TOSSICITA' DEGLI OSSIDI DEL COLESTEROLO

ovvero

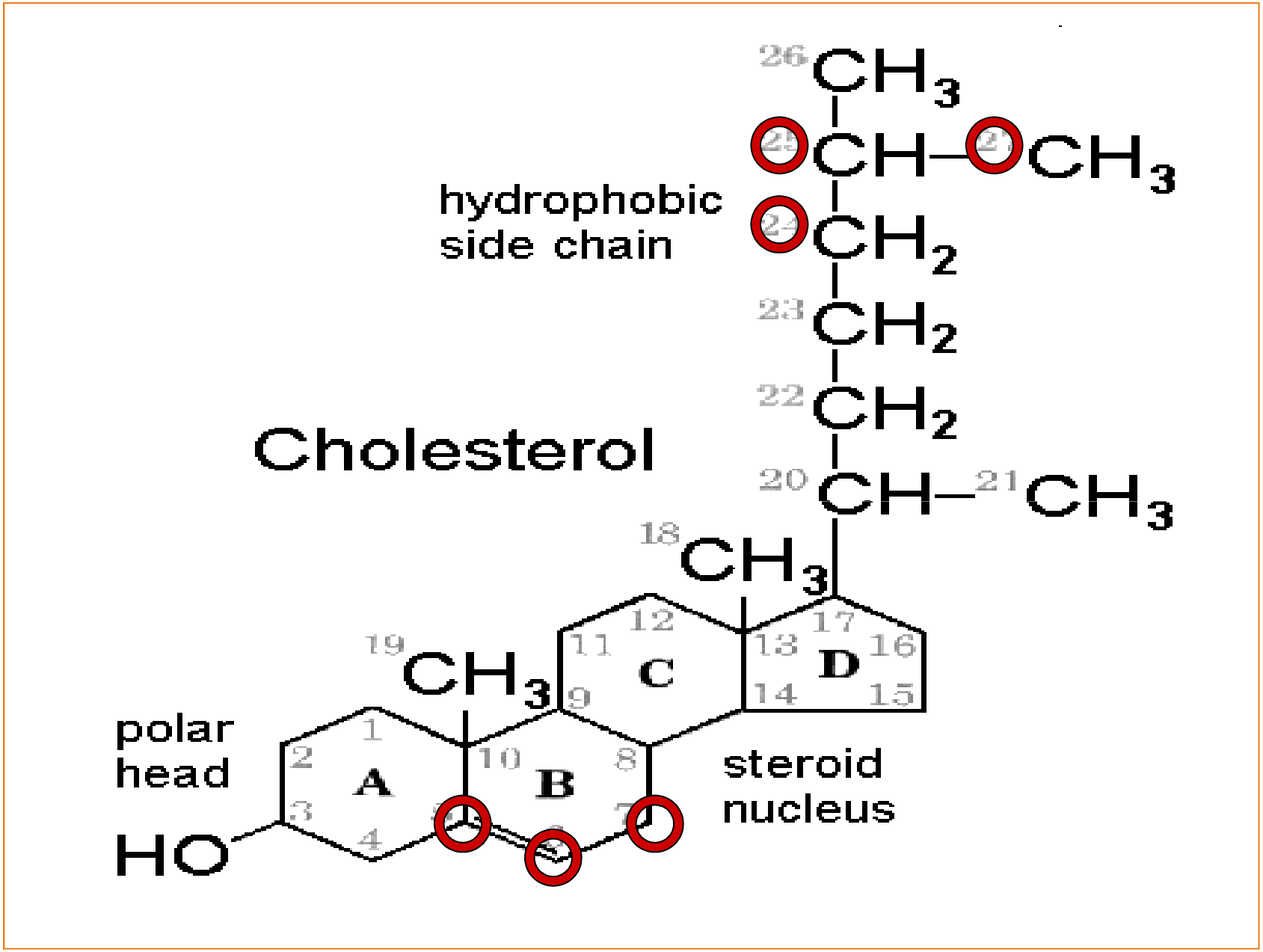
ESEMPI DI CONDIZIONI PATOLOGICHE IN CUI E' COINVOLTO L'EFFETTO PRO-INFIAMMATORIO E TOSSICO DI OSSISTEROLI

WORKSHOP SISSG
Torino, 23-24 maggio, 2013

CHOLESTEROL
essential component
of the plasmamembrane
of our cells

It is mainly localized in those areas of cell membranes, where the processes related to survival, function and interaction with external environment are taking place

HOW CHOLESTEROL COULD DEAL WITH HUMAN PATHOLOGY?


Cholesterol
hydrophobic
side chain
polar
head
HO
steroid
nucleus
A
B
C
D
18 CH3
19 CH3
20 CH
21 CH3
22 CH2
23 CH2
24 CH2
25 CH
26 CH3
27 CH3

ORIGIN OF PLASMA AND TISSUE OXYSTEROLS

From the Diet

Already present in food (red meat. egg, milk, cheese, ham..)

