LAD)

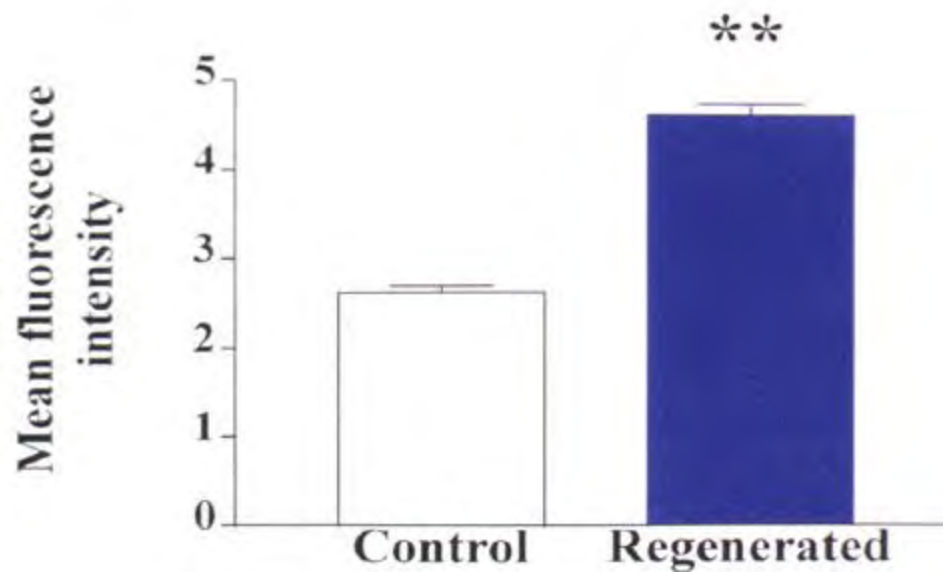
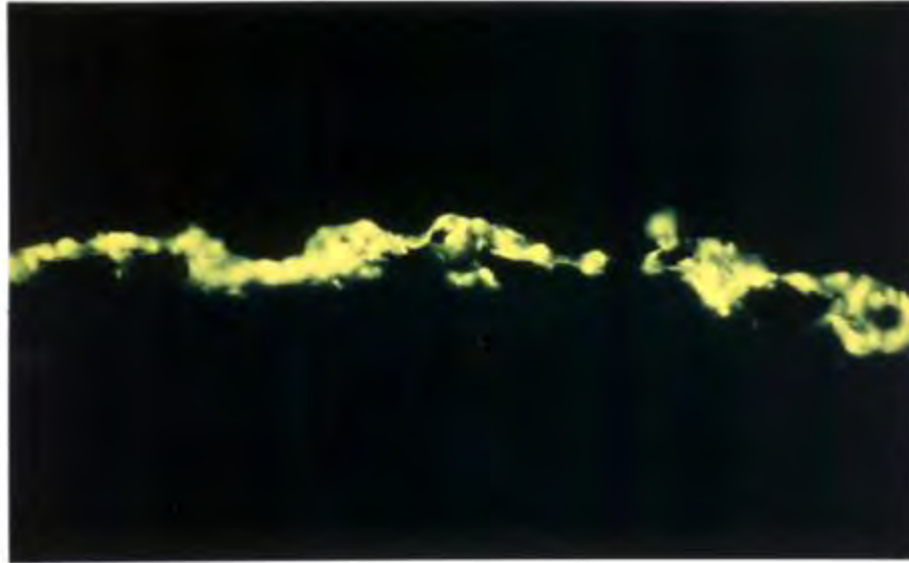
3: erythrocytes

2: control endothelial cells (LCX)

