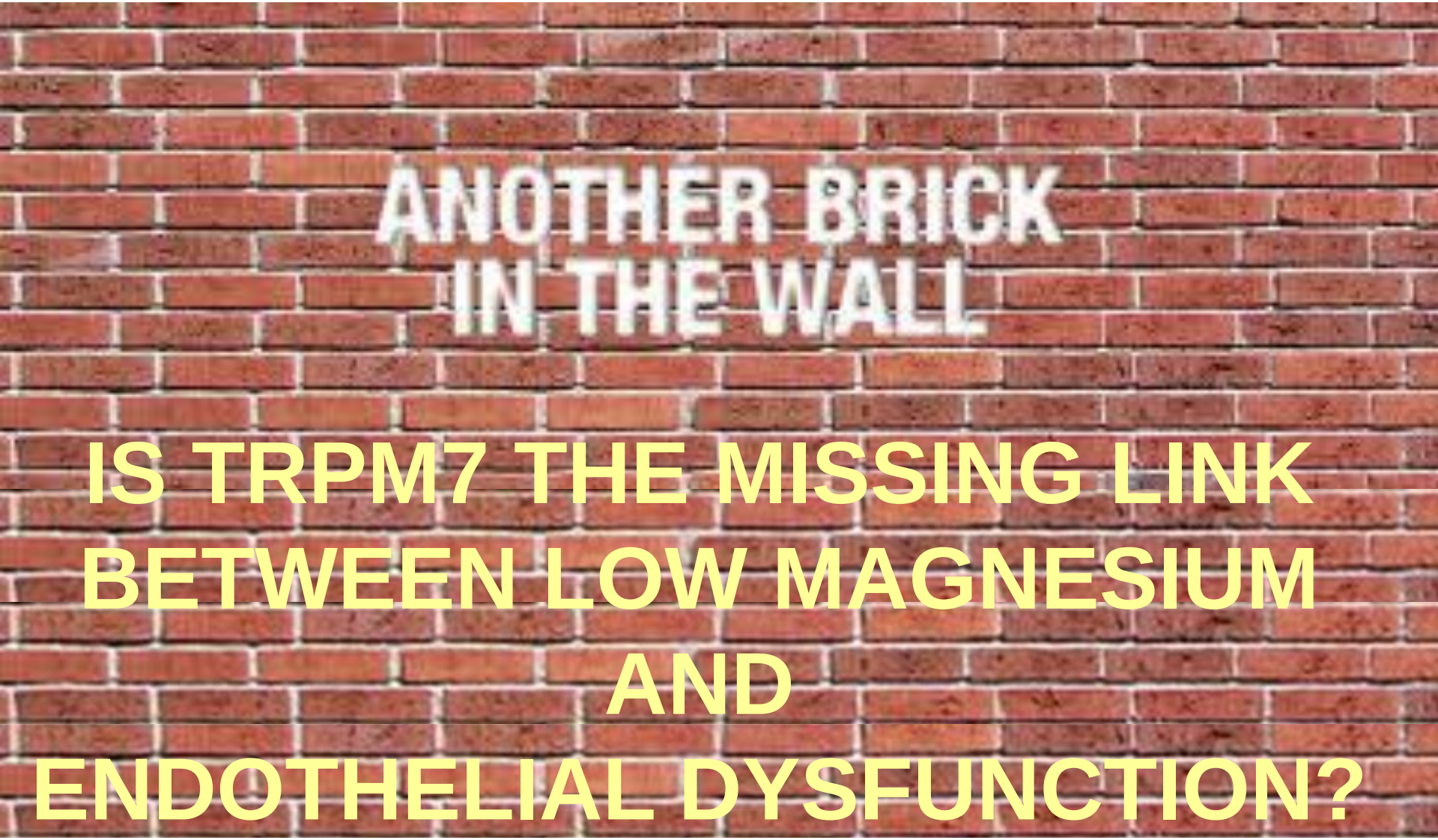


IS TRPM7 THE MISSING LINK BETWEEN LOW MAGNESIUM AND ENDOTHELIAL DYSFUNCTION?

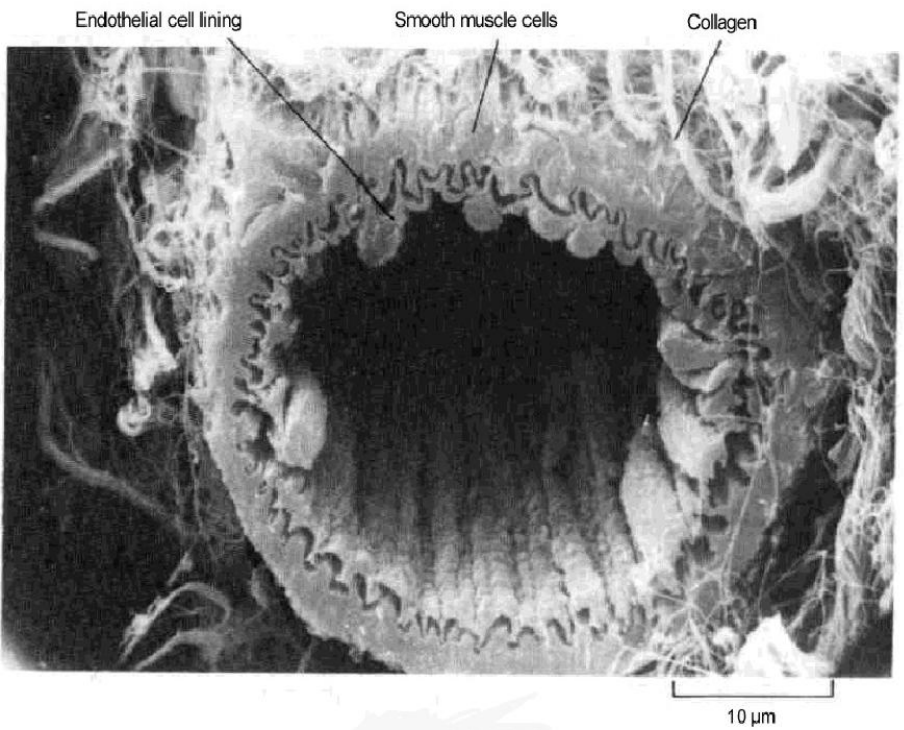
Jeanette Maier

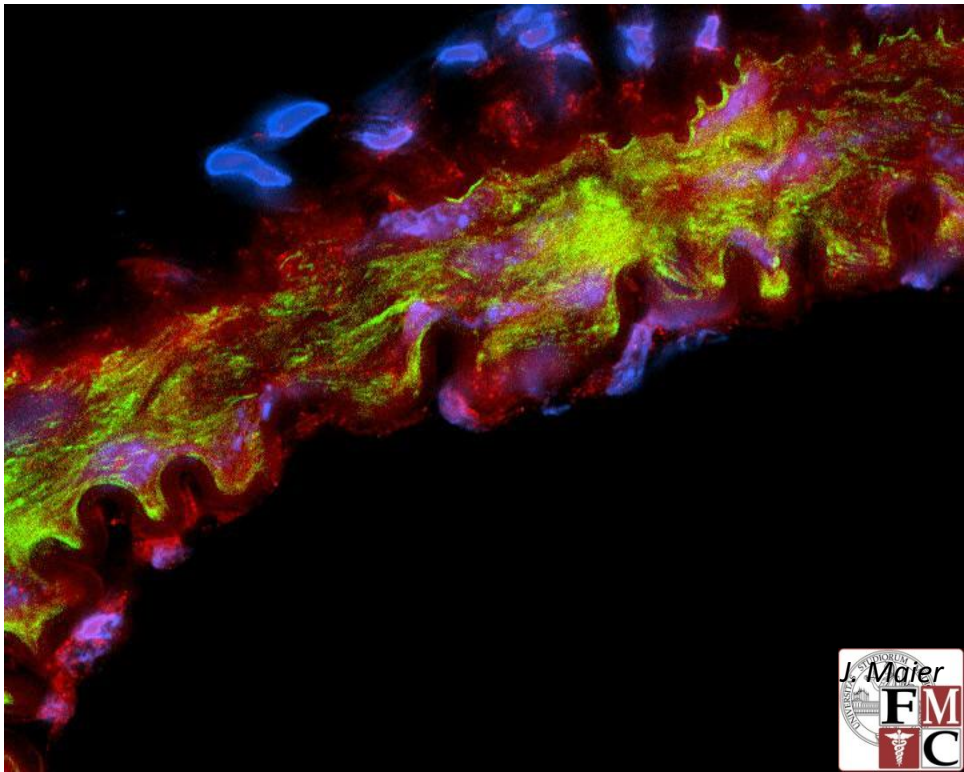
Università degli Studi di Milano

Facoltà di Medicina e Chirurgia

ENDOTHELIUM

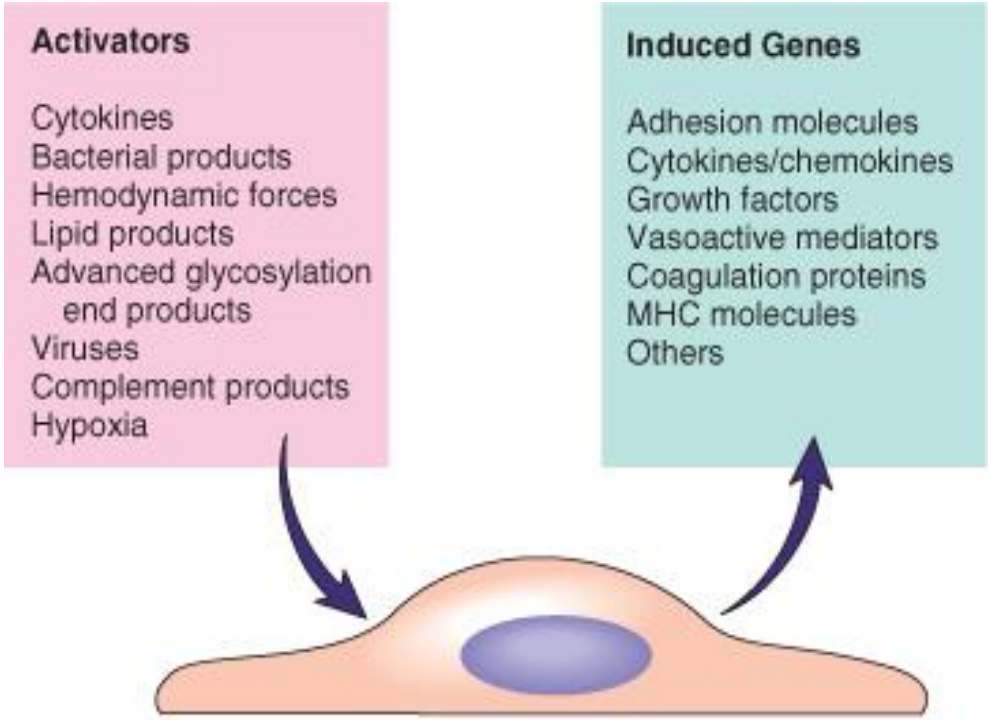
1- 6 x 10^{13} endothelial cells lining a total surface area of 4000-7000 m^2 (six tennis courts)