Formed by autoxidation of foodstuff (induced by heat, light exposure, refrigeration, freeze-drying)

Endogenous sources

1) Non enzymatic pathways

Attack by reactive oxygen species

Attack by peroxyl and alkoxyl radicals

Leukocyte/H_2O_2/HOCl system

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INFLAMMATION

Endogenous sources

1) Non enzymatic pathways

Attack by reactive oxygen species

Attack by peroxyl and alkoxyl radicals

Leukocyte/H_2O_2/HOCl system

Endogenous sources

2) Enzymatic pathways

Cholesterol oxidation is mediated by the enzymes cholesterol 24-hydroxylase (CYP46A1) (MAINLY BRAIN)

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24S-OH CHOLESTEROL

and cholesterol 27-hydroxylase (CYP27A1) (VARIOUS TISSUES)

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27-OH CHOLESTEROL

INCREASE OF

OXYSTEROLS' STEADY-STATE LEVELS

A WAY INFLAMMATION

MAY INTERFERE

WITH CHOLESTEROL METABOLISM

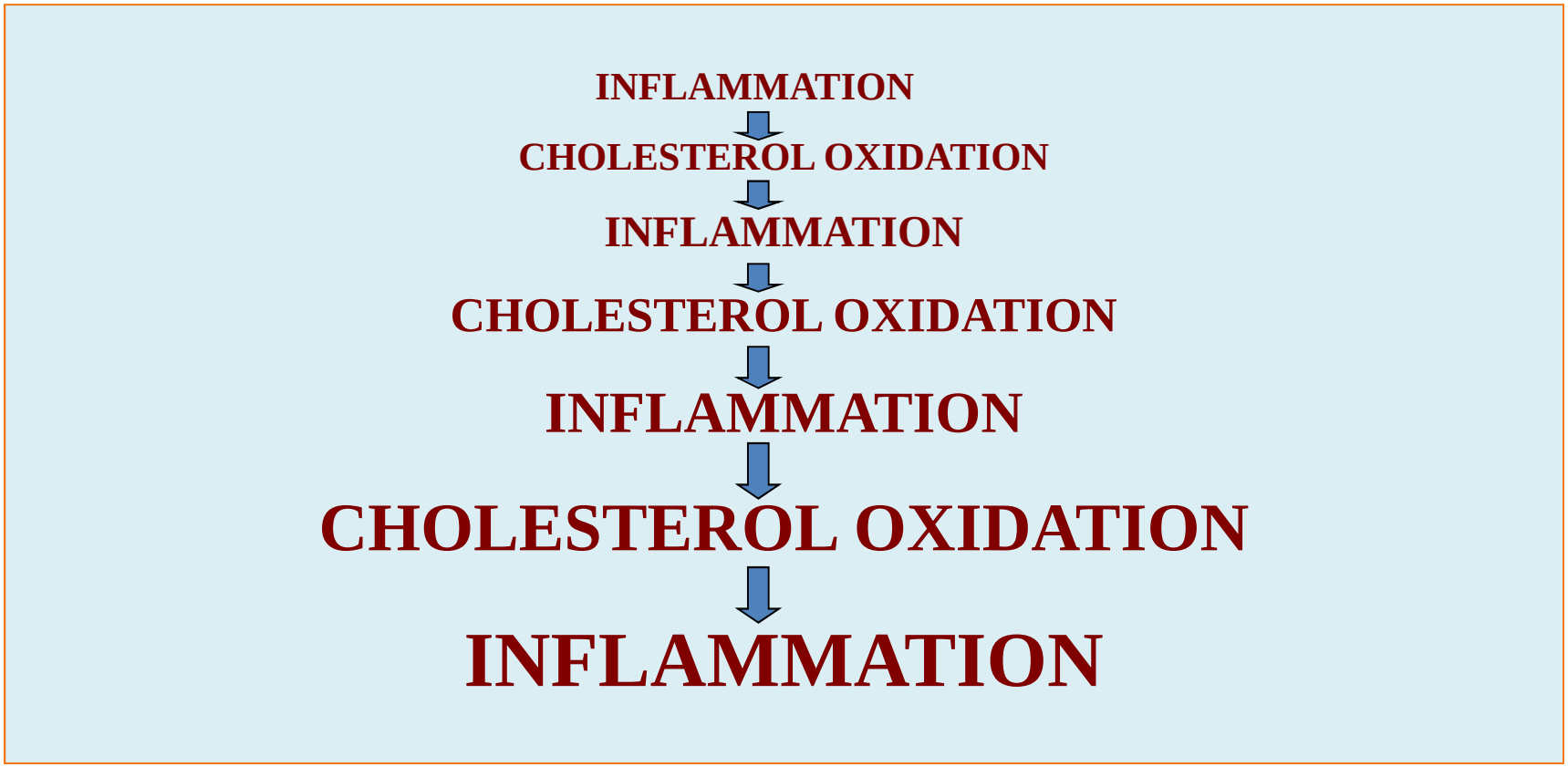
OXYSTEROLS IN HUMAN FIBROATHEROMA

7α-hydroxycholesterol (7α-OH)	4%
7β-hydroxycholesterol (7β-OH)	10%
5α,6α-epoxycholesterol (α-EPOX)	8%
5β,6β-epoxycholesterol (β-EPOX)	22%
cholestan-3β,5α,6β-triol (TRIOL)	6%
7-ketocholesterol (7K)	21%
25-hydroxycholesterol (25-OH)	1%
27-hydroxycholesterol (27-OH)	28%