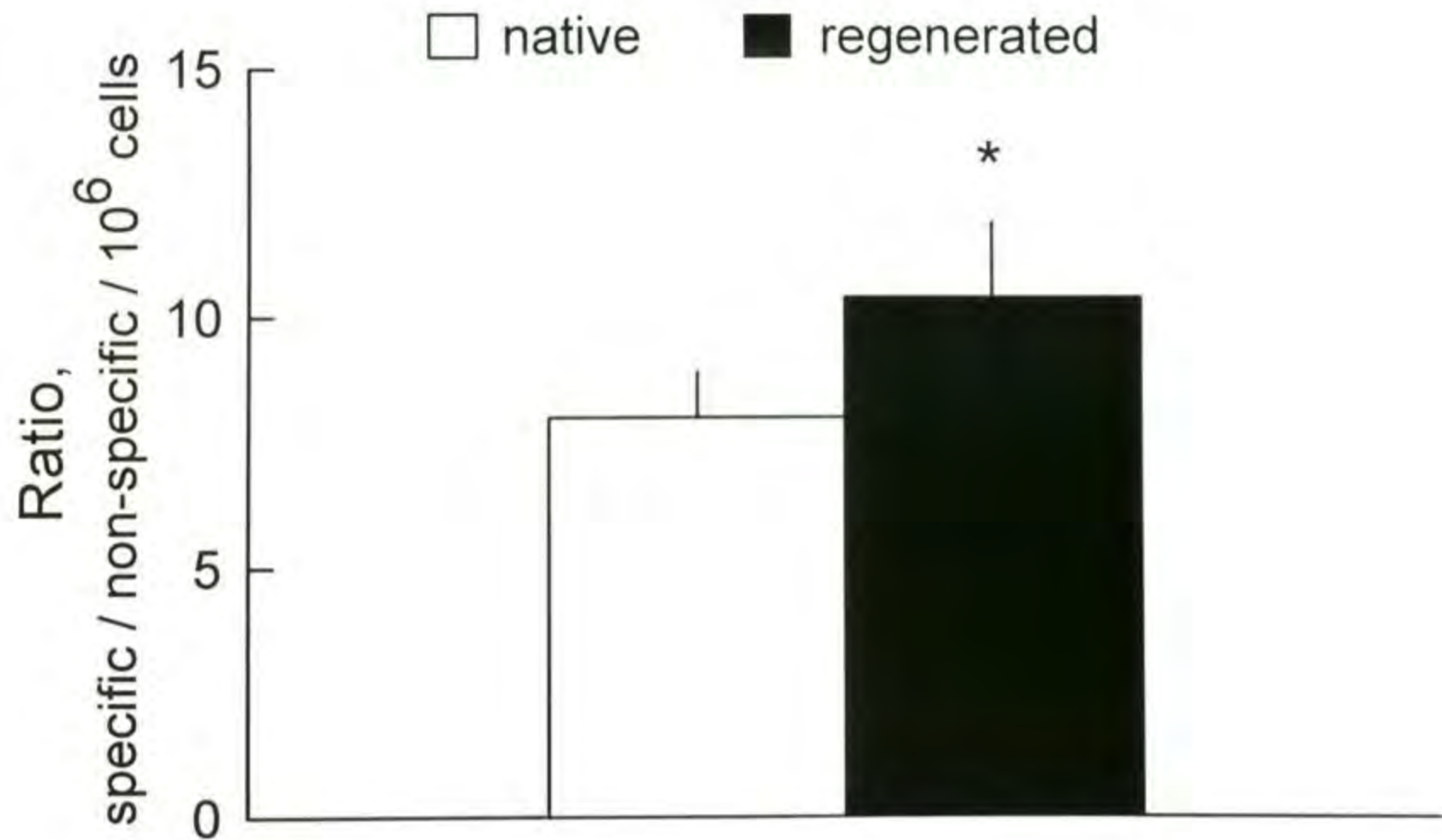
4: brain



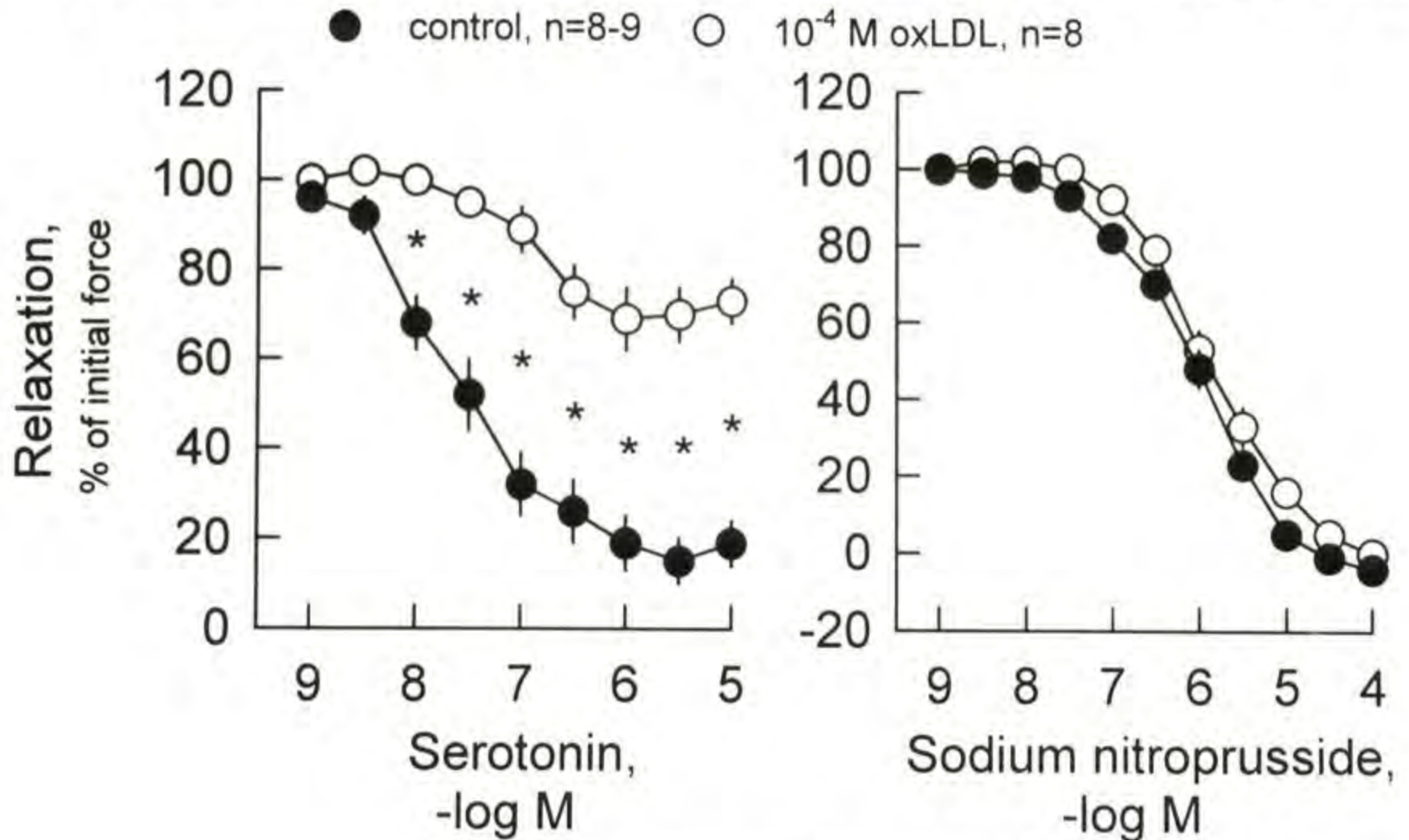
# Acetylated LDL uptake ex vivo



# OXIDIZED APO-B LIPOPROTEINS IN PORCINE ENDOTHELIAL CELLS

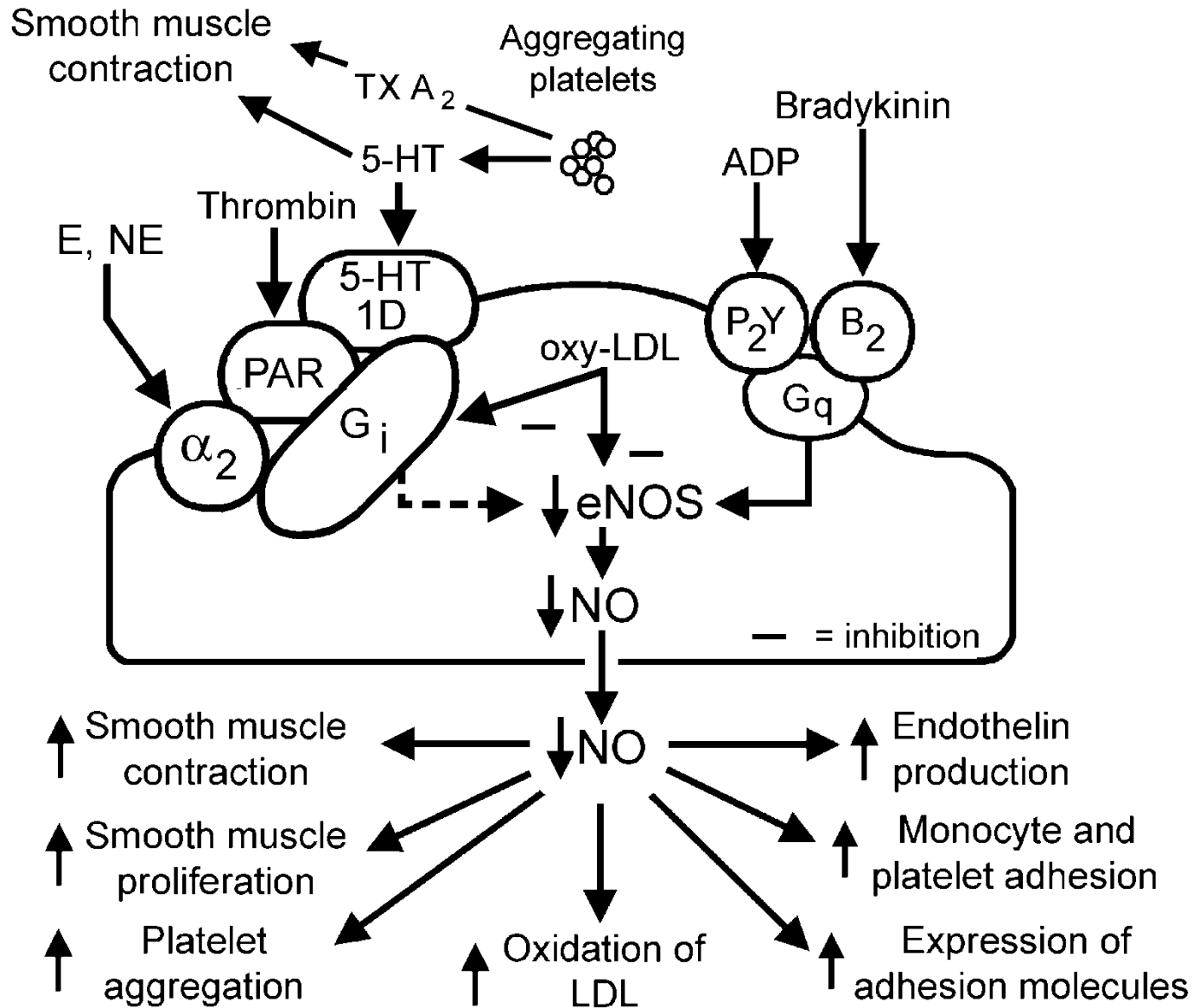


# OXIDIZED LDL AND RELAXATIONS IN PORCINE CORONARY ARTERIES WITH ENDOTHELIELIUM

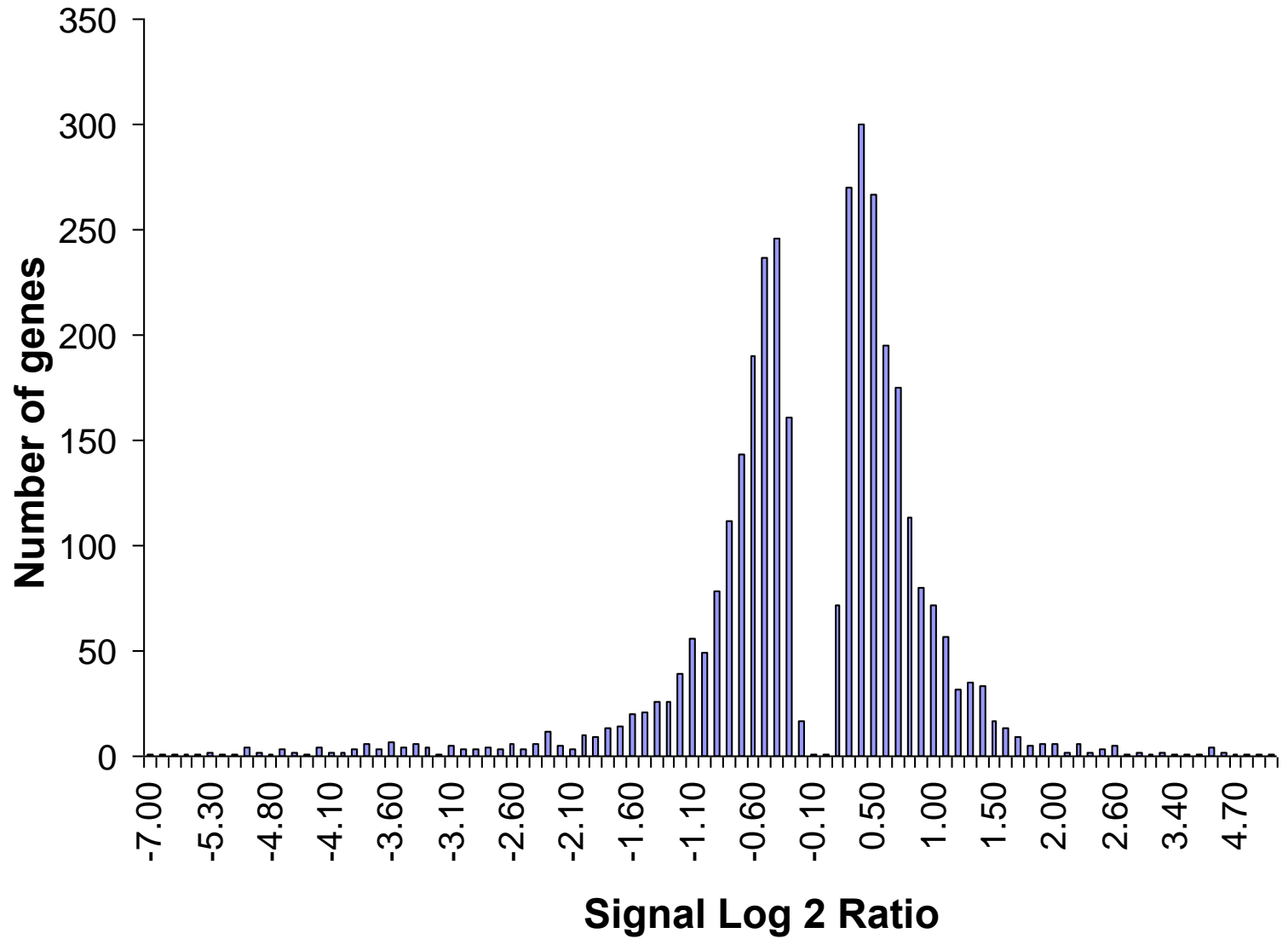


From Cox and Cohen, JPET 276:1095-1103, 1996

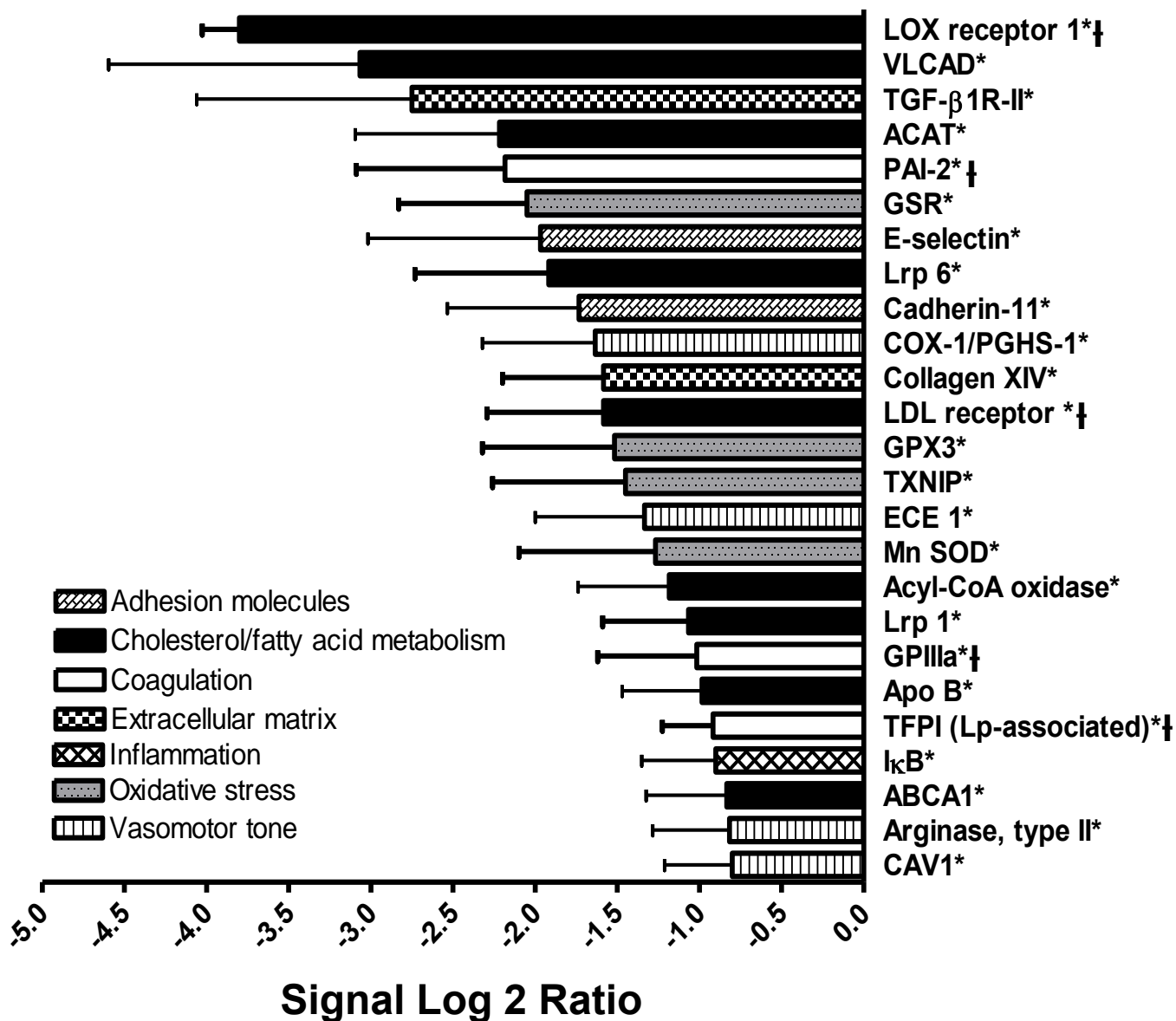
# Regenerated Endothelial Cell



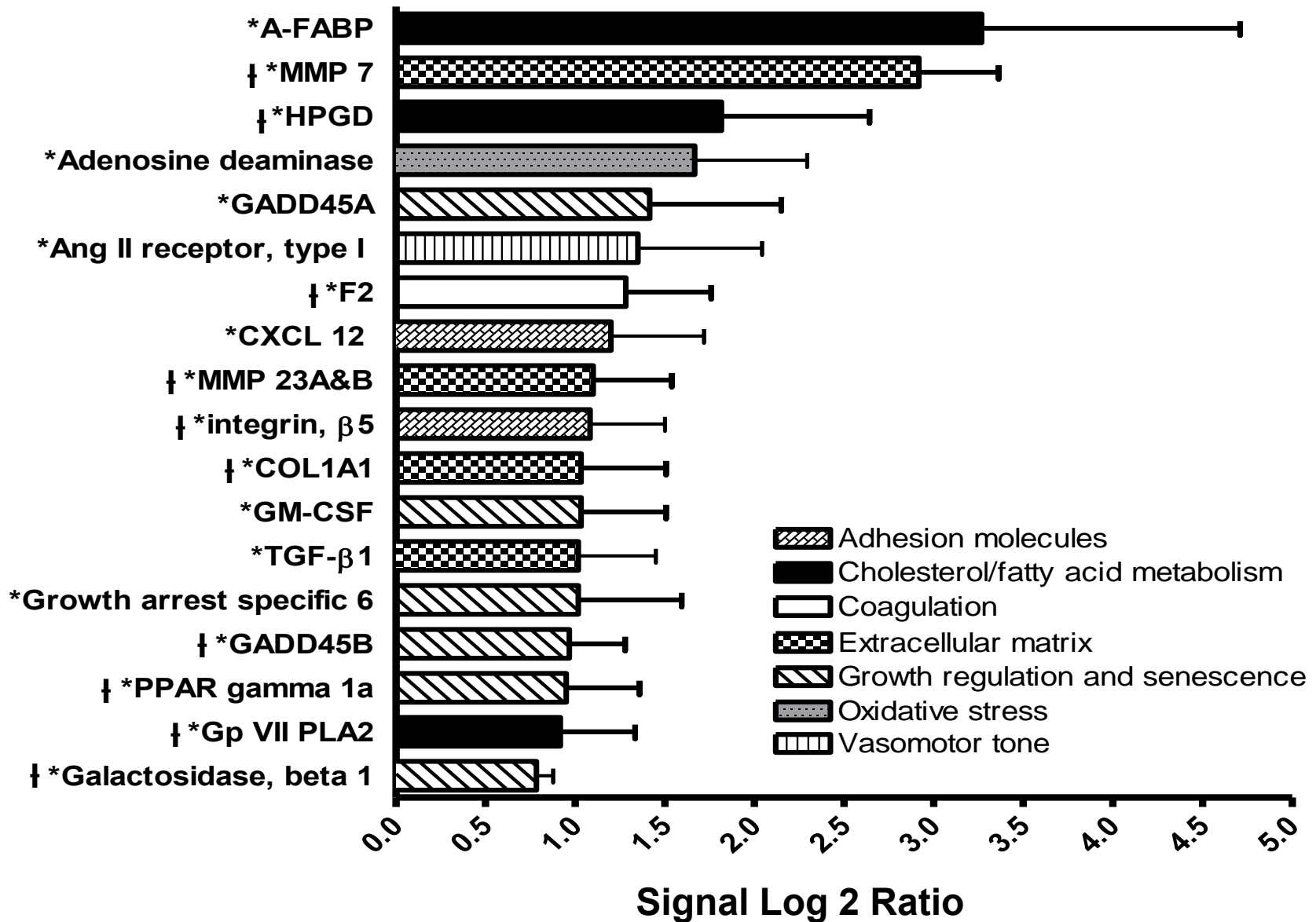
