

**Le giornate della salute e del benessere:  
Innovazione e Ricerca**

Milano, 30 Giugno - 1 Luglio



# Glutathione: relazione con stress ossidativo ed ageing; strategie per migliorarne la biodisponibilità

**Daniela Buonocore, Ph.D.**

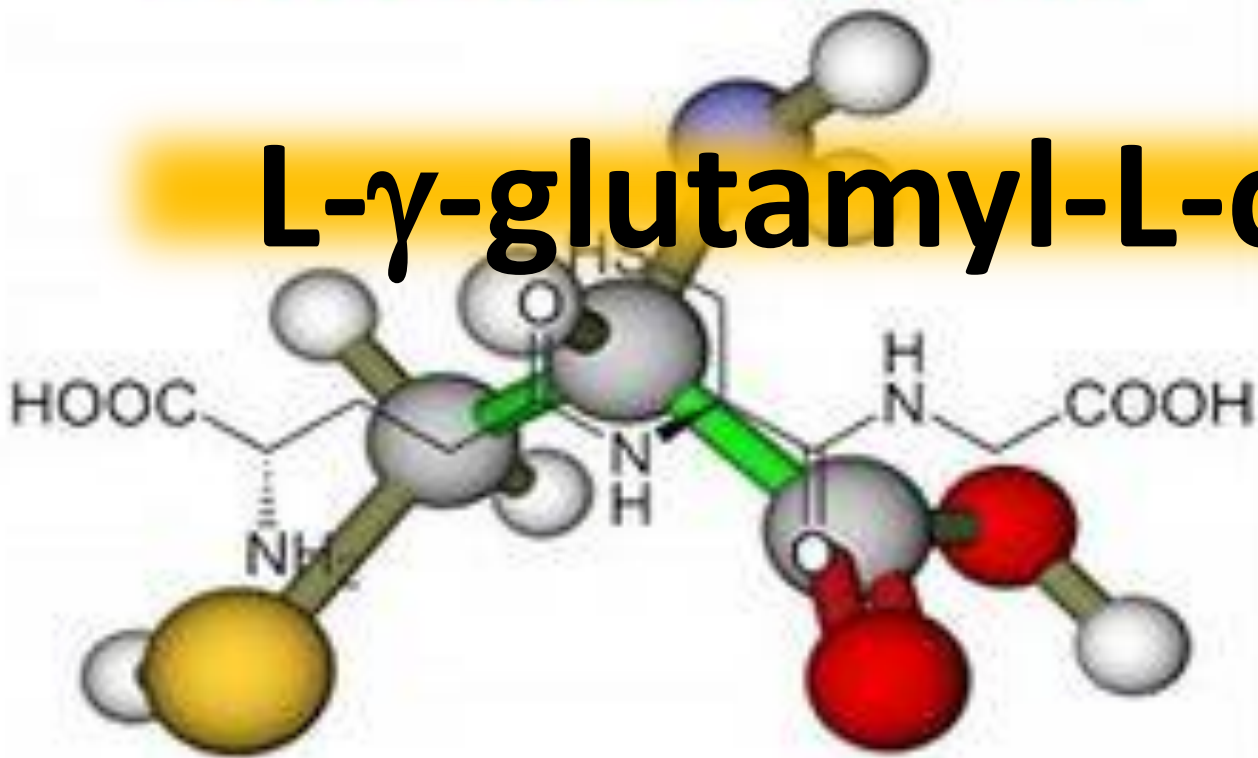
Università degli Studi di Pavia

Dipartimento di Biologia e Biotecnologie «L. Spallanzani»

Laboratorio di Farmacobiochimica, Nutrizione e Nutraceutica

# The Master Antioxidant

L- $\gamma$ -glutamyl-L-cysteinyl-glycine



«GLUTATIONE»



Cisteina

Glicina

Glutammato



Glutathione ridotto

GSH

$\gamma$ -Glu- Cys-Gly

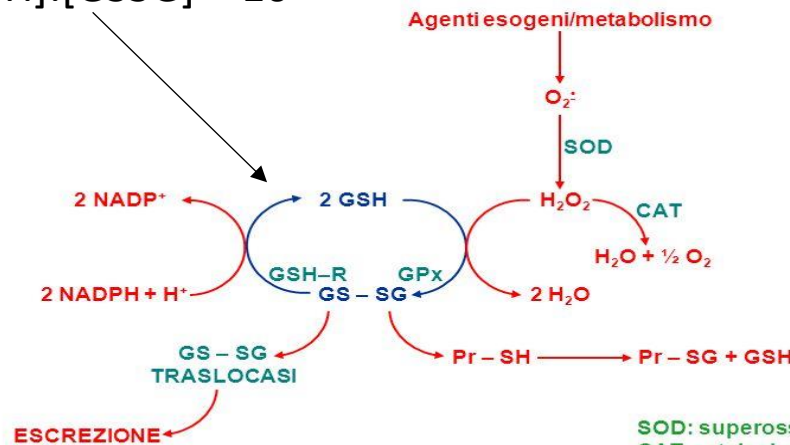
$\gamma$ -Glu- Cys-Gly

Glutathione ossidato

GSSG

### Ciclo del glutathione

[GSH]:[GSSG] > 10



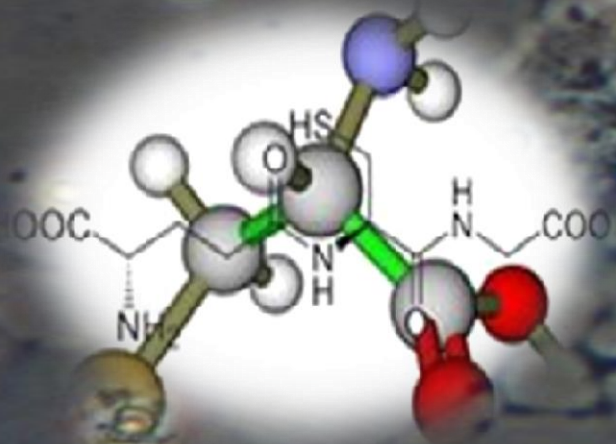
SOD: superossidodismutasi  
 CAT: catalasi  
 GPx: glutathione perossidasi  
 GSH-R: glutathione reductasi