PRO-INFLAMMATORY EFFECTS OF OXYSTEROLS

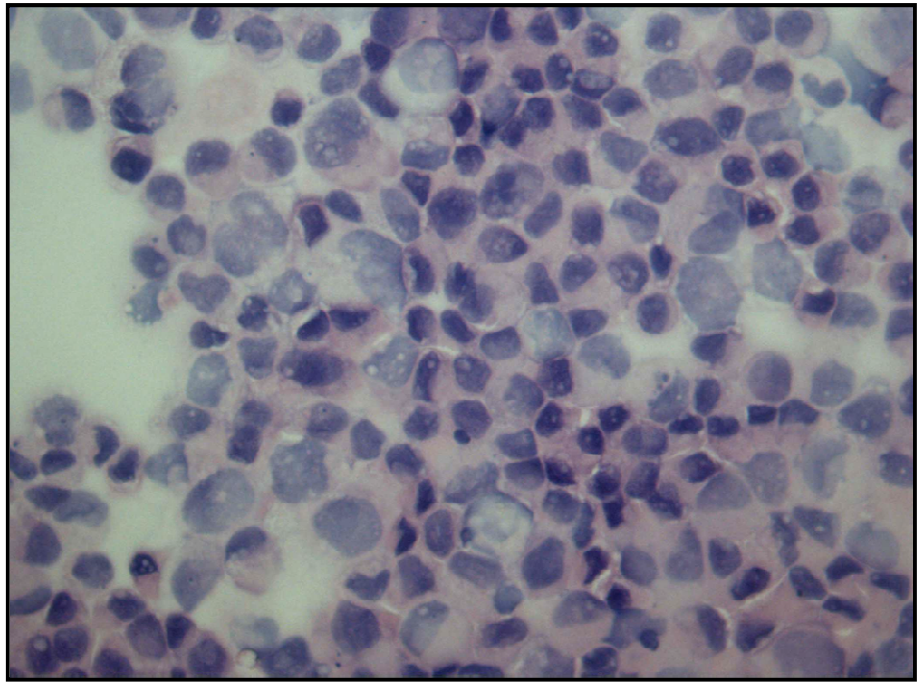
oxysterol/s	*cell type*	*induction*	
7-ketocholesterol	U937, U4 cells	IL-1β	Lizard 1997 Rosklint 2002
7-keto/7α-,7β-OHchol	HUVECs	VCAM-1 ICAM-1 E-selectin IL-1β	Lemaire 1998 Naito 2004
7-keto, 7β-OHchol, 7α-OHchol	macrophage lineage	IL-8 MCP-1 MIP-1β TNF-α	Liu 1997 Leonarduzzi 2005 Prunet 2006 Leonarduzzi 2005 Erridge 2007

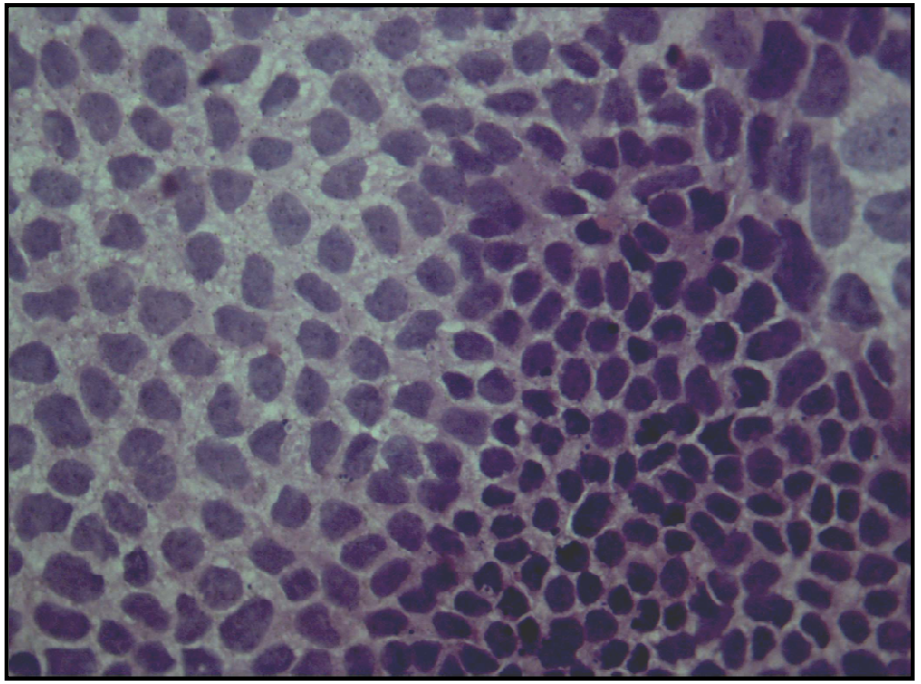
INFLAMMATION AND CHOLESTEROL OXIDATION IN A GIVEN DISEASE PROCESS: A VICIOUS CIRCLE THAT MAKES THE DISEASE PROGRESSING