**Figure 2A**



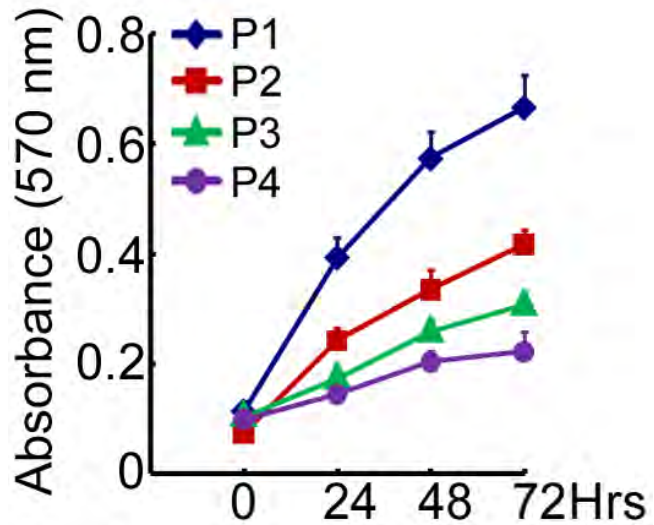
# Downregulation of genes in regenerated endothelial cells



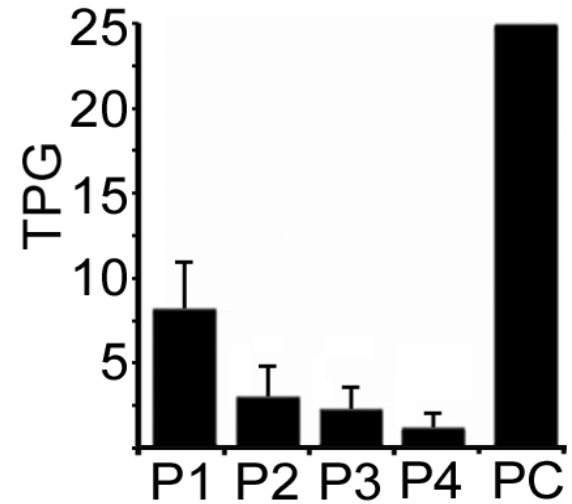
# Upregulation of genes in regenerated endothelial cells



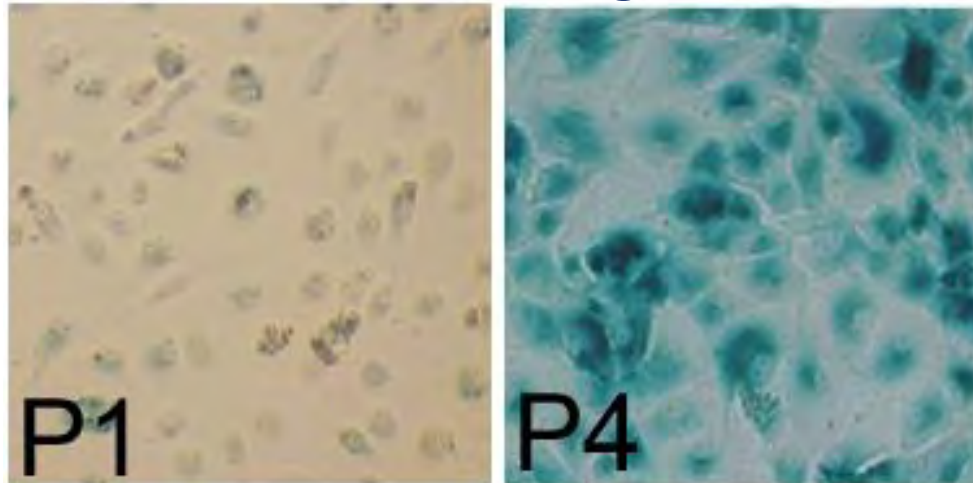
## Cell proliferation



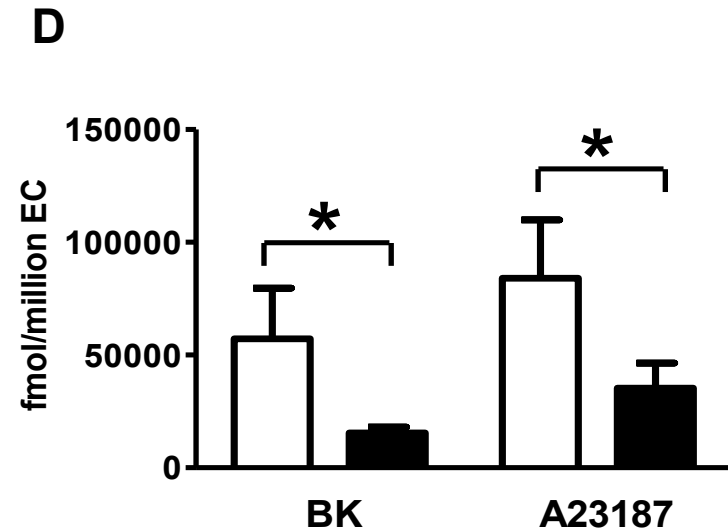
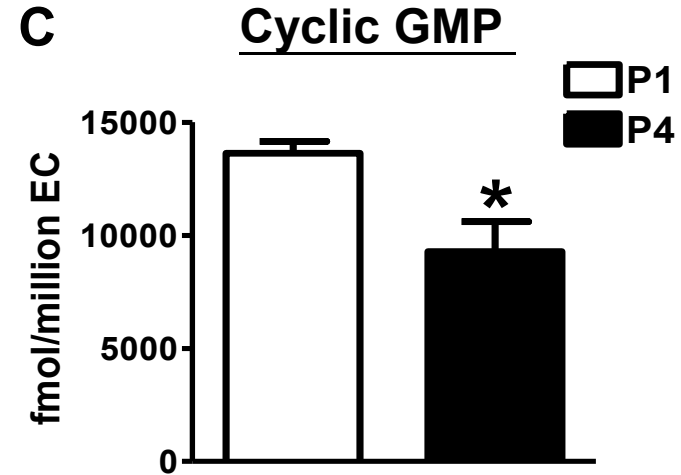
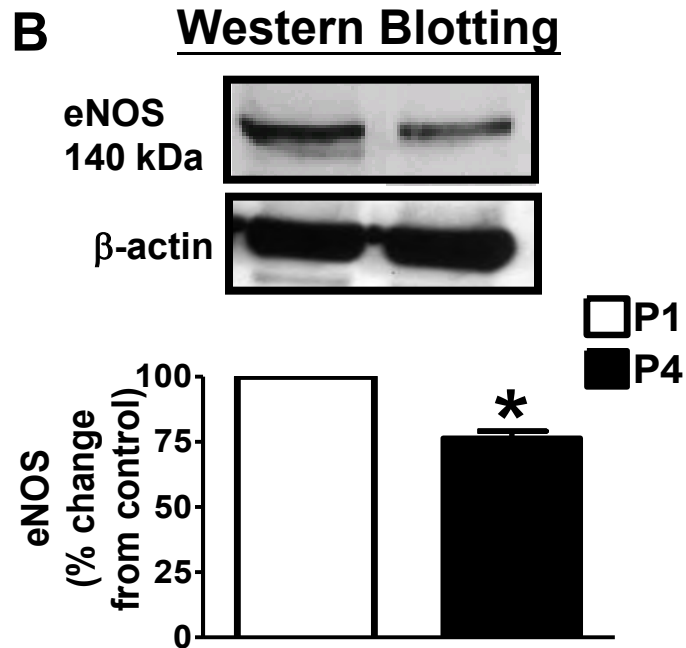
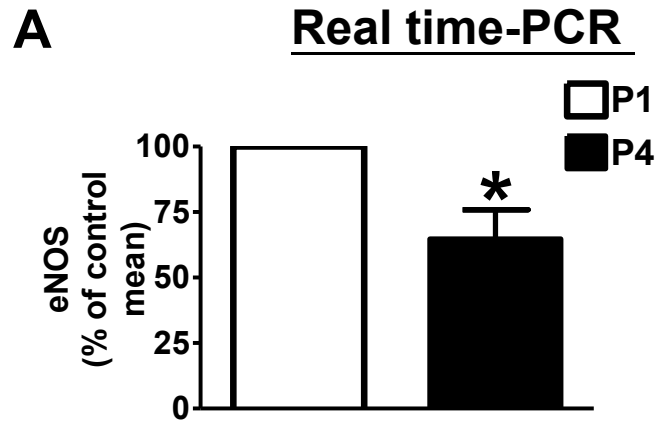
## Telomerase activity