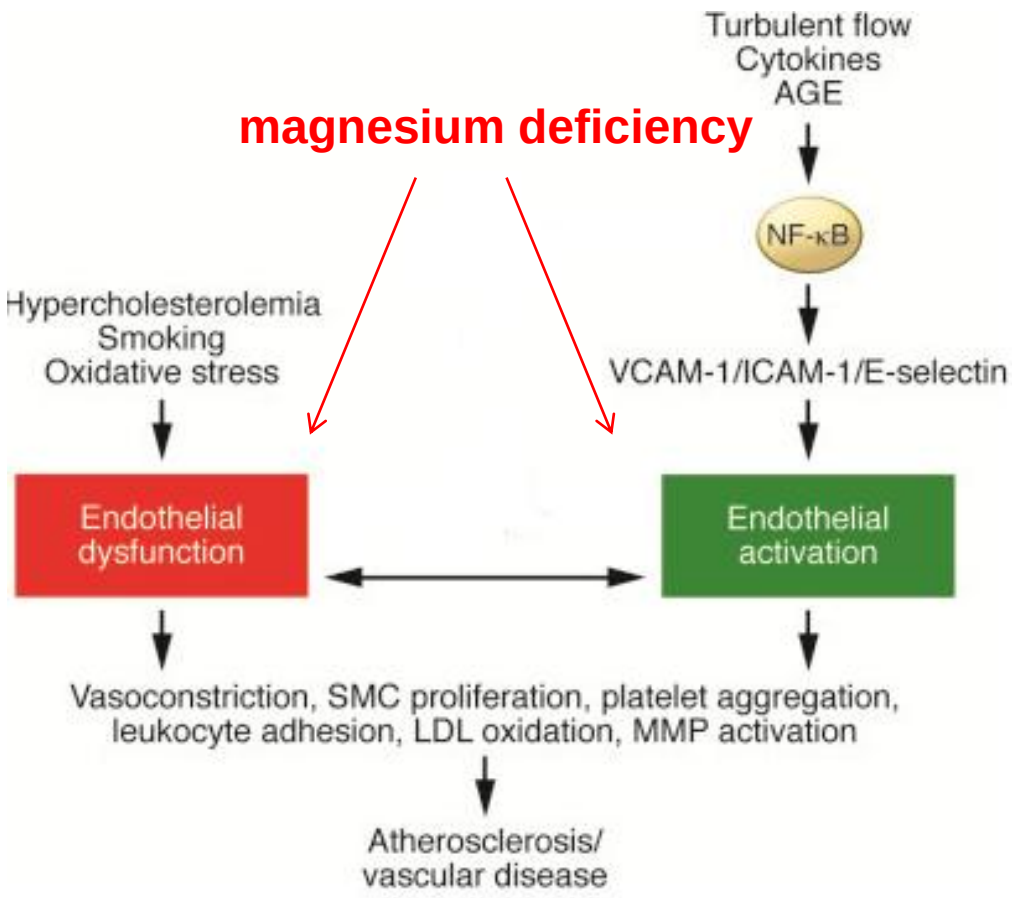


Endothelial cell properties and functions

- Maintenance of permeability barrier
- Elaboration of anticoagulant, antithrombotic, fibrinolytic molecules
- Modulation of blood flow and vascular reactivity
- Extracellular matrix production (collagen, proteoglycans)
- Regulation of the trafficking of circulating blood cells
- Regulation of inflammation and immunity

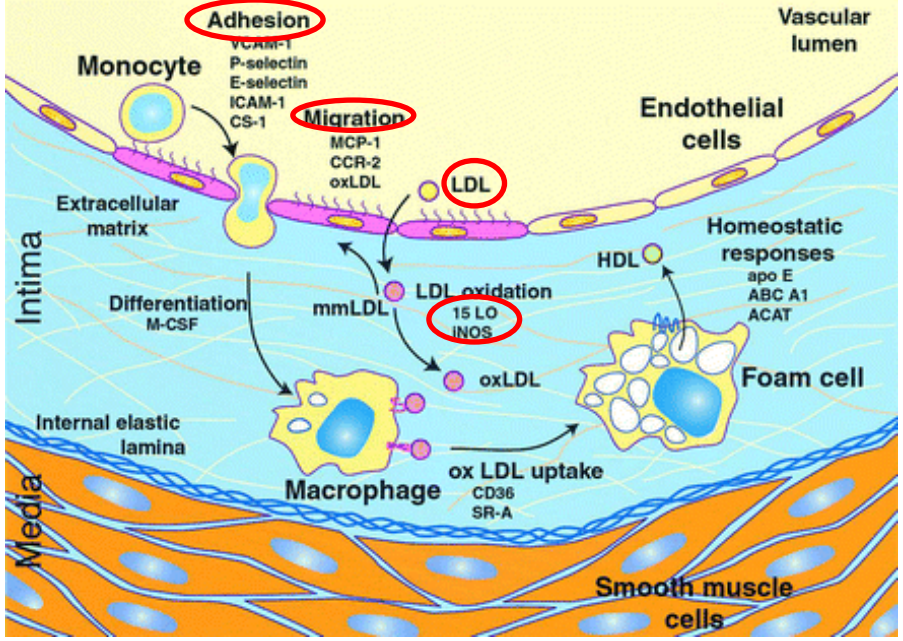


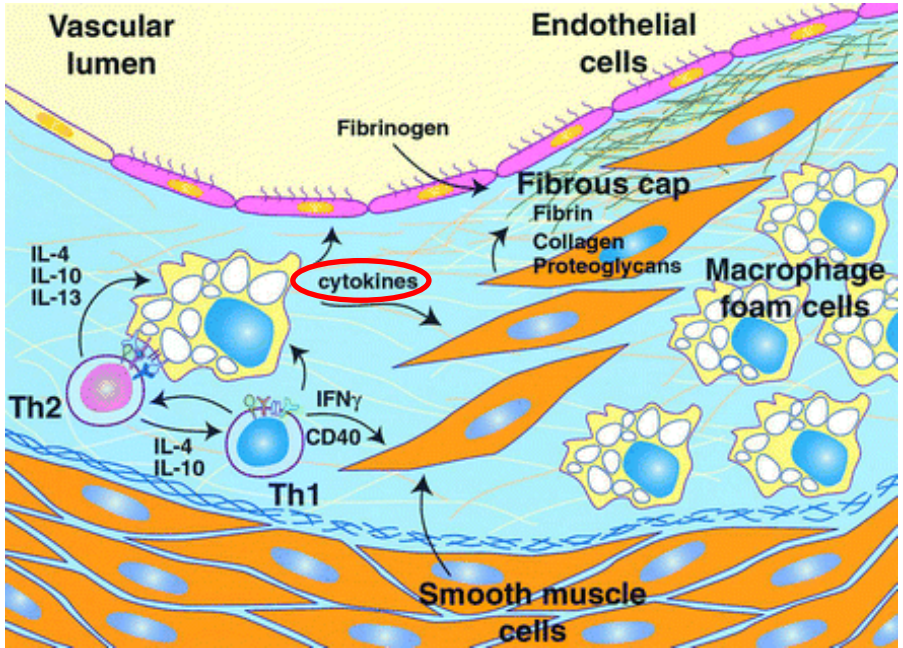
J. Maier
FMC


magnesium deficiency
Turbulent flow
Cytokines
AGE
NF-κB
Hypercholesterolemia
Smoking
Oxidative stress
VCAM-1/ICAM-1/E-selectin
Endothelial dysfunction
Endothelial activation
Vasoconstriction, SMC proliferation, platelet aggregation, leukocyte adhesion, LDL oxidation, MMP activation
Atherosclerosis/ vascular disease

J. Maier

STEPS IN ATHEROGENESIS





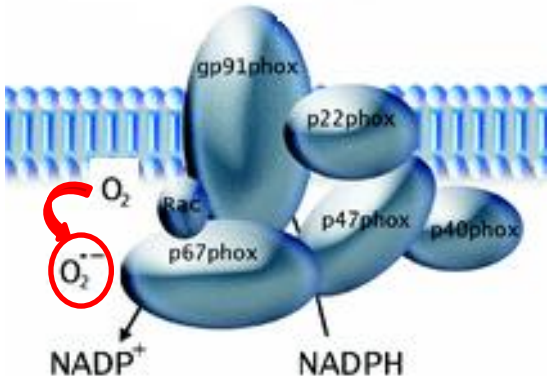
J. Maier

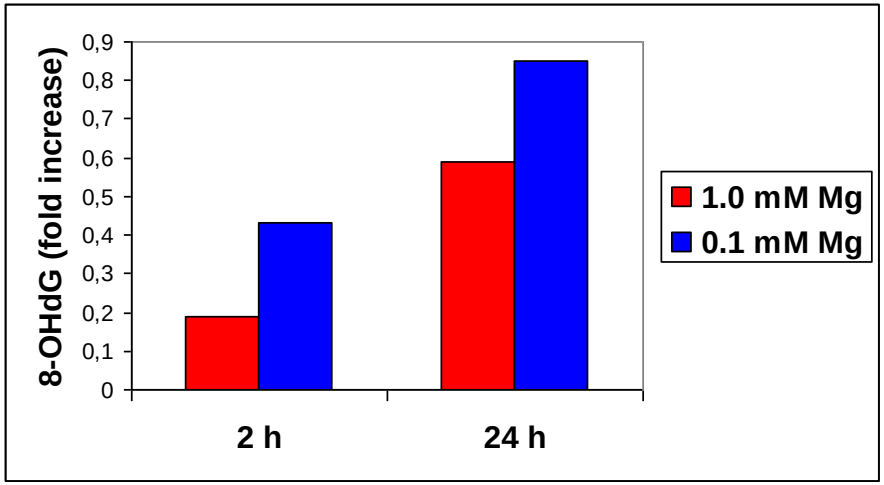
LOW MG AND ENDOTHELIAL CELLS: HOW IT ALL BEGINS