OXYSTEROLS
and
INFLAMMATORY
BOWEL DISEASES

HUMAN COLON ADENOCARCINOMA CELLS CaCo-2





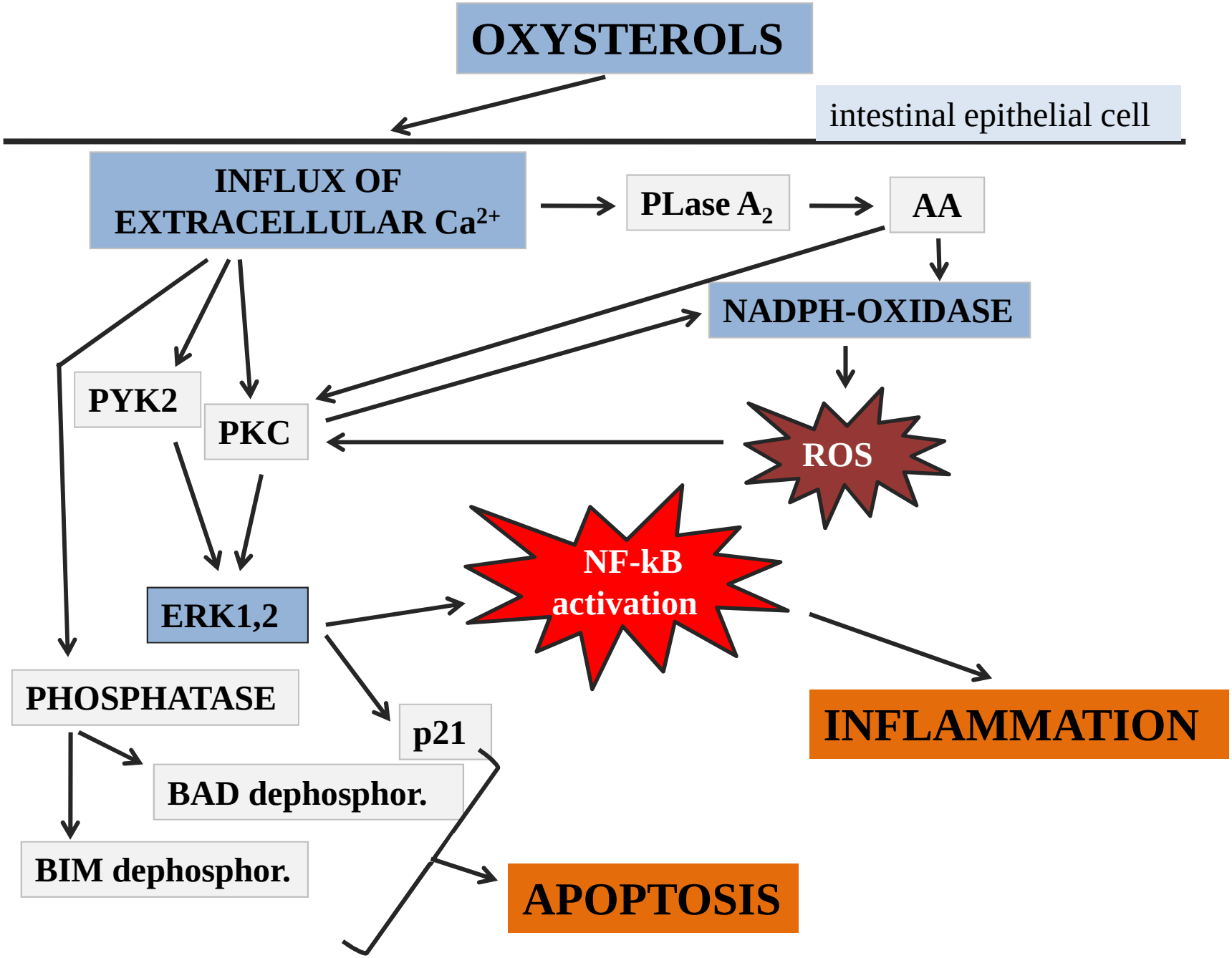
UNDIFFERENTIATED

DIFFERENTIATED (21 gg)

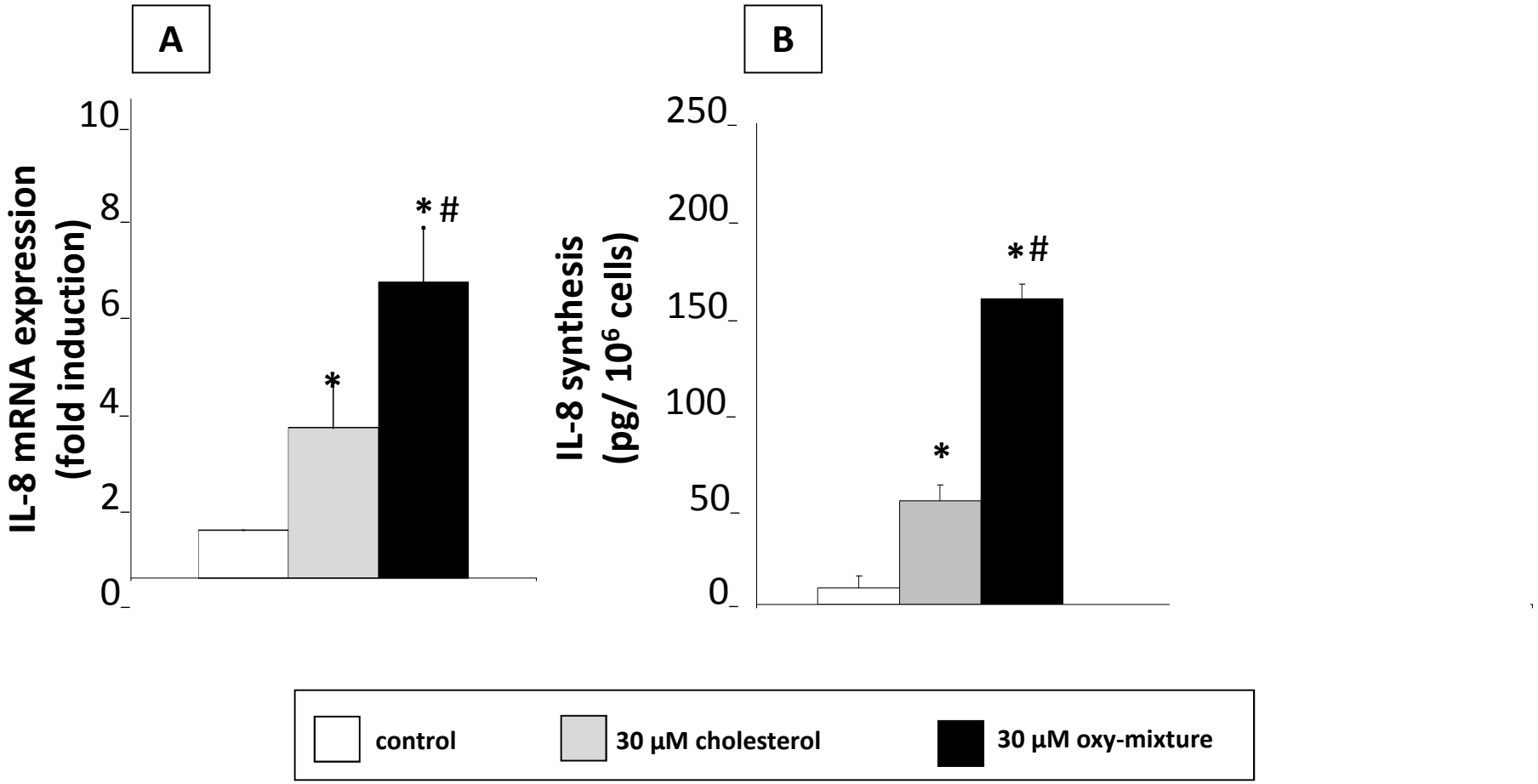
Hematoxilin-Eosin staining – Magnification 40X