## Senescence-associated beta-galactosidase staining



mRNA expression, protein presence of eNOS, cyclic GMP levels under basal conditions and upon stimulation with bradykinin (BK;  $10^{-6}$ M) and A23187 ( $10^{-6}$ M)



SIRT1



LKB1



Lysine

\* \* \* \*  
48 62 64 122

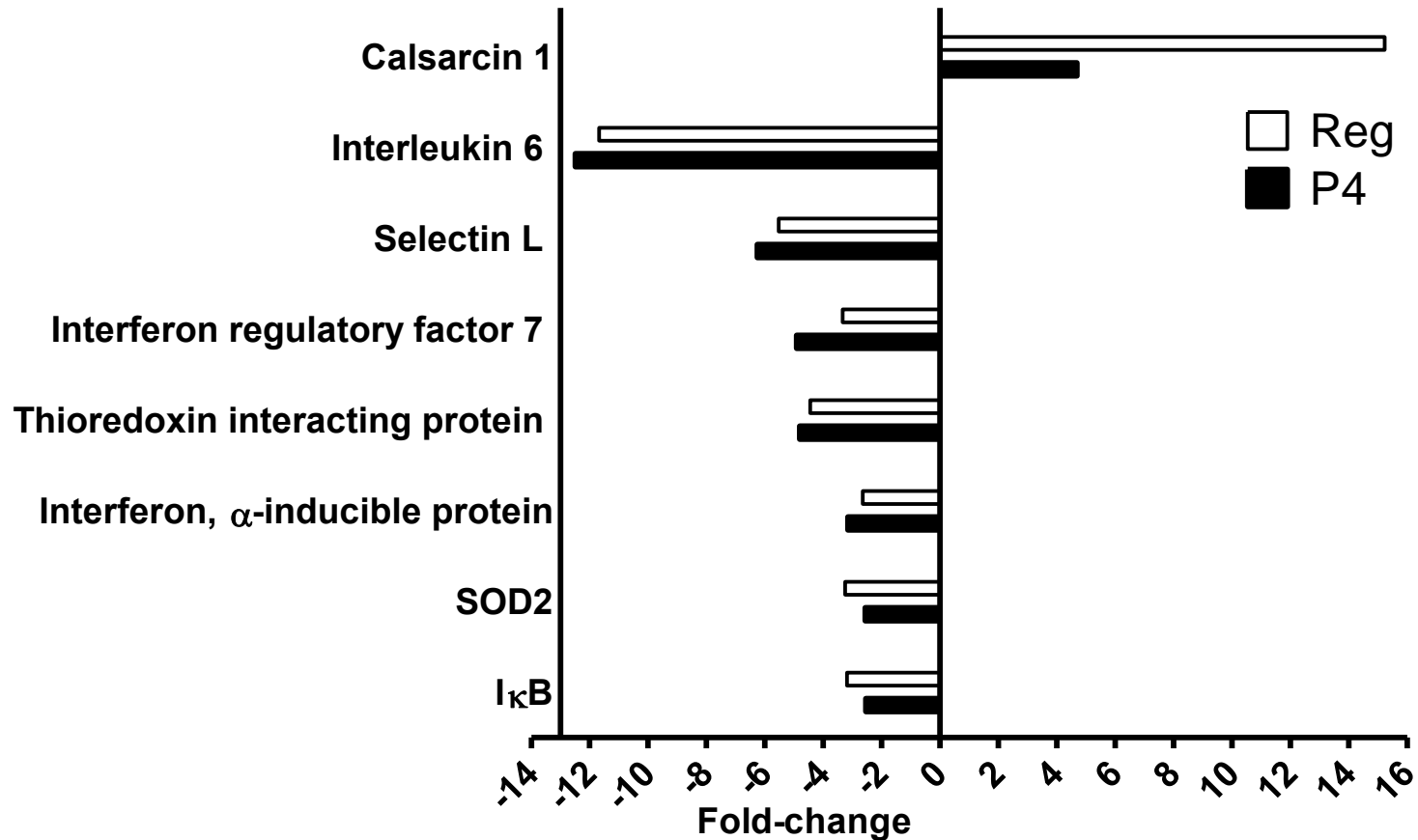


AMPK

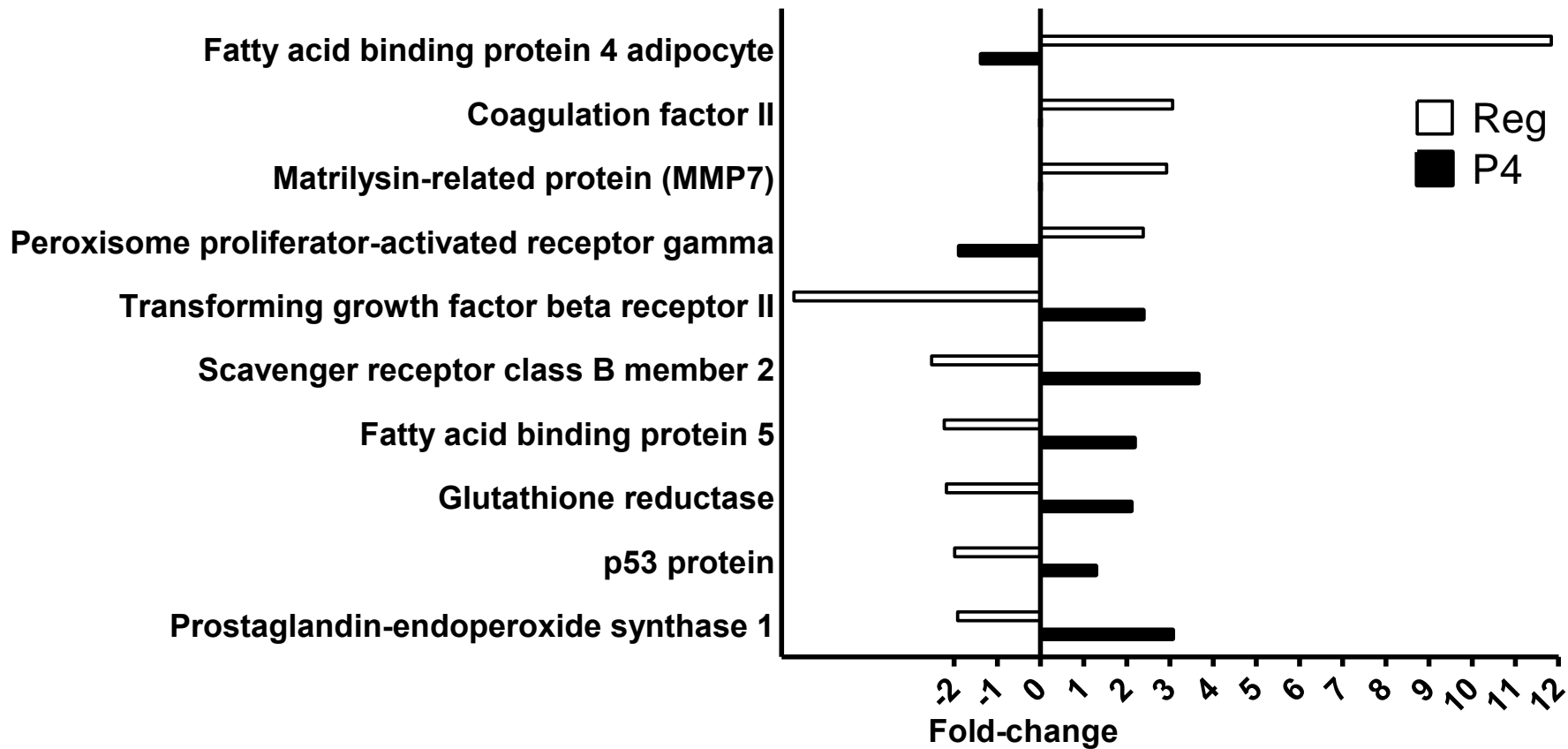


Endothelial Aging

Similar expression of genes in cultures derived from regenerated endothelial cells (Reg) and senescent endothelial cells (P4) compared to the young cells (P1)



Differential expression of genes in cultures derived from regenerated endothelial cells (Reg) and senescent endothelial cells (P4) compared to the young cells (P1)

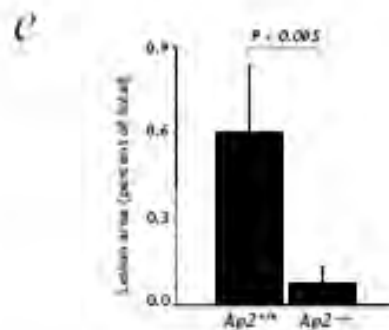
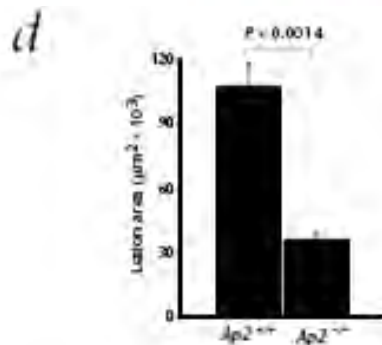
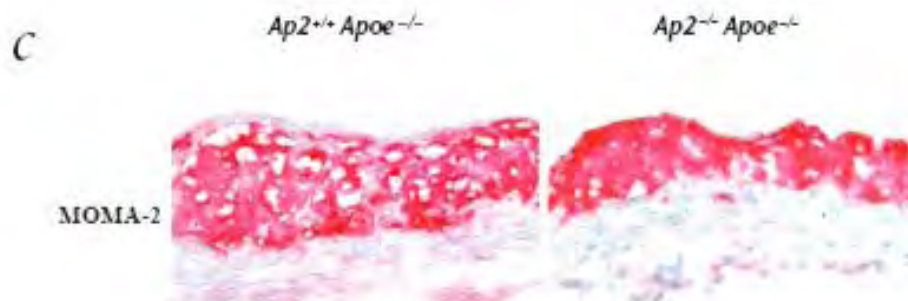


**Regenerate *in vivo* to be old...**

# A-FABP and atherosclerosis (1)

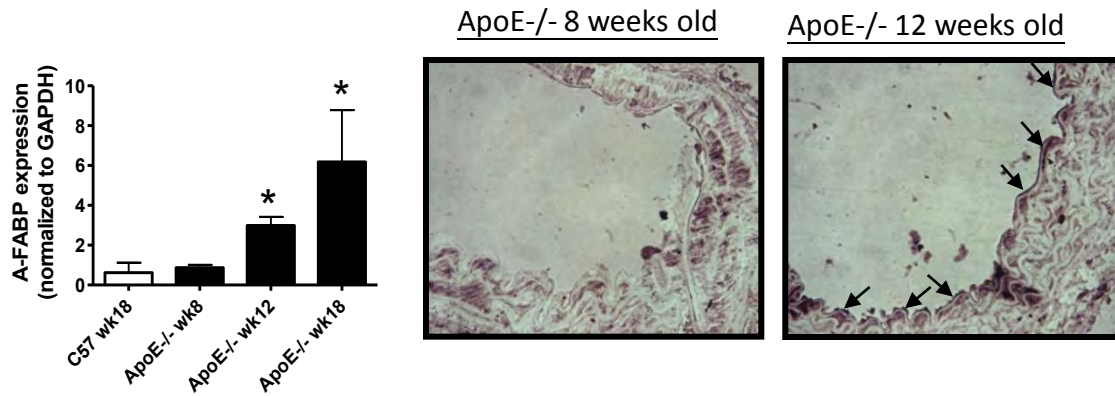
Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis

LIZA MAKOWSKI<sup>1</sup>, JEFFREY B. BOORD<sup>2</sup>, KAZUHISA MAEDA<sup>1</sup>, VLADIMIR R. BABAEV<sup>2</sup>,  
K. TEOMAN UYSAL<sup>1</sup>, MAUREEN A. MORGAN<sup>5</sup>, REX A. PARKER<sup>5</sup>, JILL SUTTLES<sup>6</sup>, SERGIO FAZIO<sup>2,3</sup>,  
GÖKHAN S. HOTAMISLIGIL<sup>1</sup> & MACRAE F. LINTON<sup>2,4</sup>