LOW MG ACTIVATES NADPH OXIDASE

↓

INCREASED PRODUCTION OF OXIDANTS



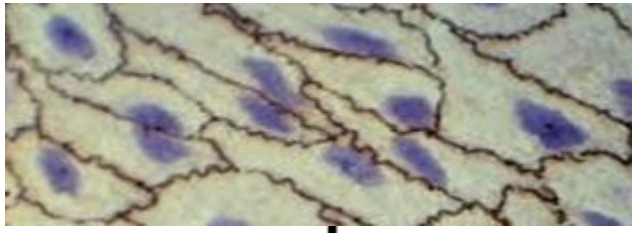


Wolf FI, 2008

J. Maier

Stimuli

- Physical
- Inflammatory
- Bioactive



Sources of ROS/RNS

- Mitochondrial electron transport chain
- NAD(P)H oxidase
- Xanthine oxidase
- Cytochrome P450
- Lipooxygenase, cyclooxygenase
- Uncoupled eNOS
- eNOS/iNOS

ROS/RNS

Regulation of EC signaling pathways:

- ↑ Protein kinases & phosphatases
- ↑ Lipid kinases & phosphatases
- ↑ Phospholipases
- ↑ p38 MAPK – MK2 – HSP 27
- ↑ Transcription factors
- ↑ Apoptotic cascade

- Actin polymerization
- Alteration of actin-binding proteins

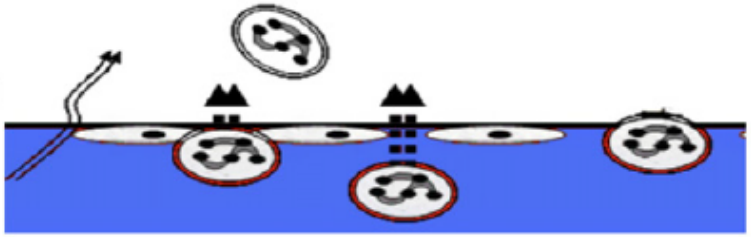
Cytoskeletal remodeling
Paracellular gap formation

- ↓ occludin/ZO-1
- ↓ cadherin/catenin

EC/Cell surface events:

- ↑ adhesion molecules
- Endothelial contraction
- Junctional remodeling

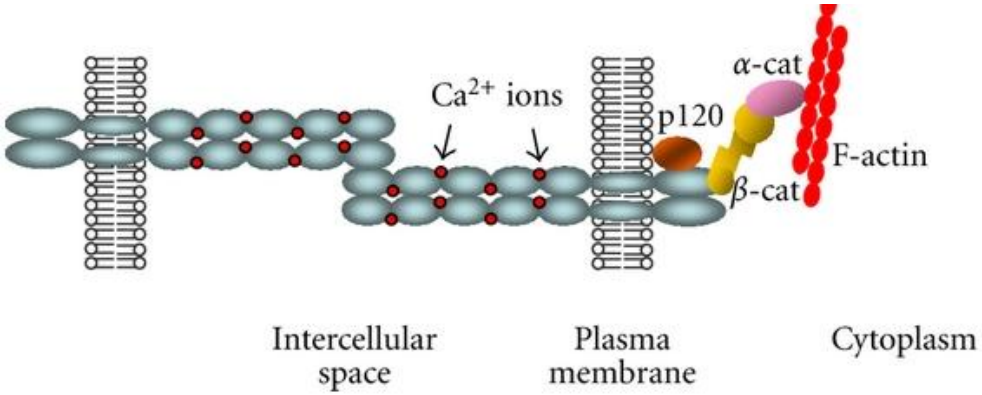
Leukocyte extravasation



Endothelial barrier dysfunction

J. Maier

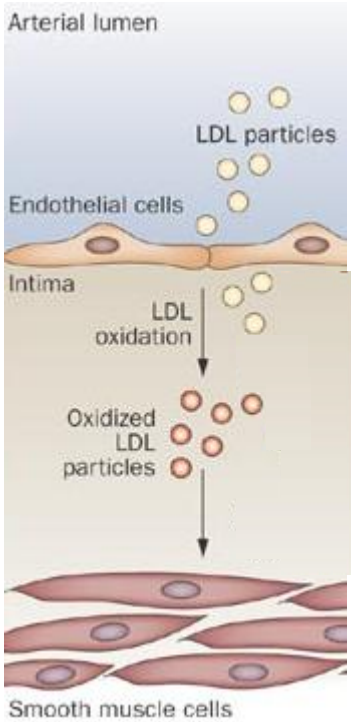
LOW MG INCREASES PERMEABILITY BY ALTERING VE- CADHERIN DISTRIBUTION



VE-cadherin is implicated in the opening and closing of the endothelial barrier, and is central in permeability changes

J. Maier

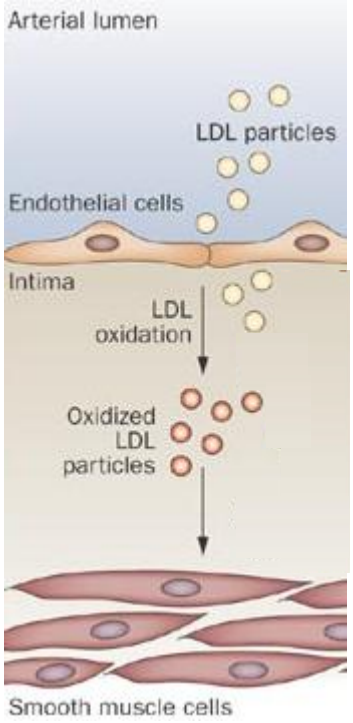
AN INCREASE OF ENDOTHELIAL PERMEABILITY PROMOTES ATHEROGENESIS

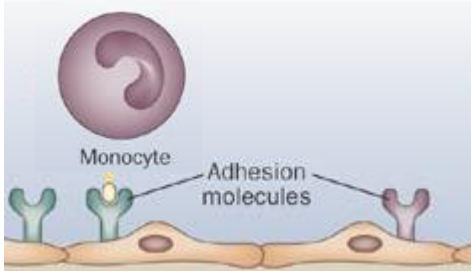


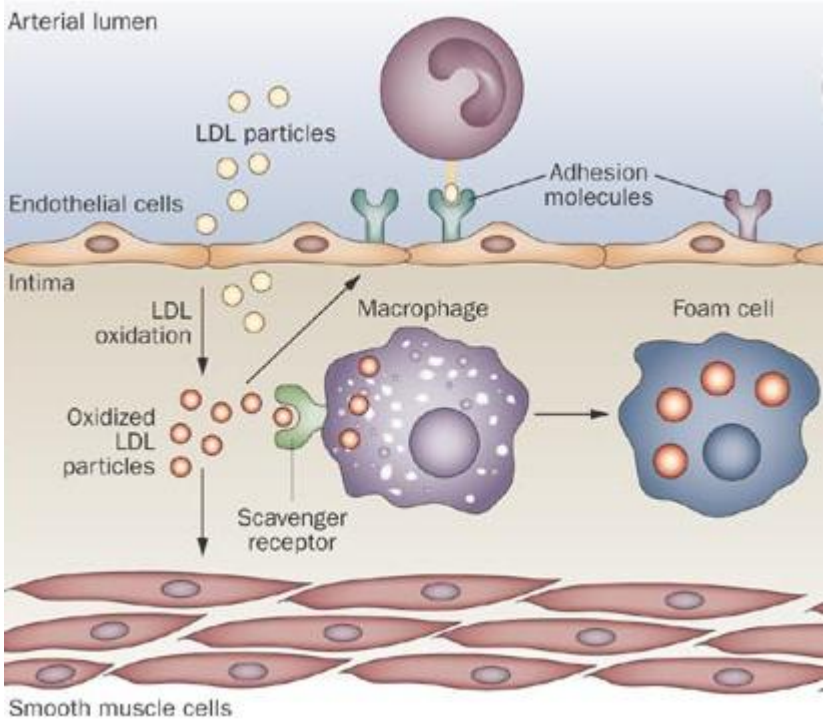
Magnesium deficiency increases LDL transport across the endothelial monolayer (*Suttorp N, 1989; Yokoyama S, 1994*)

Once LDLs reach the subendothelial space, they are trapped by matrix proteoglycans and become an easy target for oxidant species generated by low-Mg-stressed endothelial cells (*Yokoyama S, 1994*)

J. Maier


Arterial lumen
LDL particles
Endothelial cells
Intima
LDL
oxidation
Oxidized
LDL
particles
Smooth muscle cells

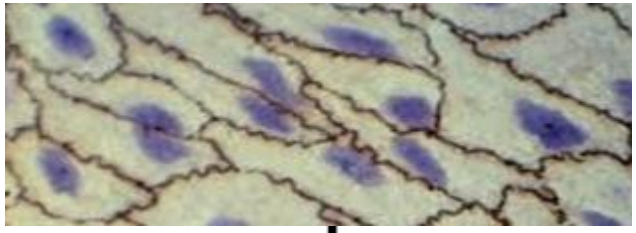

Monocyte
Adhesion
molecules


Arterial lumen
LDL particles
Adhesion
molecules
Endothelial cells
Intima
LDL
oxidation
Macrophage
Foam cell
Oxidized
LDL
particles
Scavenger
receptor
Smooth muscle cells