Oxysterols generated by heat-dependent oxidation of dietary cholesterol

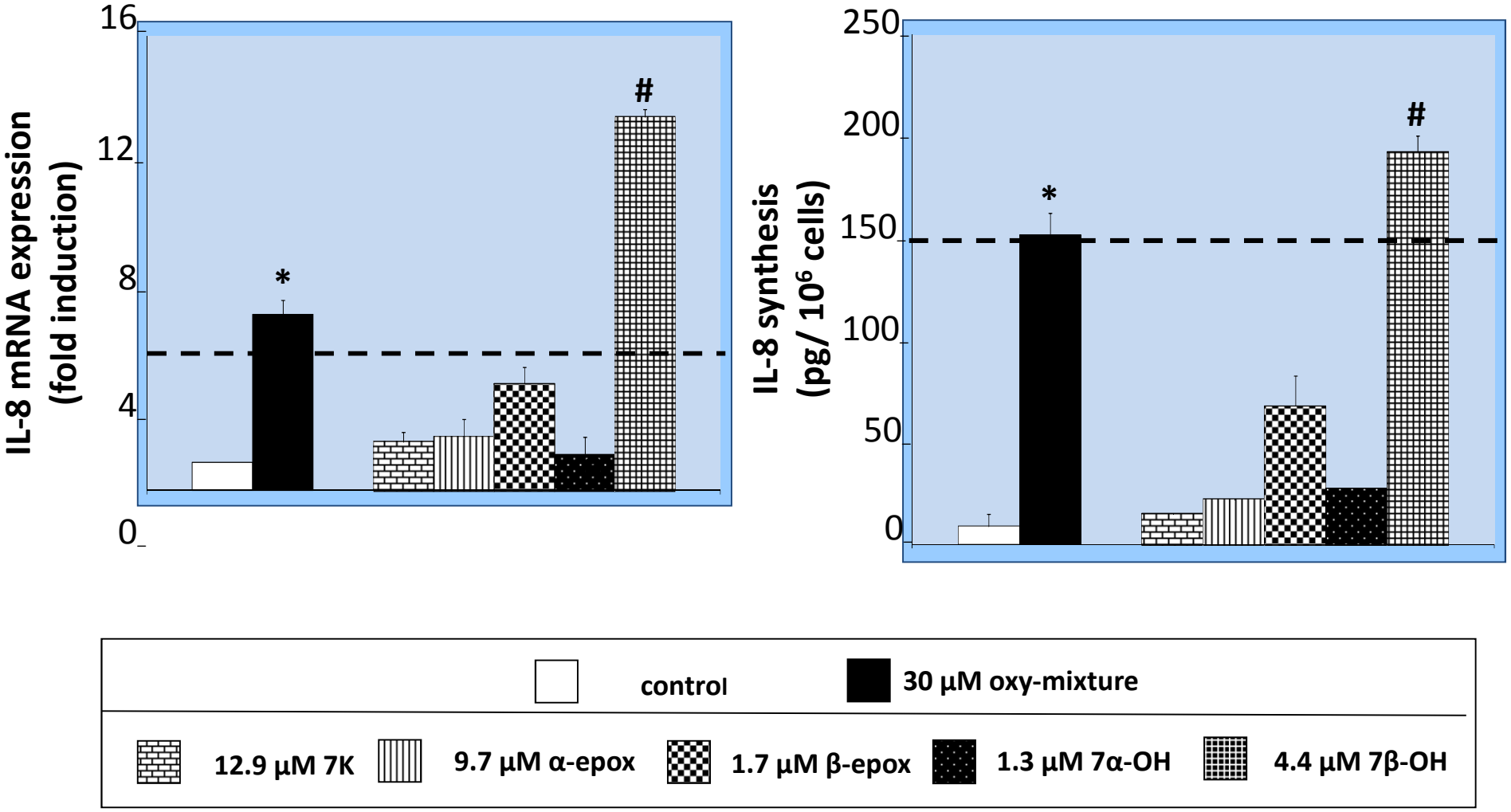
7-ketocholesterol (7K)	(42.96 %)
5α,6α-epoxycholesterol (α-epox)	(32.3%)
5β,6β-epoxycholesterol (β-epox)	(5.76%)
7α-hydroxycholesterol (7α-OH)	(4.26%)
7β-hydroxycholesterol (7β-OH)	(14.71%)