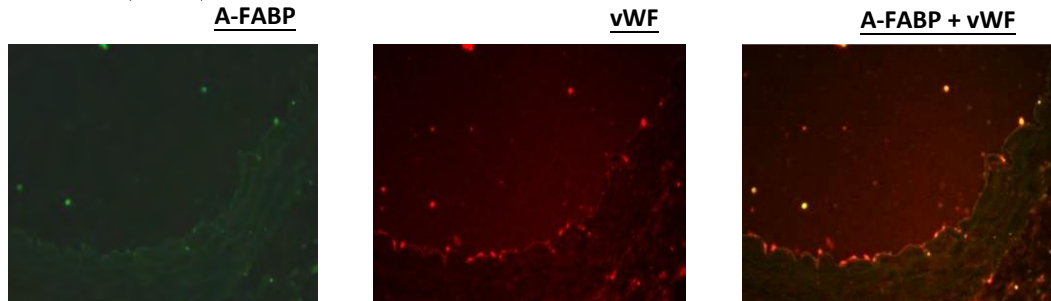


Makowski L; Nature Medicine, 2001

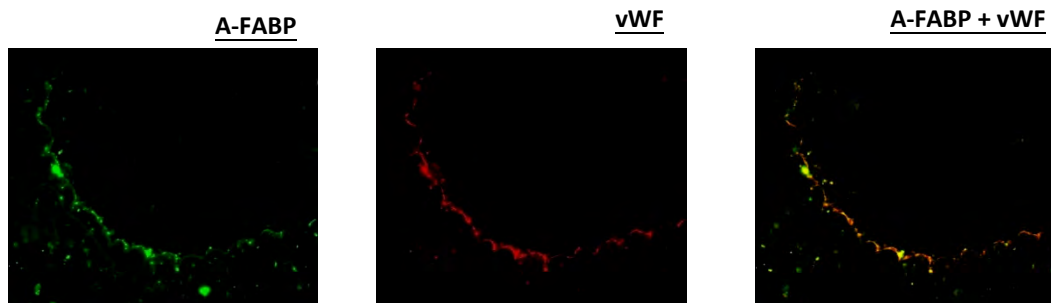
# Identification of A-FABP in mouse aortae by quantitative RT-PCR, immunohistochemical and immunofluorescent staining



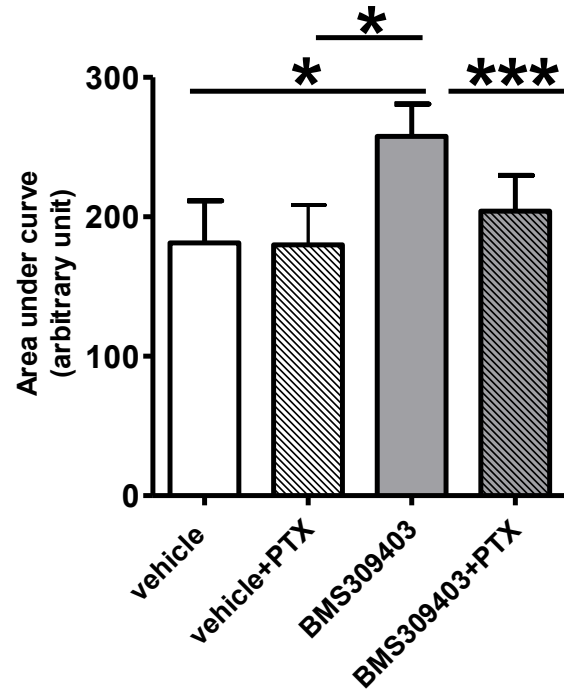
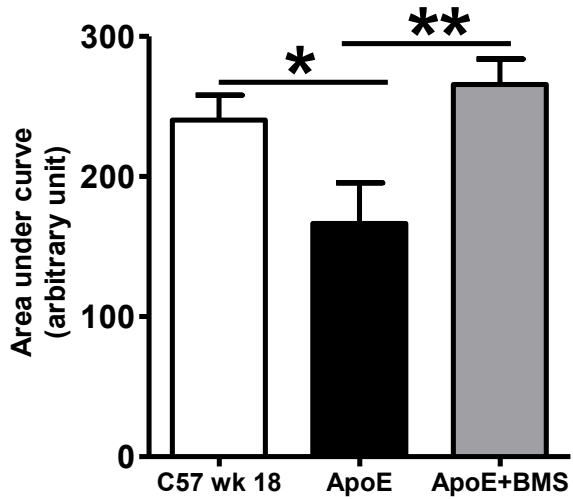
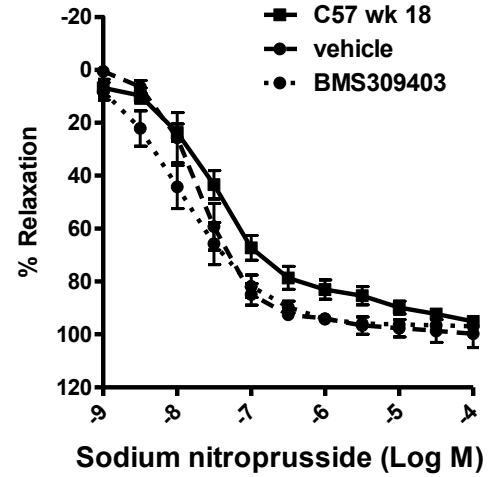
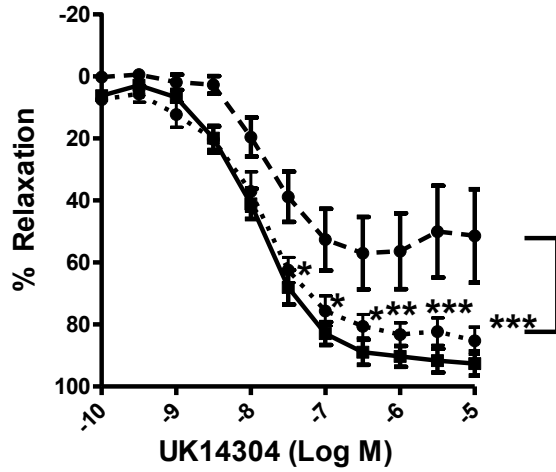
C57 WT 12 weeks old



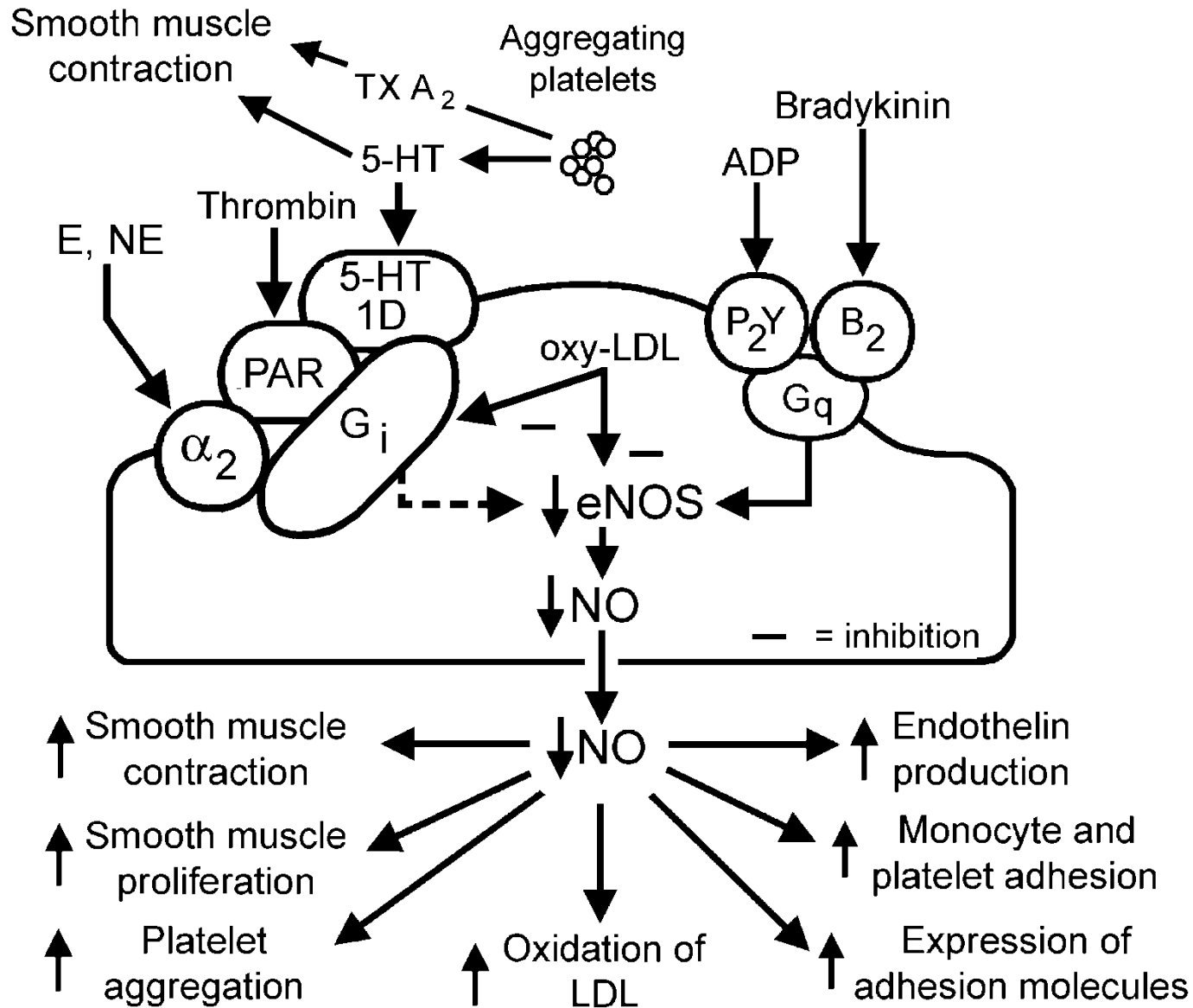
ApoE-/- 12 weeks old



**Relaxations in aortae (with endothelium) of 18 weeks old ApoE<sup>-/-</sup> mice treated with BMS309403 (15 mg/kg/day; ApoE-BMS) or vehicle for six weeks. Effect of pertussis toxin (PTX; 400ng/ml)**



# Regenerated Endothelial Cell



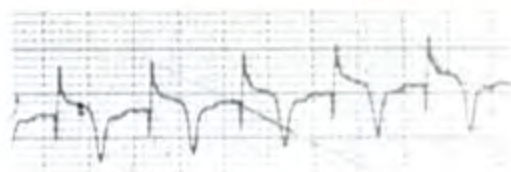
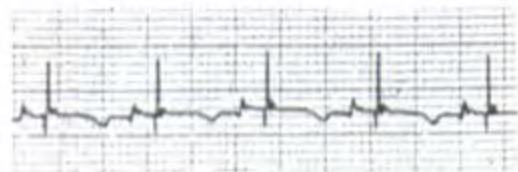
# SEROTONIN-INDUCED SPASM IN PORCINE CORONARY ARTERY WITH REGENERATED ENDOTHELIUM

Control

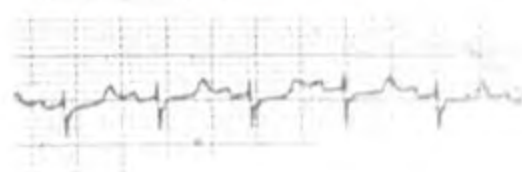
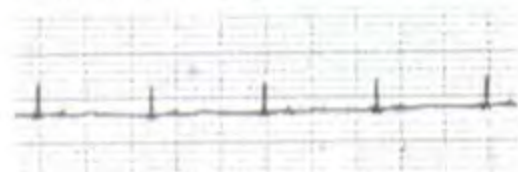
Serotonin, 10 $\mu$ g/kg i.c.