J. Maier

Stimuli

- Physical
- Inflammatory
- Bioactive



Sources of ROS/RNS

- Mitochondrial electron transport chain
- NAD(P)H oxidase
- Xanthine oxidase
- Cytochrome P450
- Lipooxygenase, cyclooxygenase
- Uncoupled eNOS
- eNOS/iNOS

ROS/RNS

Regulation of EC signaling pathways:

- ↑ Protein kinases & phosphatases
- ↑ Lipid kinases & phosphatases
- ↑ Phospholipases
- ↑ p38 MAPK – MK2 – HSP 27
- ↑ Transcription factors
- ↑ Apoptotic cascade

- Actin polymerization
- Alteration of actin-binding proteins

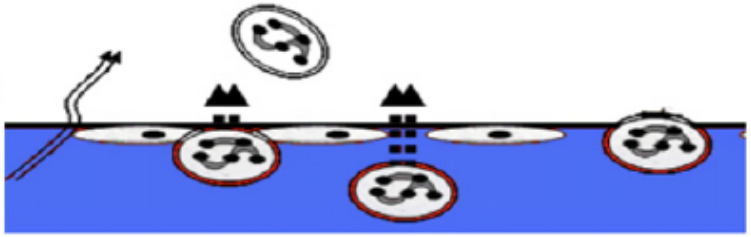
- ↓ occludin/ZO-1
- ↓ cadherin/catenin

Cytoskeletal remodeling
Paracellular gap formation

EC/Cell surface events:

- ↑ adhesion molecules
- Endothelial contraction
- Junctional remodeling

Leukocyte extravasation

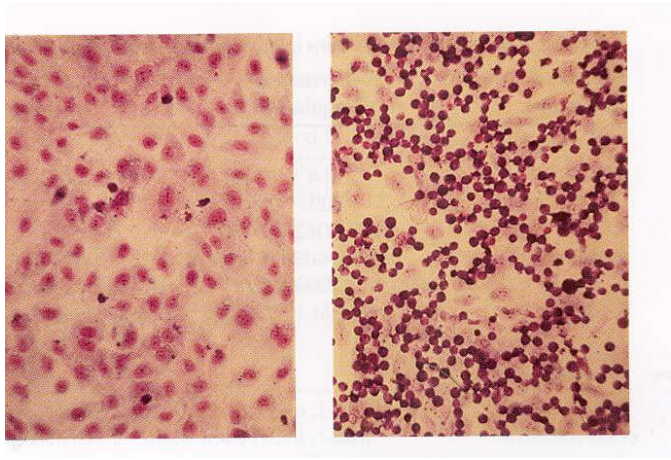


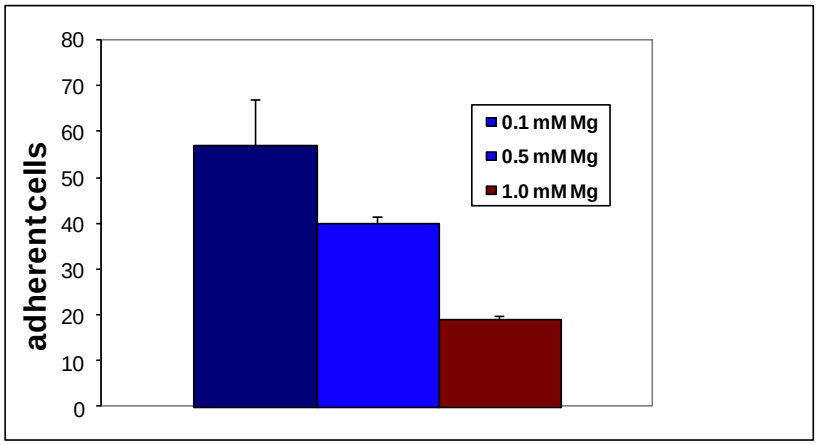
Endothelial barrier dysfunction

J. Maier

Low magnesium promotes monocyte/endothelial interactions

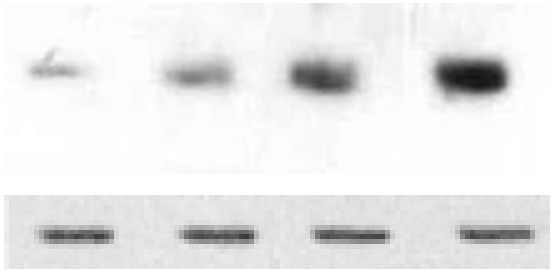
1. 0 0.1 mM Mg





Low magnesium promotes monocyte/endothelial interactions by inducing VCAM

1.0 0.5 0.1 IL-1



VCAM

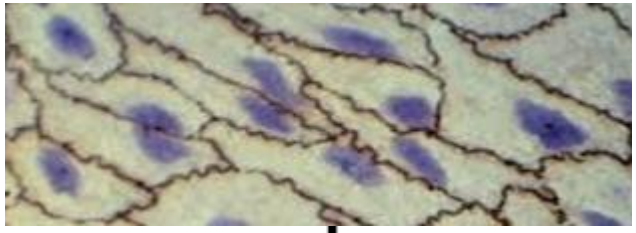
GAPDH

Maier 2004

J. Maier

Stimuli

- Physical
- Inflammatory
- Bioactive



Sources of ROS/RNS

- Mitochondrial electron transport chain
- NAD(P)H oxidase
- Xanthine oxidase
- Cytochrome P450
- Lipooxygenase, cyclooxygenase
- Uncoupled eNOS
- eNOS/iNOS

ROS/RNS

Regulation of EC signaling pathways:

- ↑ Protein kinases & phosphatases
- ↑ Lipid kinases & phosphatases
- ↑ Phospholipases
- ↑ p38 MAPK – MK2 – HSP 27
- ↑ Transcription factors
- ↑ Apoptotic cascade

EC/Cell surface events:

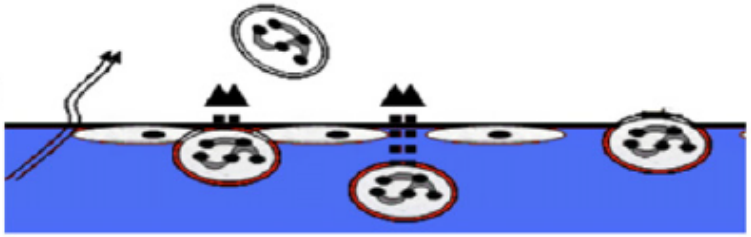
- ↑ adhesion molecules
- Endothelial contraction
- Junctional remodeling

Leukocyte extravasation

- Actin polymerization
- Alteration of actin-binding proteins

- ↓ occludin/ZO-1
- ↓ cadherin/catenin

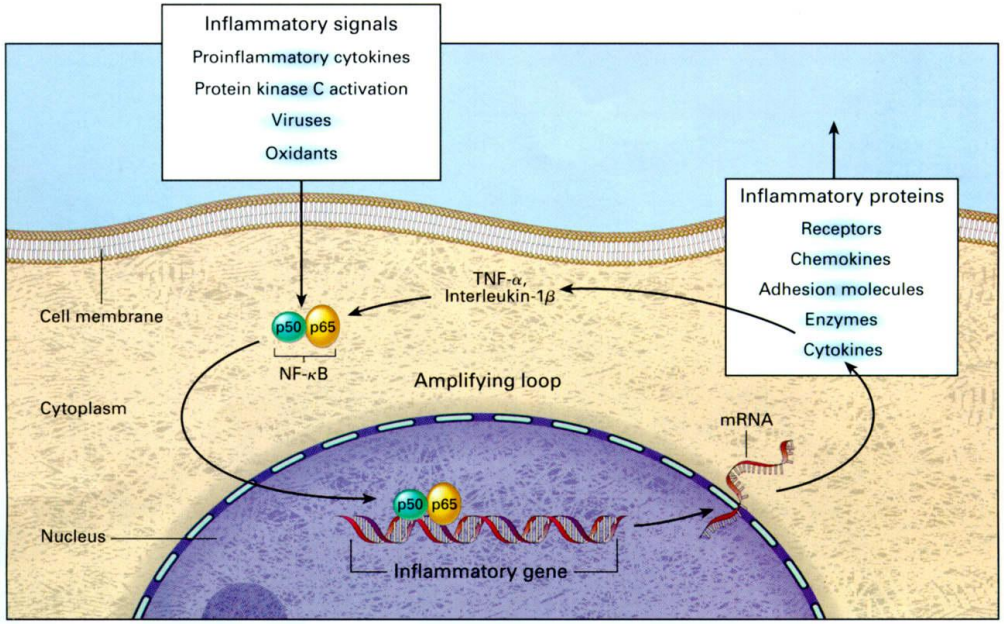
Cytoskeletal remodeling
Paracellular gap formation



Endothelial barrier dysfunction

J. Maier

LOW MG INDUCES NFkB ACTIVATION



Canonical pathway of nuclear factor κB activation selectively regulates proinflammatory and prothrombotic responses in human atherosclerosis

Claudia Monaco*, Evangelos Andreakos*, Serafim Kiriakidis*, Claudia Mauri*†, Colin Bicknell‡, Brian Foxwell*, Nicholas Cheshire‡, Ewa Paleolog*, and Marc Feldmann*§

5634–5639 | PNAS | April 13, 2004 | vol. 101

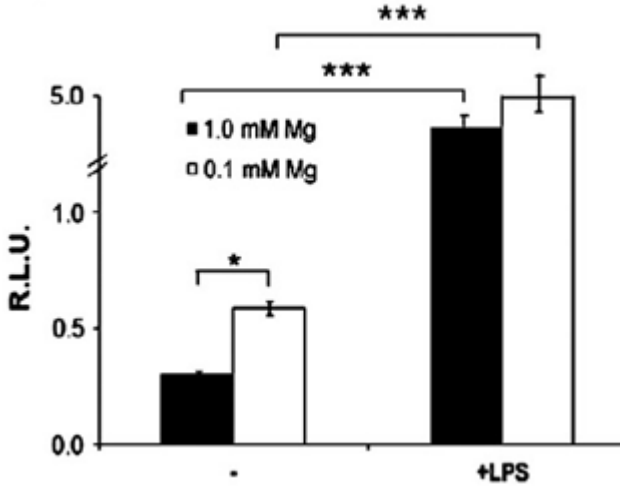
5308–5319 *Nucleic Acids Research, 2005, Vol. 33, No. 16*
doi:10.1093/nar/gki836