OXYSTEROLS
intestinal epithelial cell
INFLUX OF EXTRACELLULAR Ca2+
PLase A2
AA
NADPH-OXIDASE
PYK2
PKC
ROS
NF-kB activation
ERK1,2
PHOSPHATASE
p21
INFLAMMATION
BAD dephosphor.
BIM dephosphor.
APOPTOSIS

A representative mixture of oxysterols of dietary origin induces IL-8 expression and synthesis in differentiated CaCo-2 cells more efficiently than unoxidized cholesterol



Effect on IL-8 production displayed by the single components of the oxysterol mixture



OXYSTEROLS
and
ALZHEIMER'S DISEASE

BACKGROUND

OXYSTEROLS and ALZHEIMER'S DISEASE

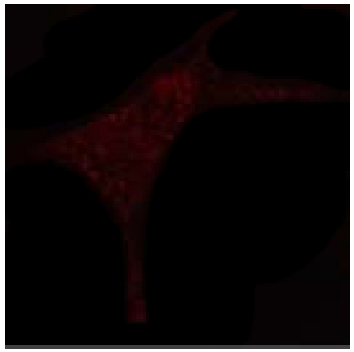
In vivo evidence

- **Higher levels of 24-hydroxycholesterol (24OH) were found in the cerebrospinal fluid of AD patients (Schönknecht et al., 2002)** (27OH not investigated)
- **Increased levels of 27-hydroxycholesterol but not of 24OH in the frontal cortex of AD (Heverin et al., 2004)** (disease staging was not considered)
- **The two enzymes 24-hydroxylase and 27-hydroxylase showed an abnormal pattern in AD brain (Brown 3rd et al. 2004)**

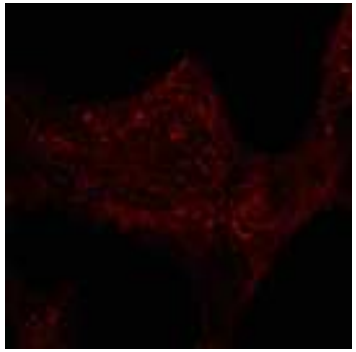
In vitro evidence

- **Increased APP level in primary cultures of human neuronal and glial cells, induced by 24OH (10 µM) (Alexandrov et al, 2005)** (27OH not investigated)
- **In SH-SY5Y cell line (undifferentiated) 27OH (10 µM) was significantly able to up-regulate cell APP level and β-secretase activity, while identical concentrations of 24OH, did not (Prasanthi et al,, 2009)**

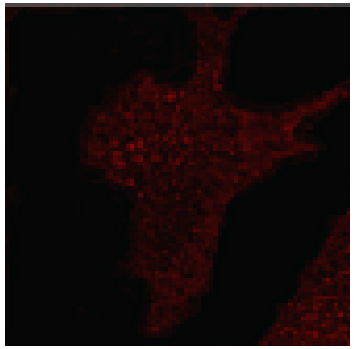
OXYSTEROL-INDUCED UP-REGULATION OF CD36 AND β1-INTEGRIN ON CELL PLASMAMEMBRANE ENHANCES BINDING AND UPTAKE OF Aβ $_{1-42}$