III

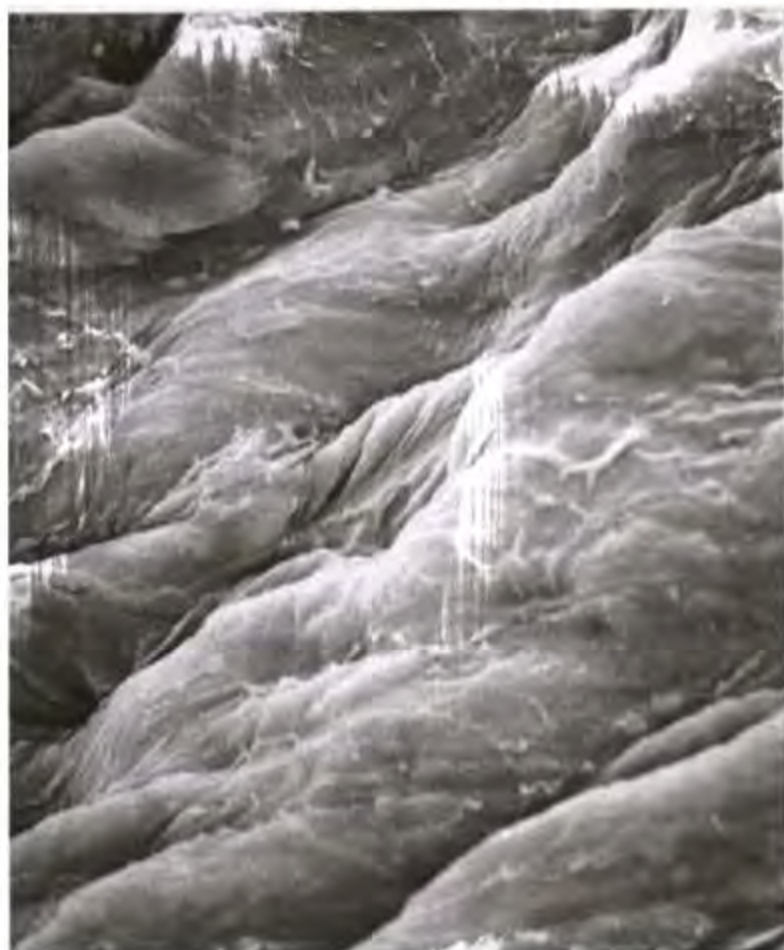


aVL

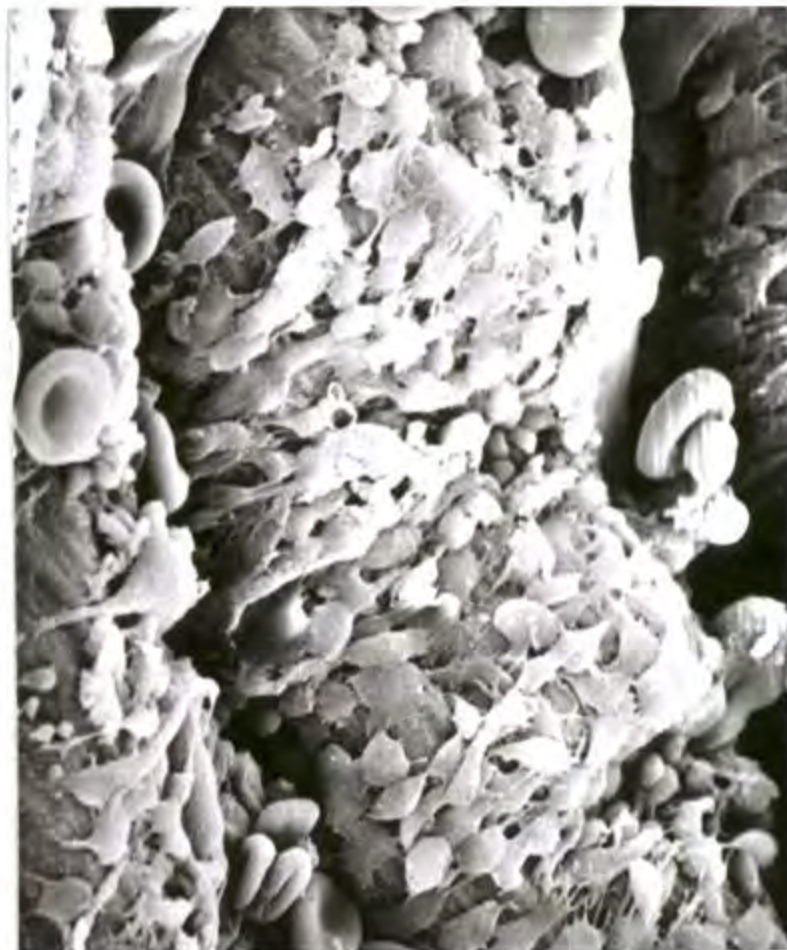


# Chronic Reperfusion and Canine Coronary Endothelium

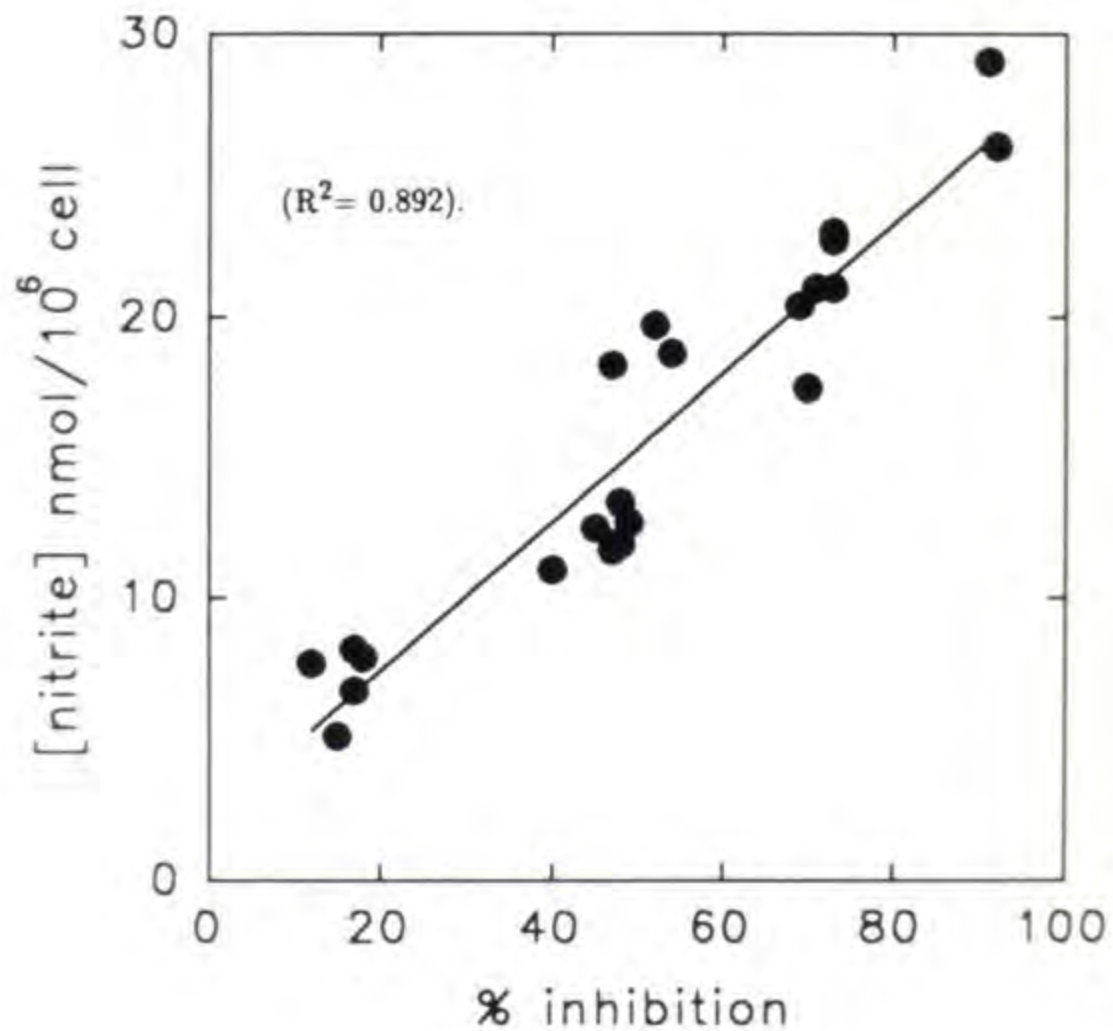
**Control**



**Chronic Reperfusion**



REGRESSION ANALYSIS OF LEVELS OF NITRITE IN CONDITIONED MEDIUM  
VS % INHIBITION OF [<sup>3</sup>H]-THYMIDINE INCORPORATION BY rVSMC  
STIMULATED WITH FGF OR EGF.  
[IN PRESENCE OF 10 μM INDOMETHACIN]

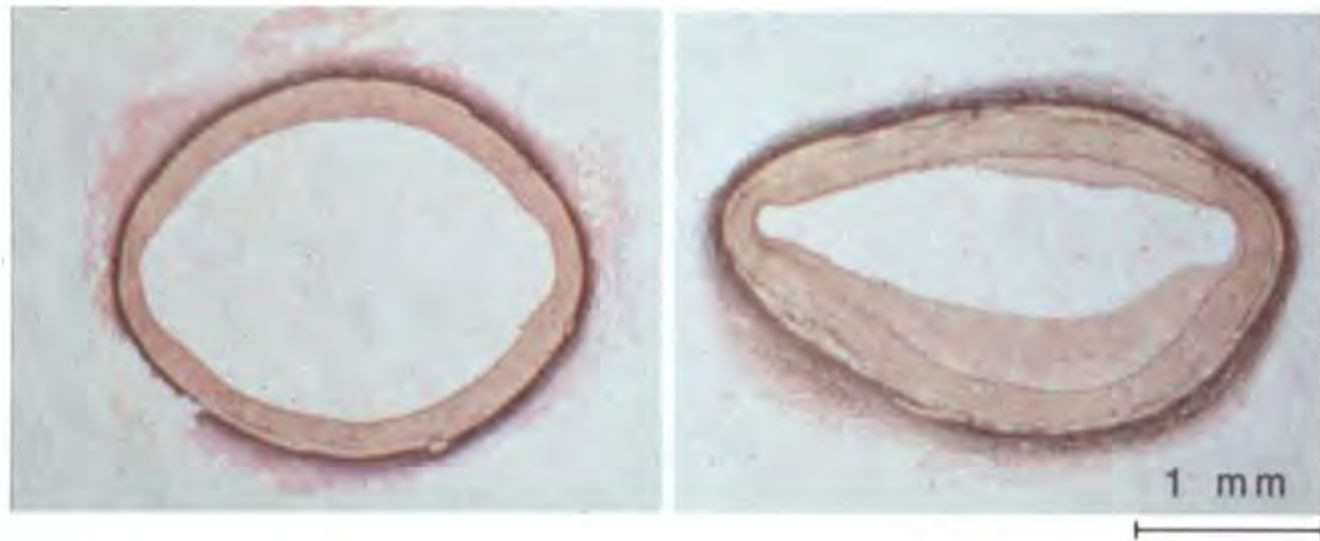


# HISTOLOGY OF NORMAL AND ATHEROSCLEROTIC PORCINE CORONARY ARTERIES

( Elastic Van Gieson staining )

Control  
( LAD )

Atherosclerosis  
( LAD )