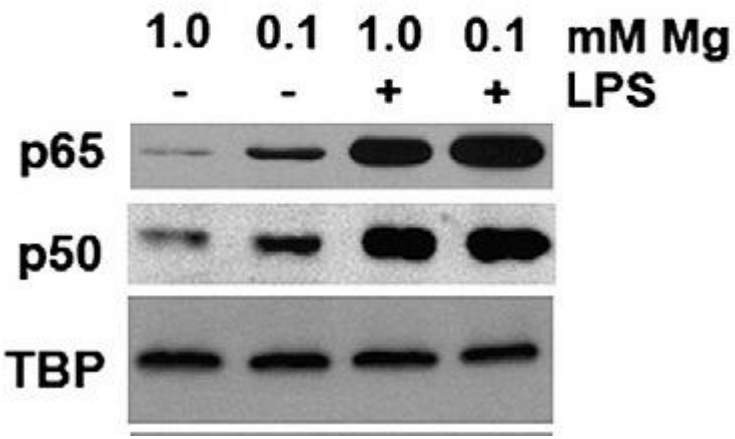
NF-κB controls the global pro-inflammatory response in endothelial cells: evidence for the regulation of a pro-atherogenic program

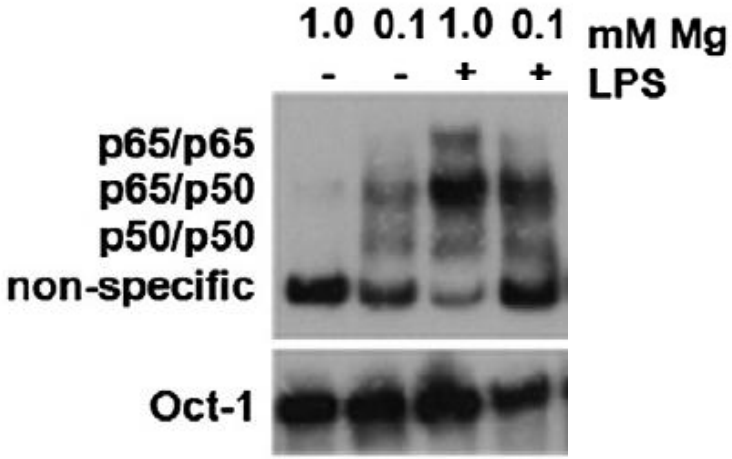
Sybille Kempe, Hans Kestler[1], Andrea Lasar and Thomas Wirth*

J. Maier

LOW MG INDUCES NFkB ACTIVATION



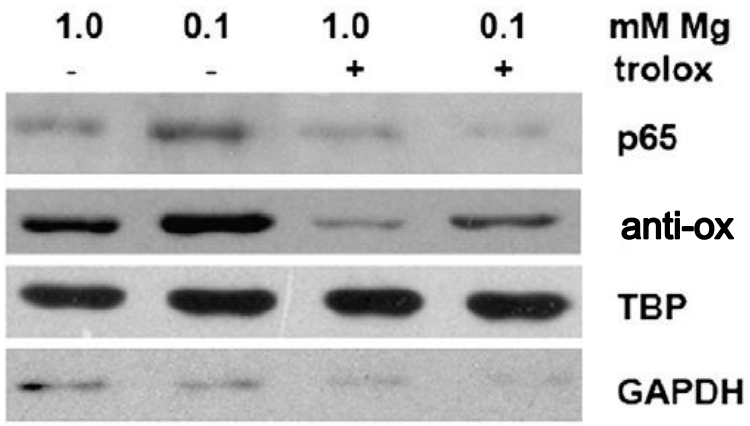


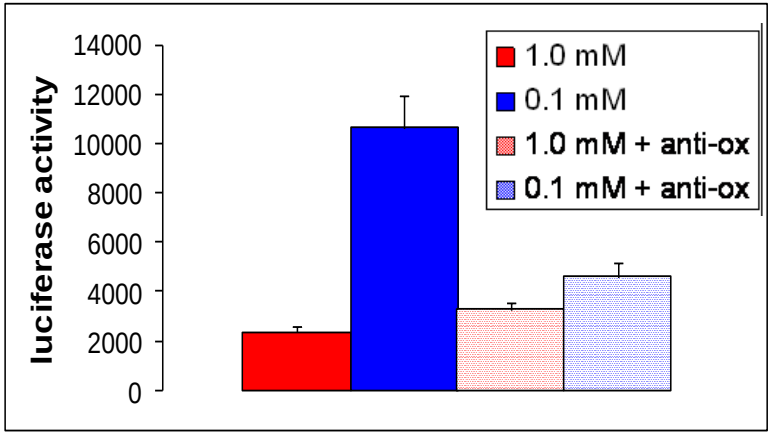


Ferré 2010

J. Maier

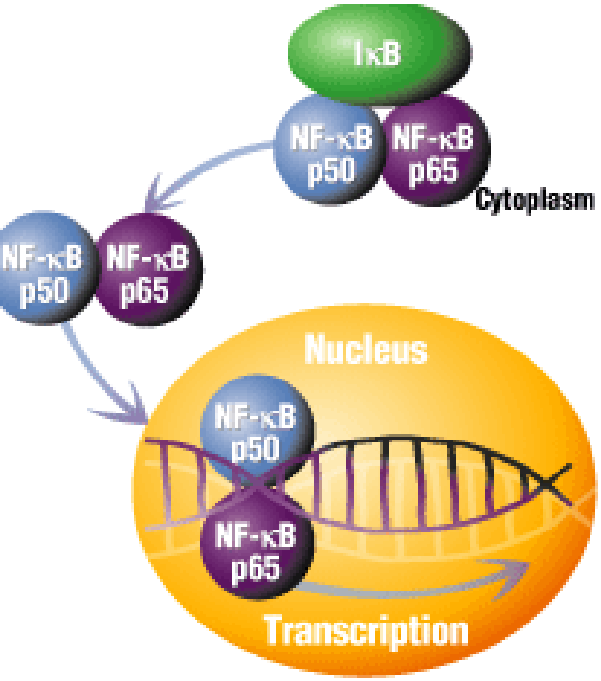
ANTI-OXIDANTS PREVENT NFkB ACTIVATION





Ferré 2010

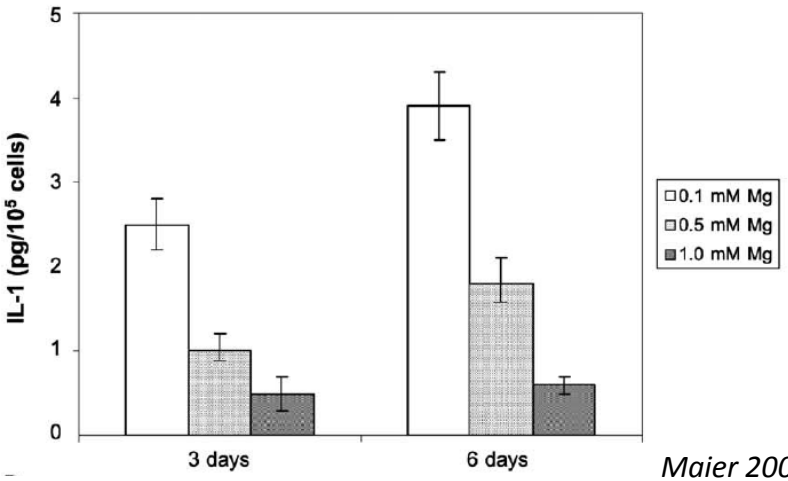
LOW MG ACTIVATES NFkB BY INDUCING PROTEASOME ACTIVITY



Mg (mM)	proteasome activity (pM)
1.0	9.7 ÷ 0.7
0.1	23.8 ÷ 1.1
1.0 + Trolox	11.4 ÷ 0.9
0.1 + Trolox	10.8 ÷ 1.4

Ferré 2007, 2010

J. Maier



Maier 2004

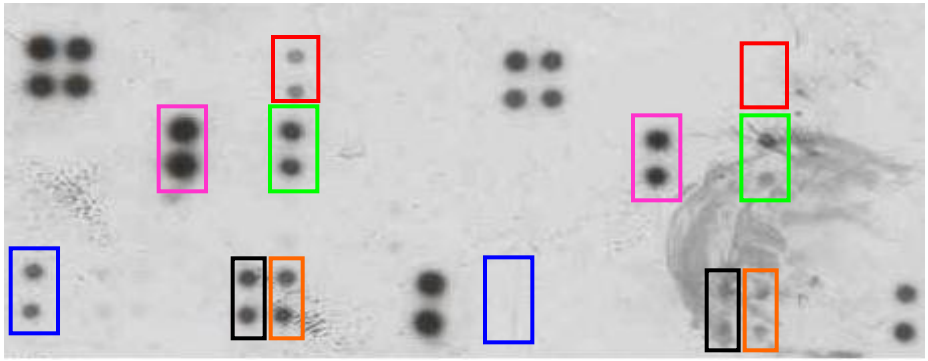
IL-1α

- inhibition of endothelial growth and promotion of senescence
- increase of endothelial permeability
- induction of endothelial/leukocytes interactions
- alterations of blood flow and vascular reactivity
- induction of the synthesis of several cytokines/chemokines
- induction of a pro-thrombotic state