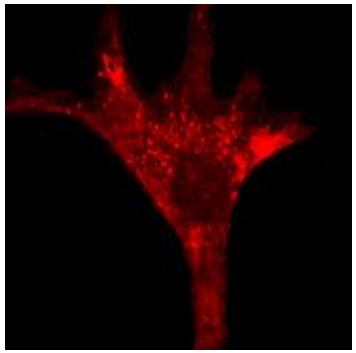
control



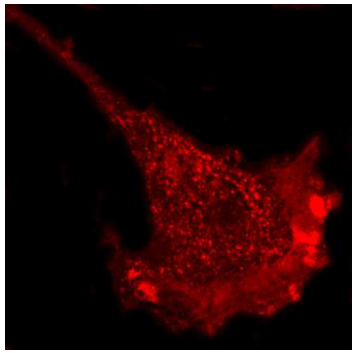
$A\beta_{1-42}$



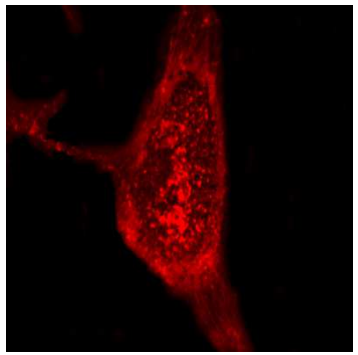
cholesterol + $A\beta_{1-42}$



27-OH + $A\beta_{1-42}$



7β-OH + $A\beta_{1-42}$

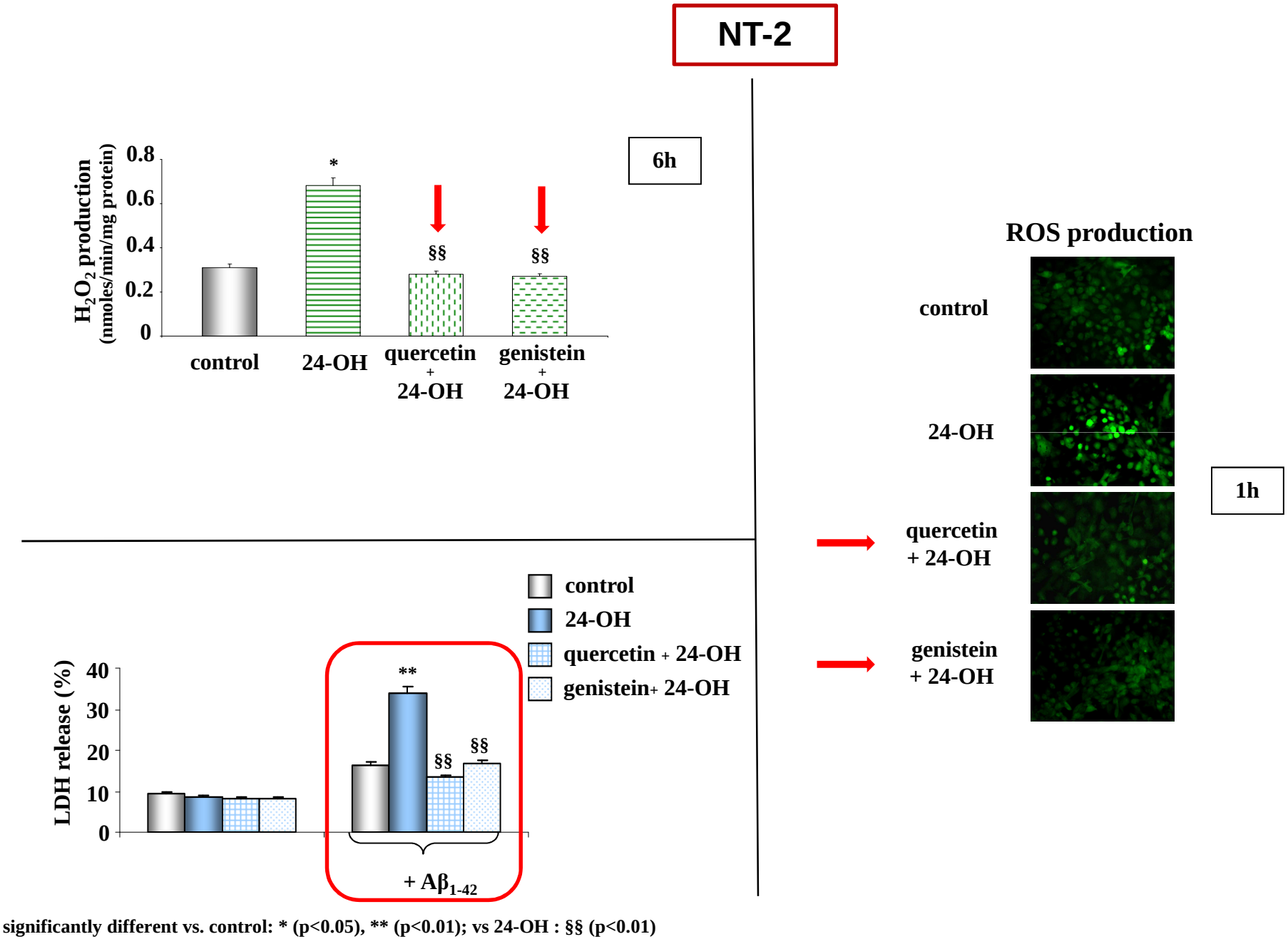


24-OH + $A\beta_{1-42}$

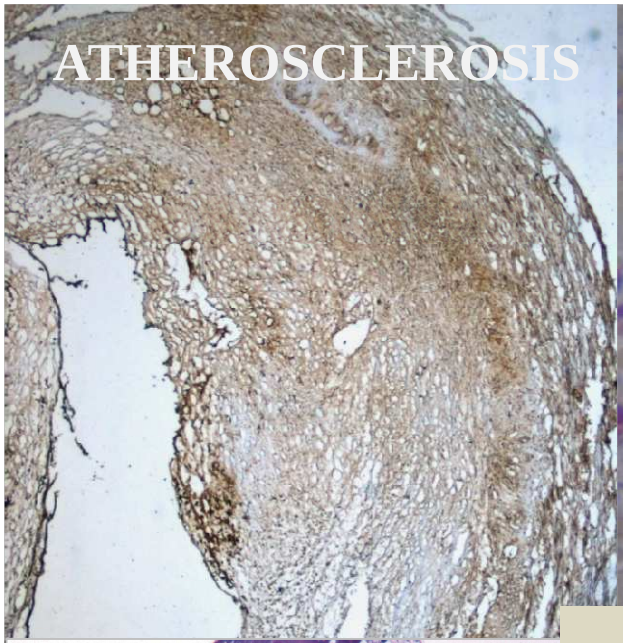
Differentiated SK-N-BE cell line - Congo Red staining