Intimal cross-sectional area

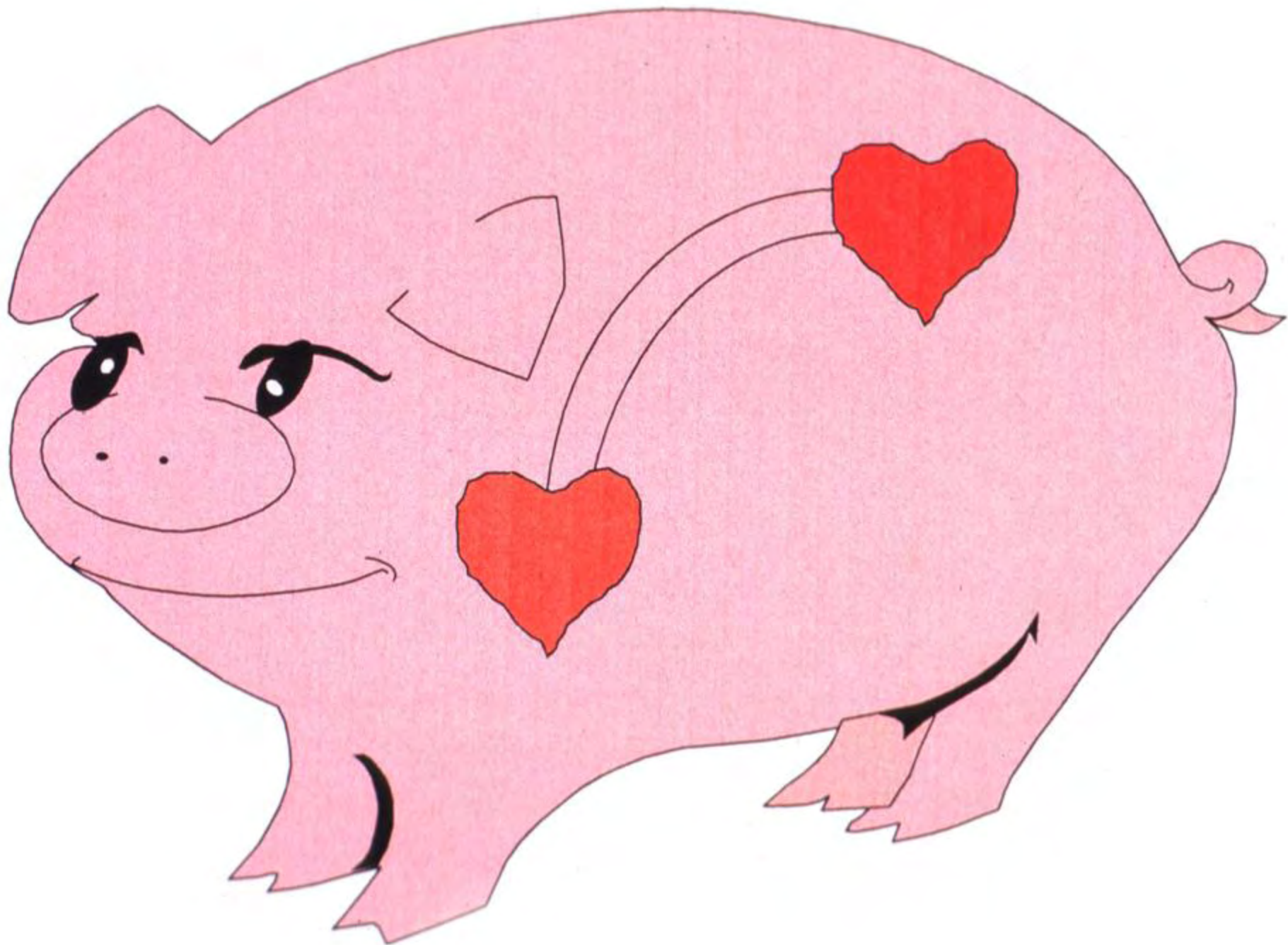
0 mm<sup>2</sup>

0.814 mm<sup>2</sup>

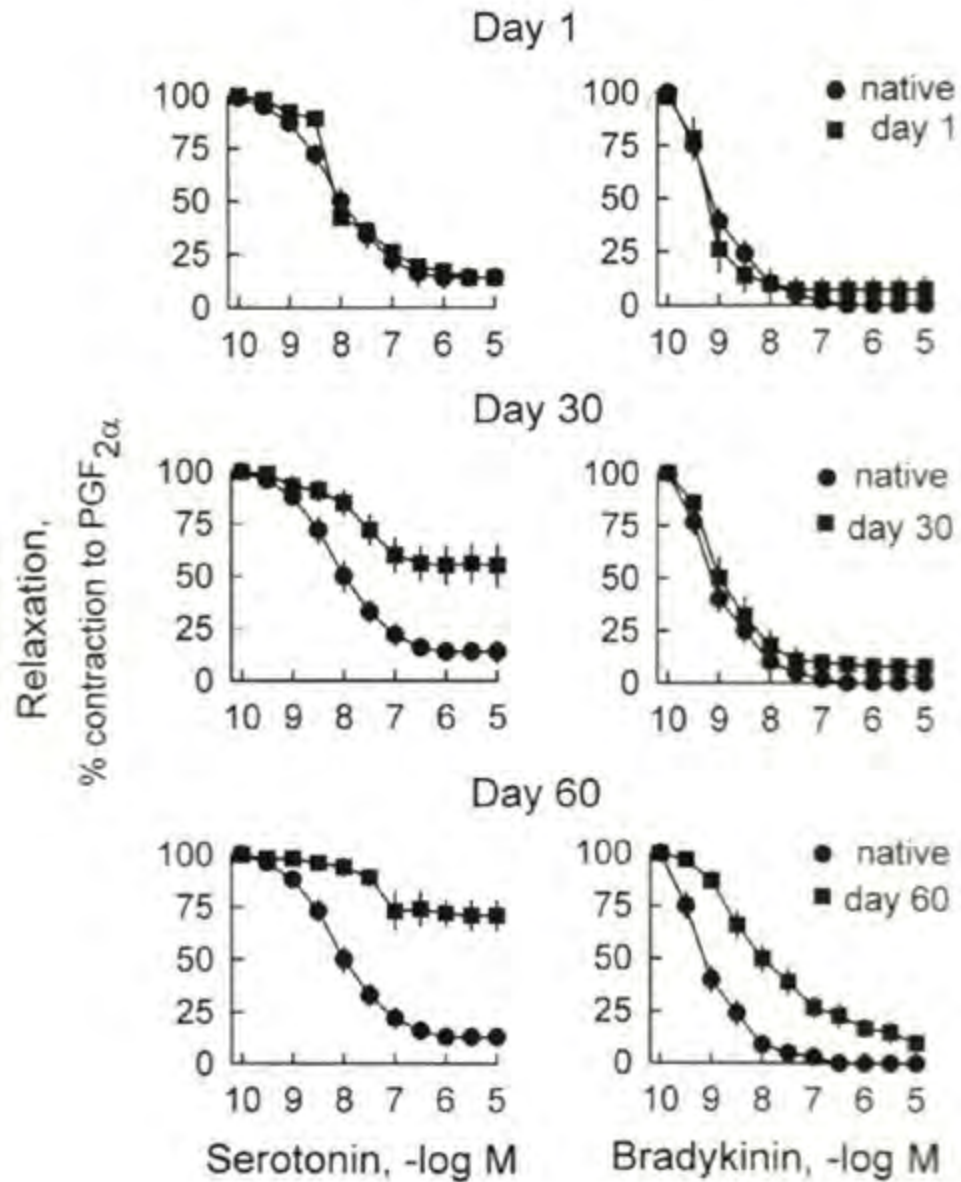
% Medial involvement

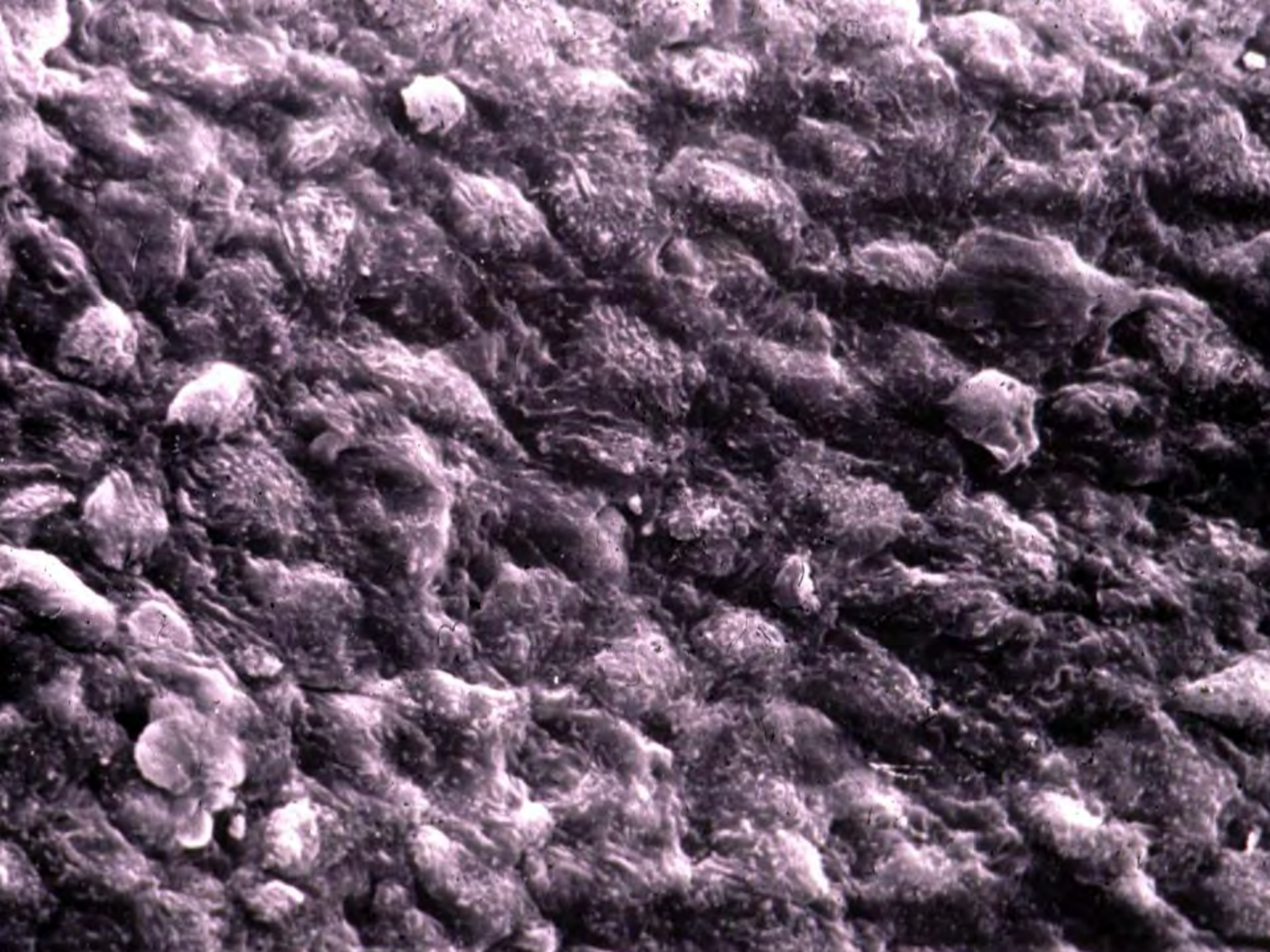
0 %

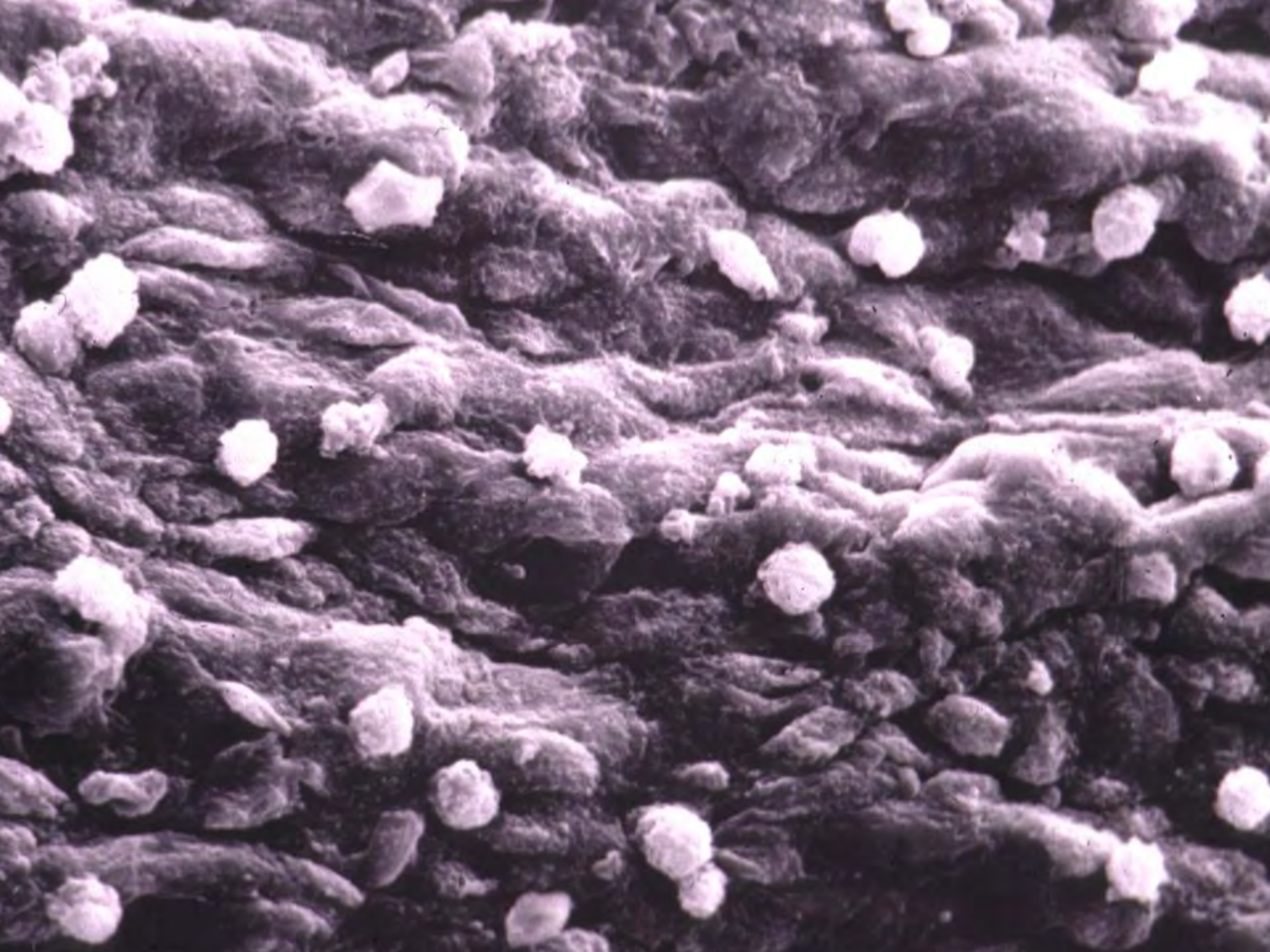
82 %



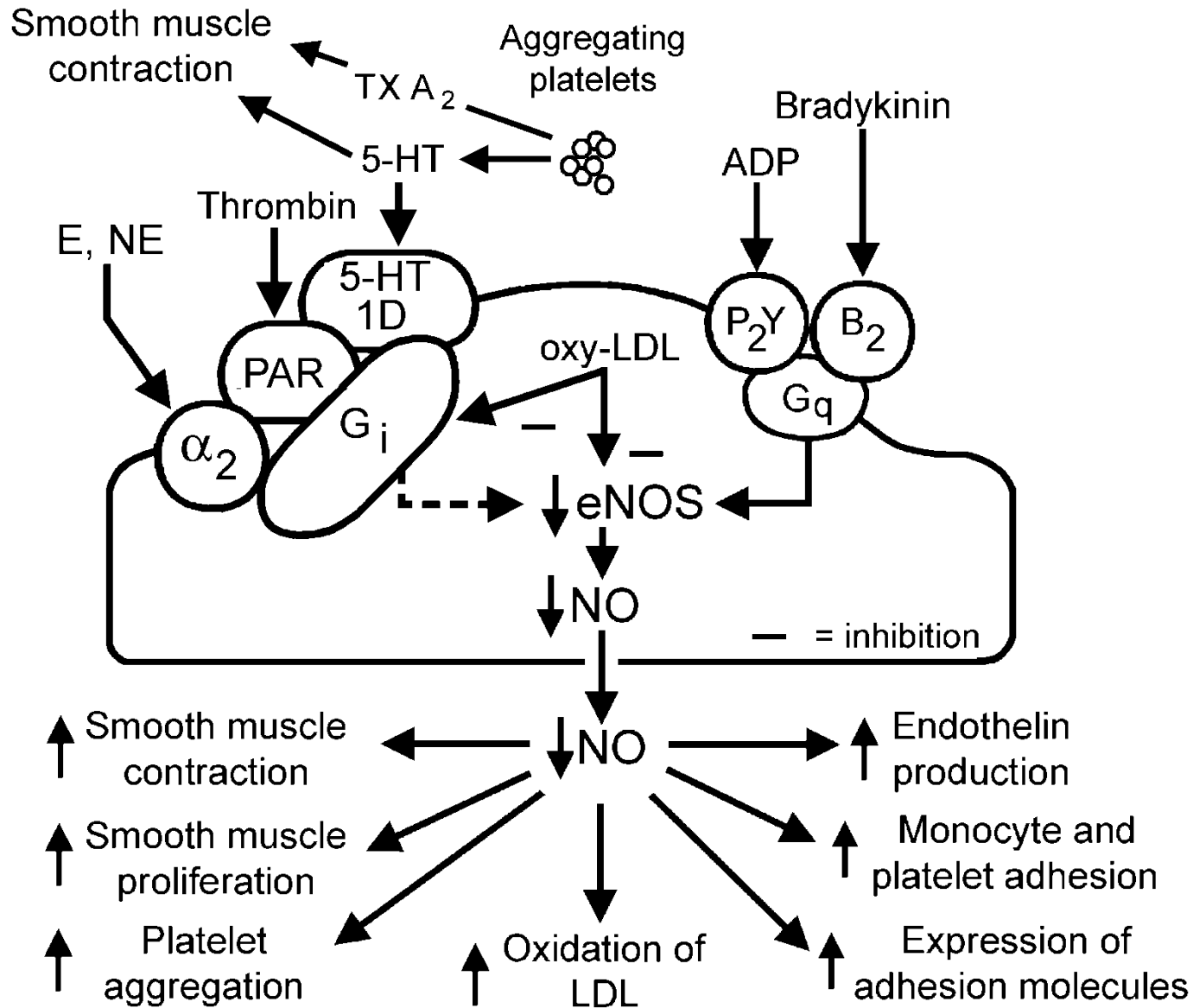
# RELAXATIONS OF PORCINE CORONARY ARTERIES WITH ENDOTHELIUM AFTER HEART TRANSPLANTATION

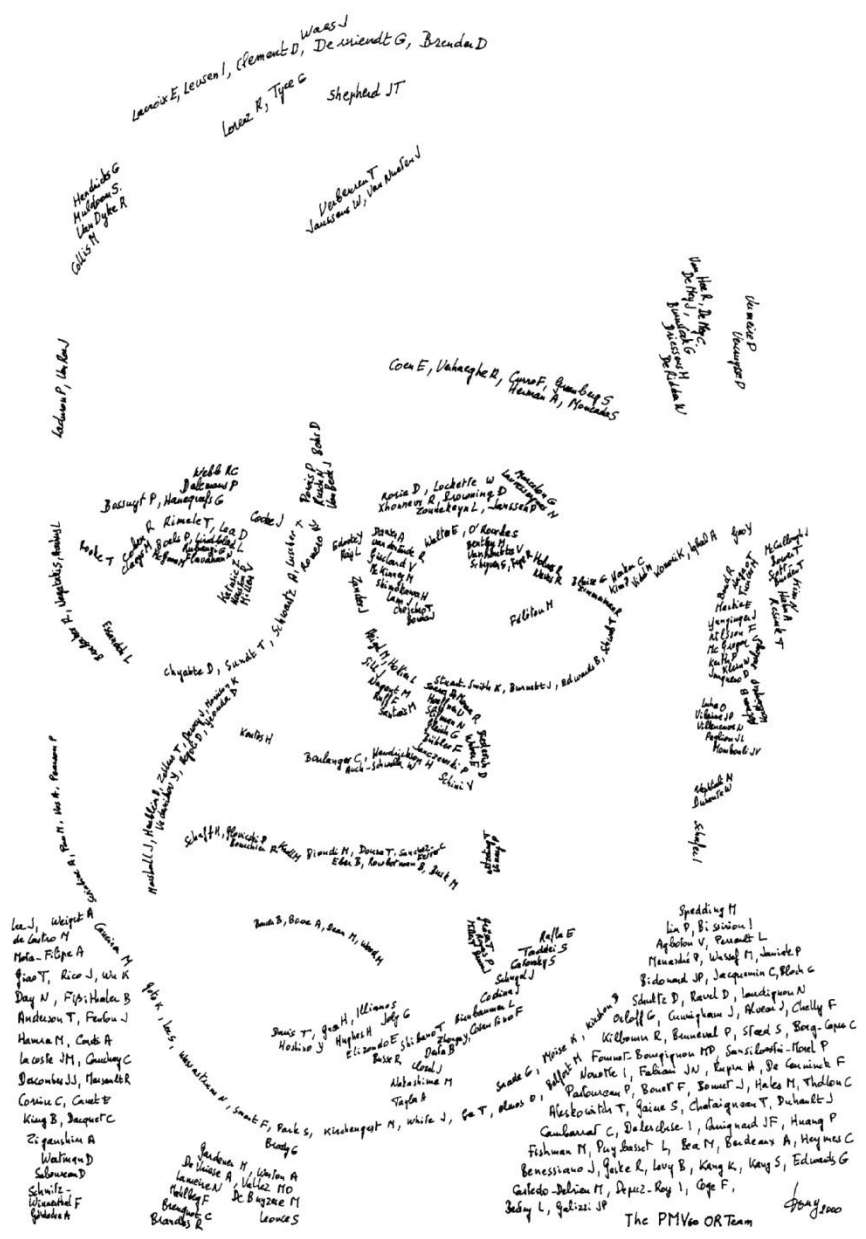






# Regenerated Endothelial Cell





The PMV or Tm Team



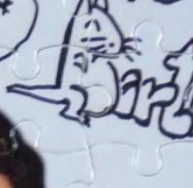
Je te souhaite  
une bonne sante  
Aron

HAPPY EVERY DAY  
to our beloved boss  
"V" for Vanhoutte  
"V" for Victory!  
Winnie

YOU ARE ALWAYS  
HUGGABLE & CHEERFUL!  
MY BELOVED BOSS!

Happy  
Wife

A belated gift  
from us all.  
Wish you happy  
everyday  
Calvin



Mary  
(HKW)

Bon  
Anniversaire

Aileen  
8

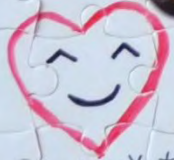
I LOVE YOU  
MY BOSS  
Michael



Happy

Birthday

NOV 12 2009



QIAN

Heureux tous les jours

Sophie: Merci beaucoup!  
You are the "god" of Lab :)