J. Maier

Low magnesium promotes a pro-inflammatory phenotype in EC

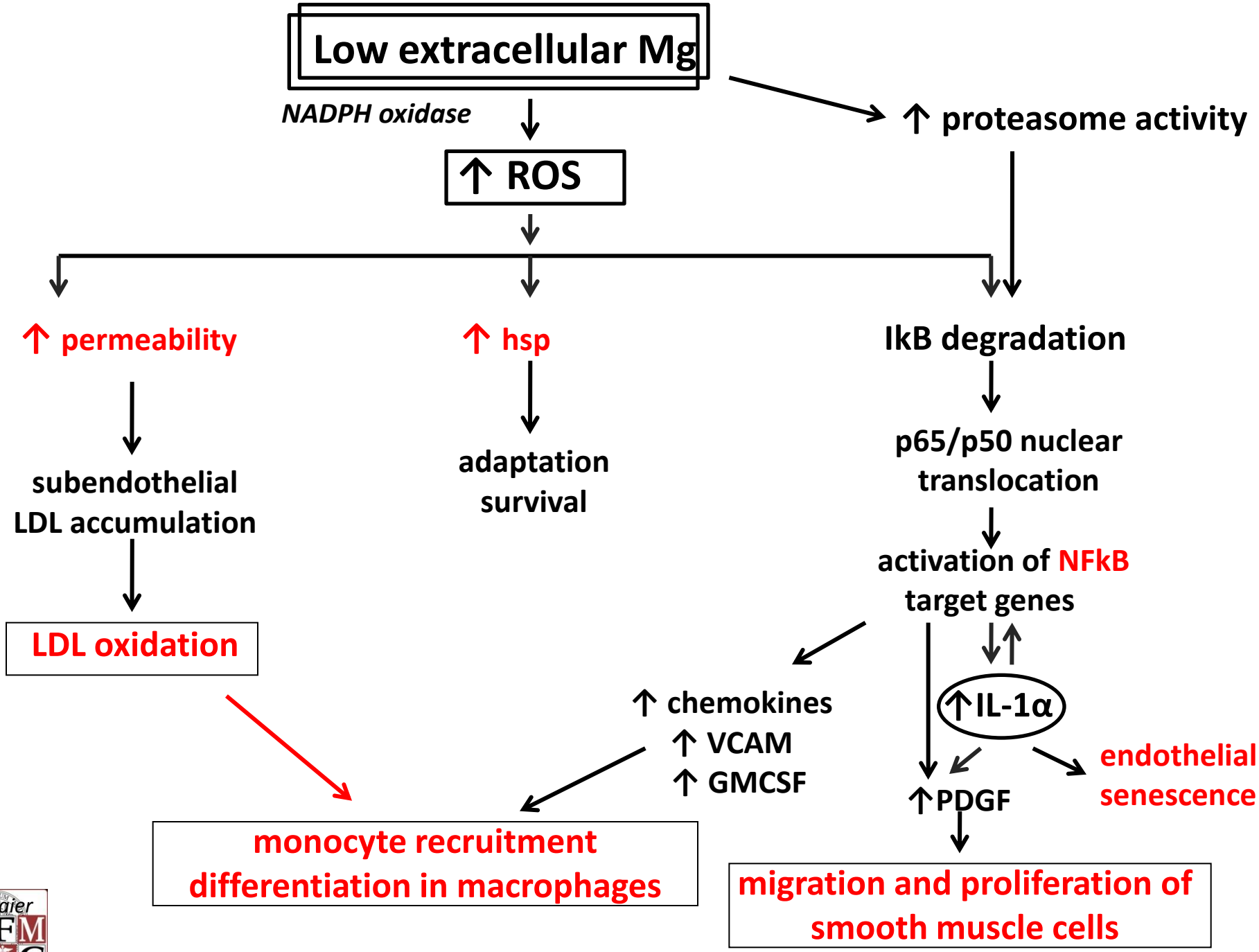
0.1 mM Mg | 1.0 mM Mg



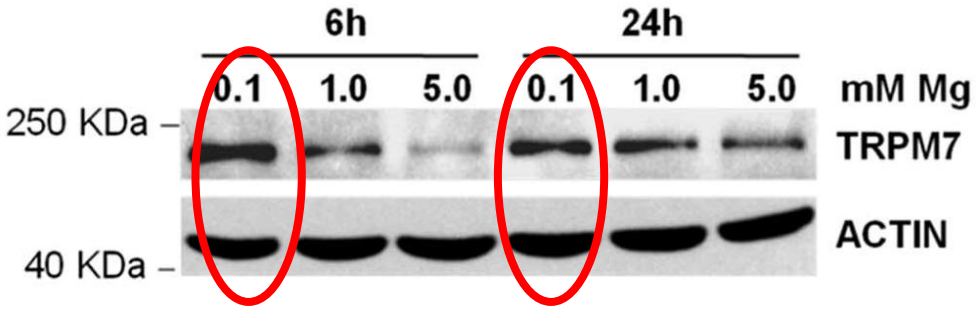
↑ IL-6
↑ RANTES
↑ IL-8
↑ GM-CSF
↑ TIMP-2
↑ PDGF-BB

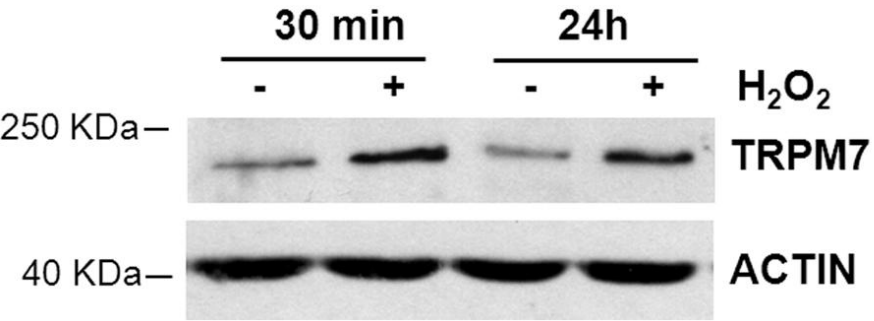
Low Mg upregulates		
	RANTES	11 x
	IL-8	9 x
	PDGF BB	9 x
	TIMP 2	7 x
	IL-6	3 x
	GM-CSF	3 x

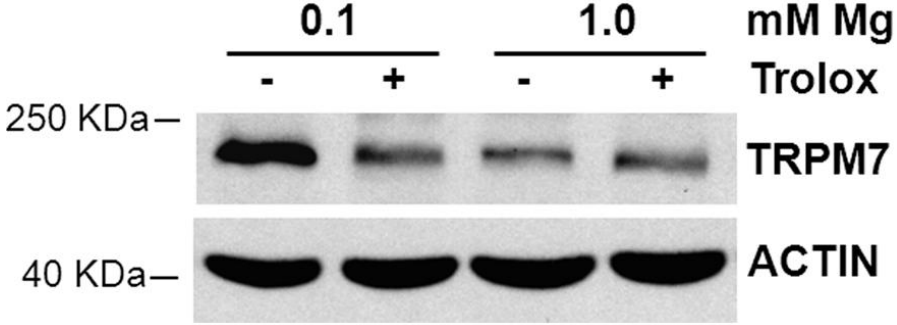
J. Maier


Low extracellular Mg
NADPH oxidase
↑ ROS
↑ proteasome activity
↑ permeability
↑ hsp
IkB degradation
subendothelial
LDL accumulation
adaptation
survival
p65/p50 nuclear
translocation
activation of NFkB
target genes
LDL oxidation
↑ chemokines
↑ VCAM
↑ GMCSF
↑IL-1α
endothelial
senescence
↑PDGF
monocyte recruitment
differentiation in macrophages
migration and proliferation of
smooth muscle cells
J. Maier
FMC