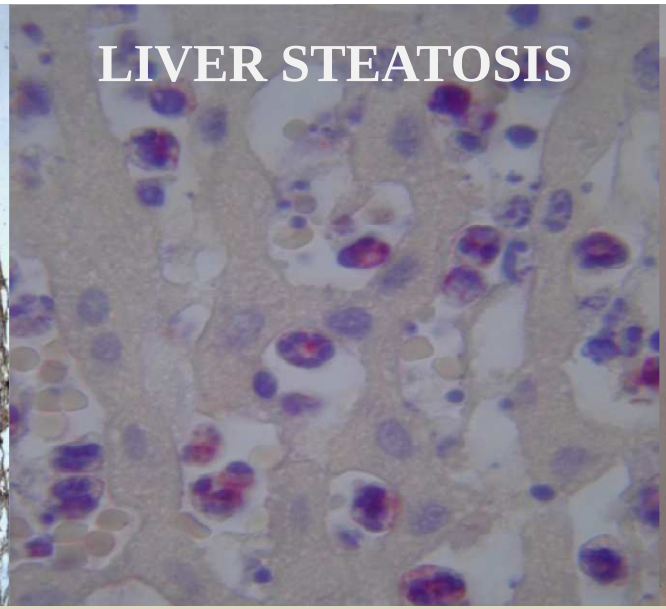
GAMBA, TESTA, LEONARDUZZI et al., 2011

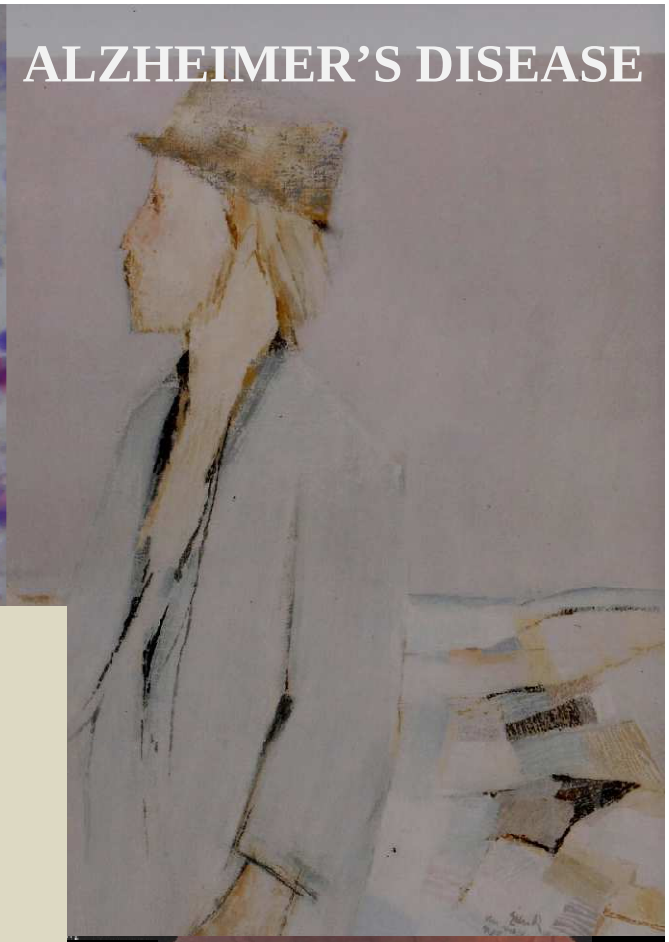
Protection exerted by flavonoids quercetin and genistein (5µM)

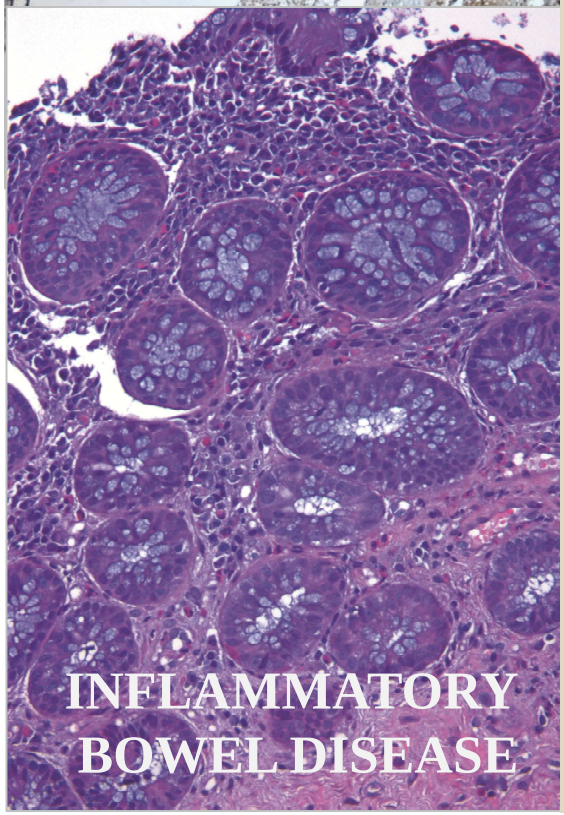


significantly different vs. control: * ($p<0.05$), ** ($p<0.01$); vs 24-OH : §§ ($p<0.01$)









OXYSTEROLS

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ROS

⇩

REDOX SIGNALING

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NF-kB, AP-1...

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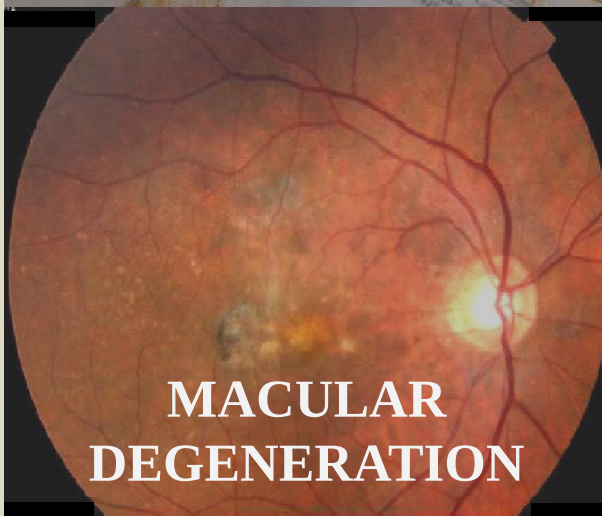
CD36, ICAM-1, MMP-9
IL-8, MCP-1, IL-1β etc.



GRAZIE !

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