LOW MG INDUCES TRPM7: MECHANISMS



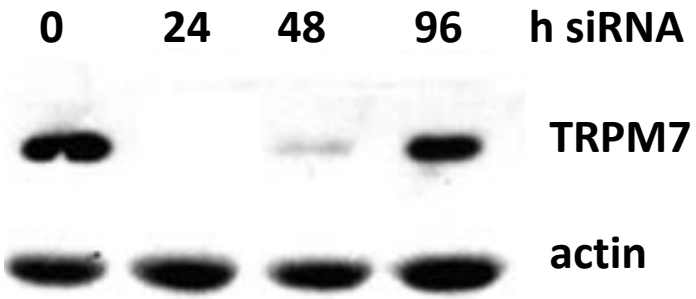


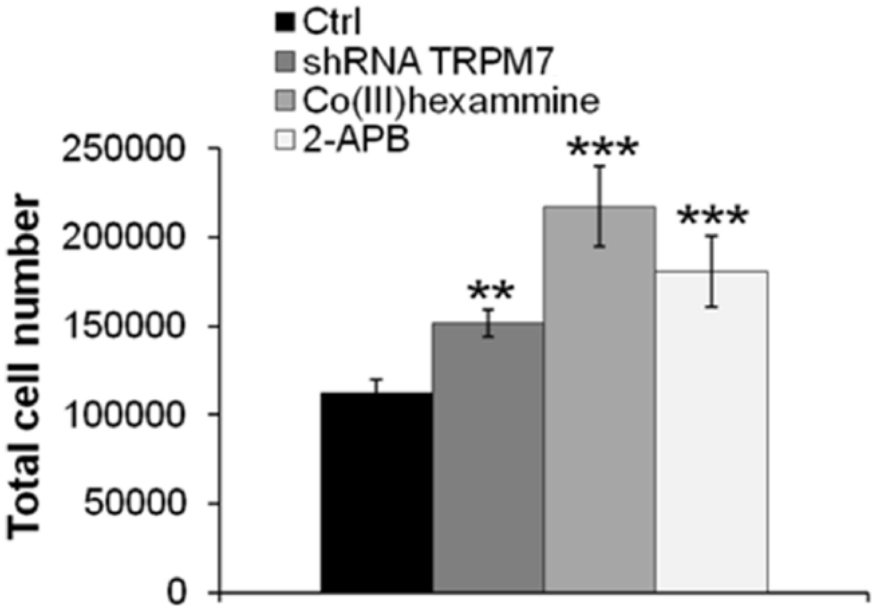


Baldoli 2013

J. Maier

EFFECTS OF TRPM7 INHIBITION ON HUVEC
1. CELL GROWTH

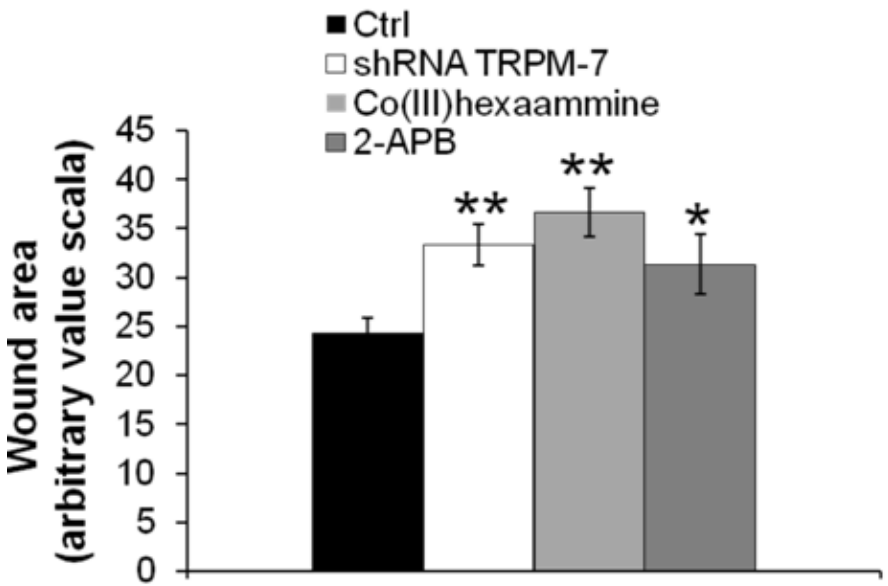


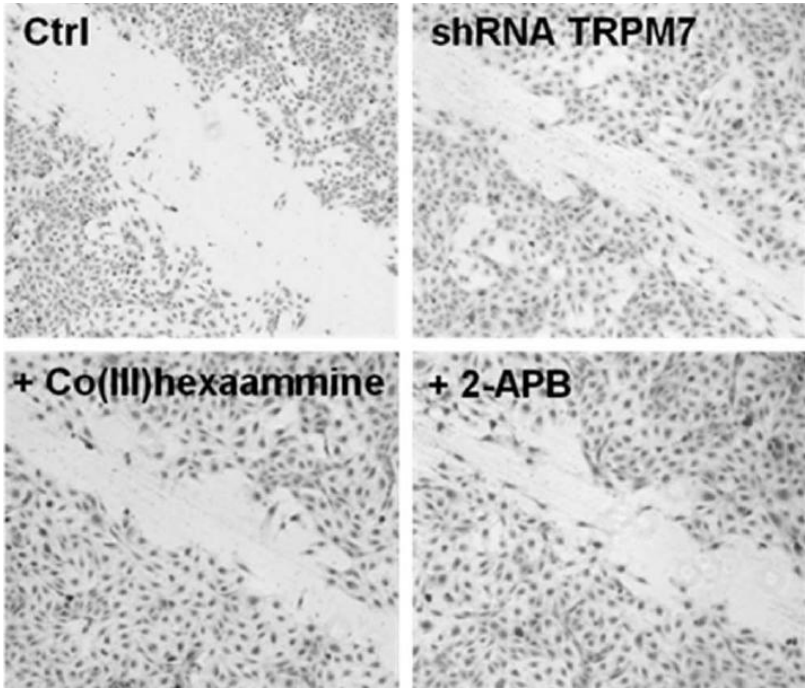


Baldoli 2013

J. Maier

EFFECTS OF TRPM7 INHIBITION ON HUVEC
2. CELL MIGRATION

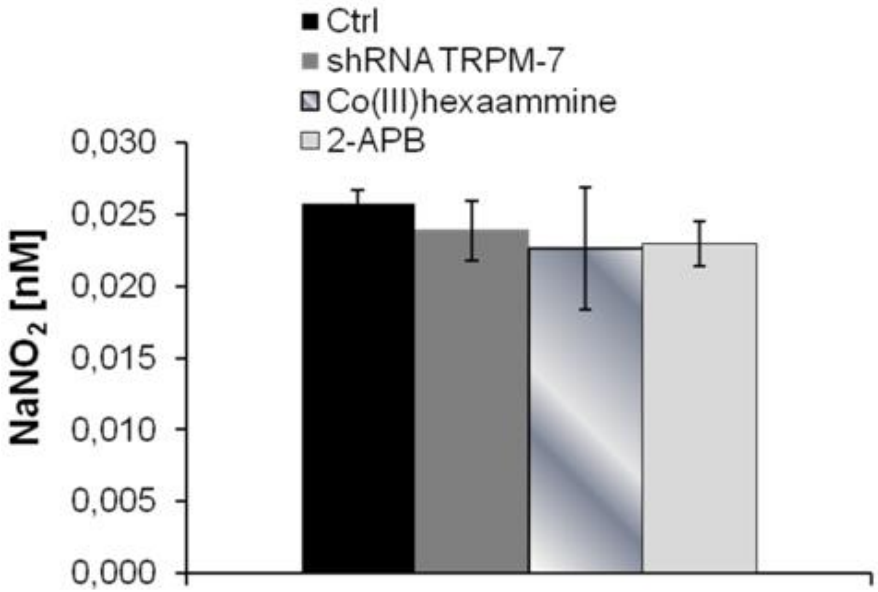




Baldoli 2013

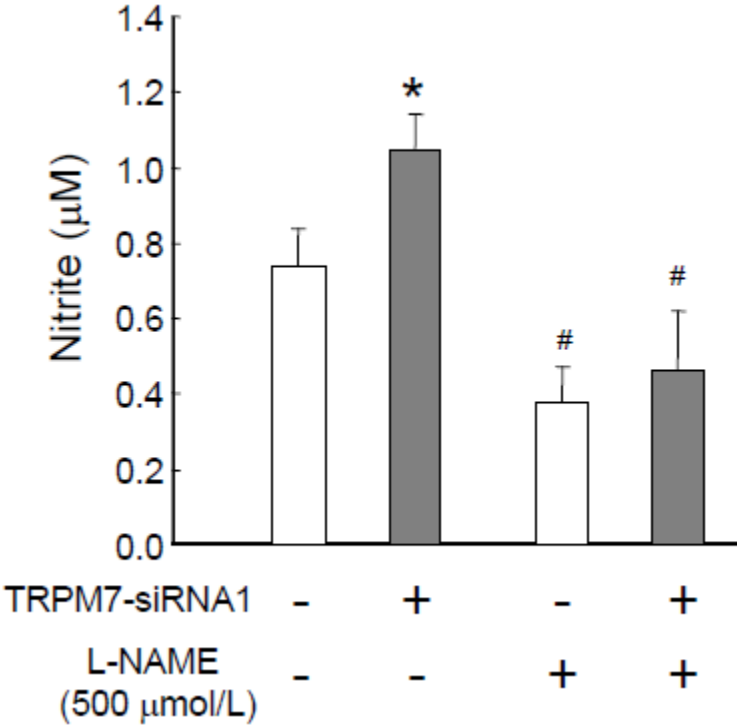
J. Maier

EFFECTS OF TRPM7 INHIBITION ON HUVEC
3. NITRIC OXIDE SYNTHESIS

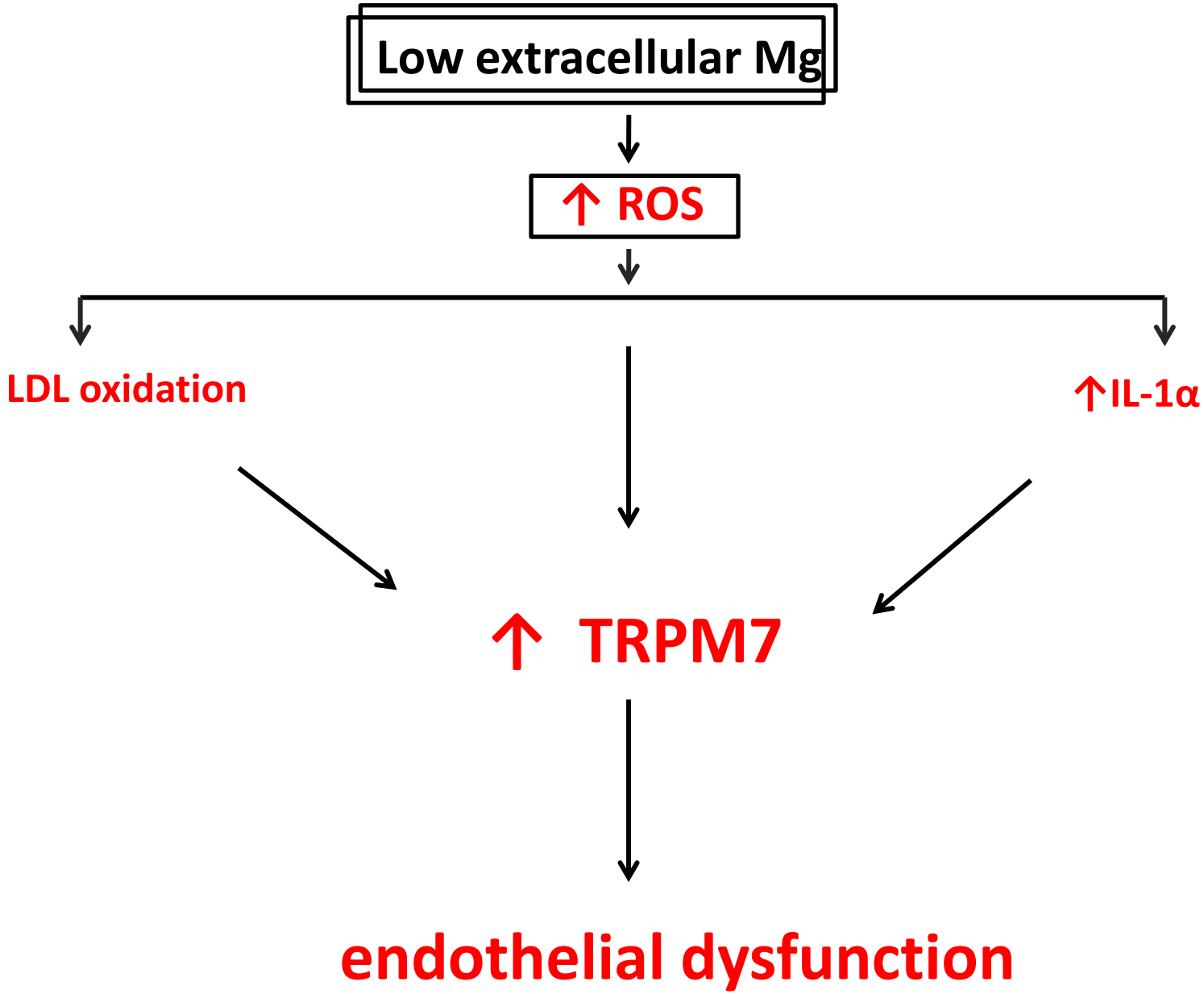


Baldoli 2013

Inoue K, 2009



J. Maier

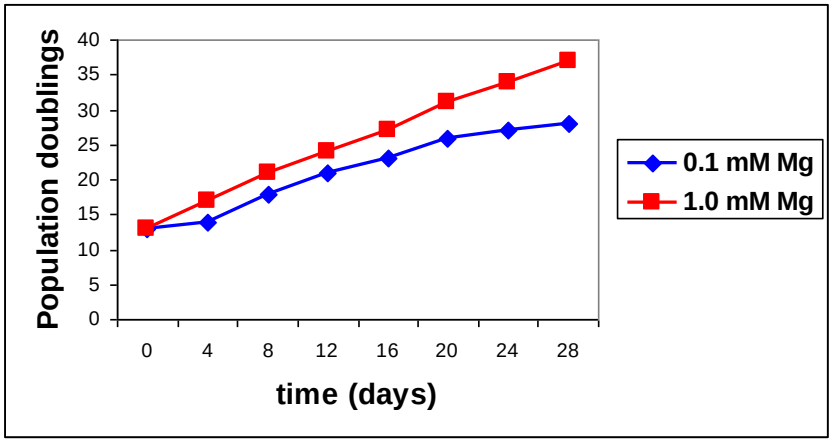

Low extracellular Mg
↑ ROS
LDL oxidation
↑IL-1α
↑ TRPM7
endothelial dysfunction

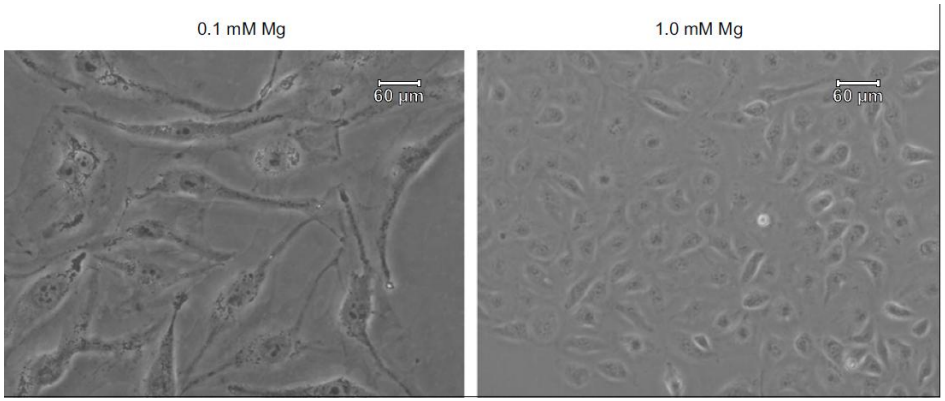
J. Maier
FMC

- Aged endothelium shows increased superoxide anions and peroxynitrite formation, enhancement of NADPH oxidase and inducible NO synthase expression.

- Aged endothelium overexpresses IL-1, VCAM, PAI1, MMP

- AGED ENDOTHELIUM IS DYSFUNCTIONAL

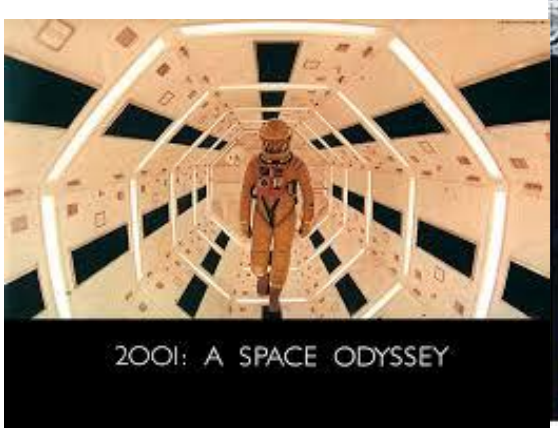


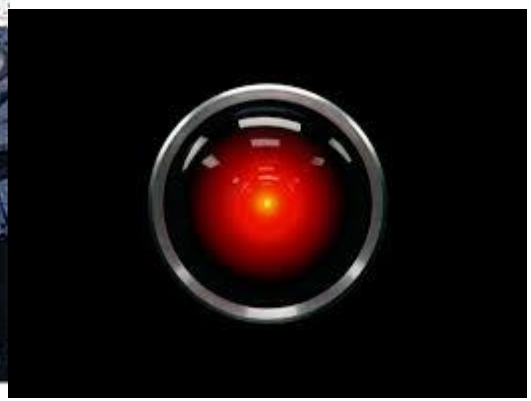


Low Mg accelerates endothelial senescence

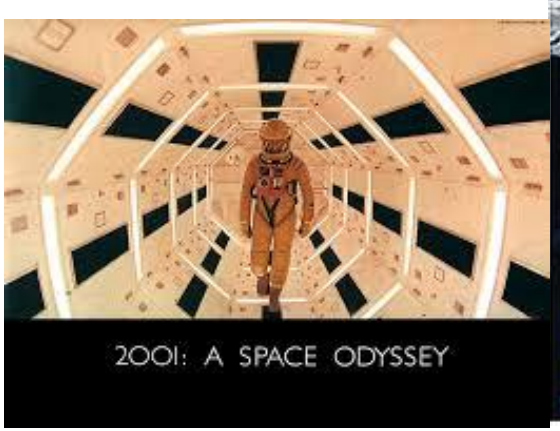
Killilea, 2008; Ferré 2007

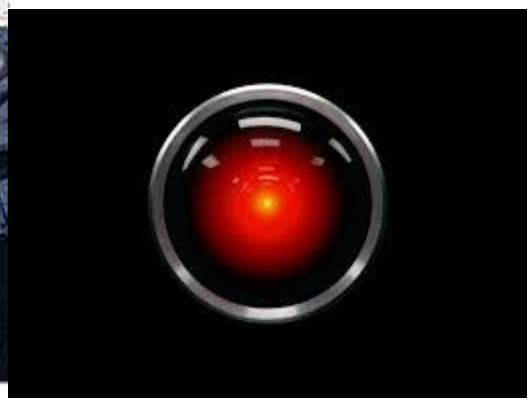
J. Maier





J. Maier





↓ **Mg** reservoir in bone and skeletal muscle

↓ **Mg** absorption in the intestine and ↑ **Mg** excretion by the kidney

hypomagnesemia

J. Maier

Microgravity related disorders

- Cardiovascular deconditioning
- Bone loss/ Muscle Atrophy
- Immune dysfunctions
- Sensory Input Changes

Spaceflight and its analogue bed rest telescope the changes observed in aging

BUT 10 TIMES FASTER THAN IN THE COURSE OF AGING!! AND FOR THE BONES, SPACE ACCELERATES 20-FOLD THE NORMAL RATE OF BONE LOSS OCCURRING IN AGING!!

J. Maier

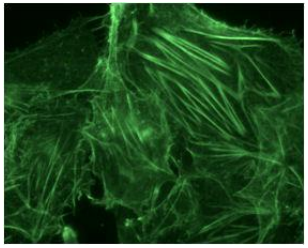
Simulated microgravity activates an adaptive response

Simulated microgravity remodels cell architecture/organization

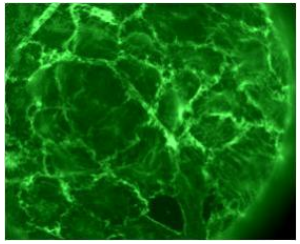
↓

- alteration of the cytoskeleton

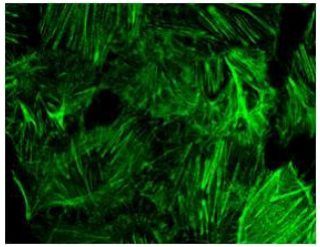
- **inhibition of actin transcription**
- **enhancement of actin degradation**



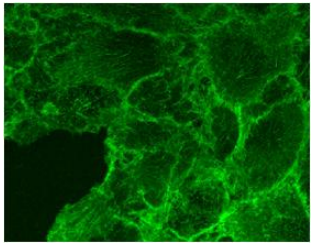
control



RWV



control



RPM

- **rapid and transient induction of stress response**
- **alteration of cell proliferation**
- **alteration of the cytokine network**
- **altered synthesis of vasoactive molecules**
- **altered gene expression**

Carlsson 2003, Versari 2007, Versari 2013

J. Maier

SPHINX

Spaceflight of HUVEC: an Integrated Experiment

Houston we have a problem:
Microgravity accelerates biological aging

"New research in* The FASEB Journal *suggests that gravitational unloading significantly impairs the function of endothelial cells, as evidenced by gene expression studies conducted in space"

Gravitational unloading significantly impairs the function of endothelial cells, as evidenced by gene expression studies conducted in space

TABLE 3. *RT-PCR of a selection of genes to validate the microarray results*

Gene	Gene assignment	Fold change RT-PCR
TXNIP	Thioredoxin-interacting protein	33.0
MIR15A	MicroRNA 15a	5.0
TP53INP1	Tumor protein p53-inducible nuclear protein 1	2.7
HSPA1B	Heat-shock 70-kDa protein 1B	−5.6
HSPA1A	Heat-shock 70-kDa protein 1A	−5.6
CLCA2	Chloride channel accessory 2	−4.1
EDN1	Endothelin 1	1.0
IL1A	Interleukin 1, α	12.6
IL6	Interleukin 6	1.0
NOS2	Nitric oxide synthase 2, inducible	2.5
NOS3	Nitric oxide synthase 3 (endothelial cell)	2.2
TNF	Tumor necrosis factor	0.9

Microgravity generates a pro-oxidative environment that activates an inflammatory response with a significant increase of interleukin 1α and β secretion, alters endothelial behavior, and promotes premature senescence

Jeanette Maier
Sara Castiglioni
Alessandra Cazzaniga
Daniela Bernardini
Silvia Ferré
Marzia Leidi

Andrzej Mazur - INRA France
Federica Wolf - Rome
Stefano Iotti - Bologna