- Gruppi sulfidrilici delle prot nello stato ridotto
- Ferro del gruppo eme allo stato ferroso (Fe<sup>2+</sup>)
- Agente riducente della glutaredossina

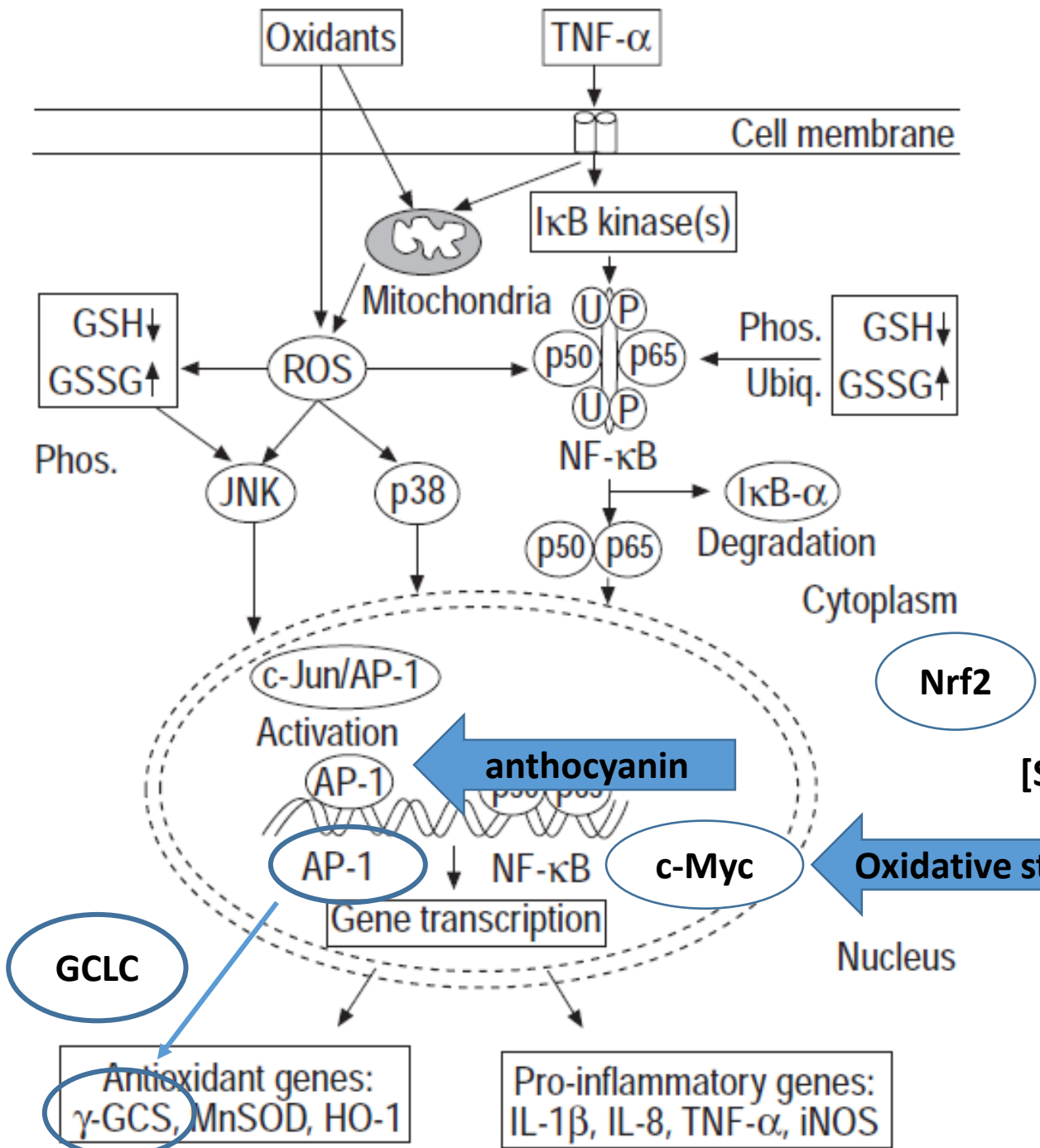


# COFATTORE DI GLUTATIONE-S-TRANSFERASI, DETOX DI XENOBIOTICI

## IMMUNO MODULAZIONE E RISPOSTE INFIAMMATORIE



- ✓ TRASDUZIONE DI SEGNALI REDOX
- ✓ REGOLAZIONE DELLA PROLIFERAZIONE CELLULARE
- ✓ ESPRESSIONE GENICA
- ✓ METABOLISMO DI NO
- ✓ RIMODELLAMENTO DELLA MATRICE EXTRACELLULARE (ECM)
- ✓ APOPTOSI
- ✓ RESPIRAZIONE MITOCONDRIALE
- ✓ GLUTATIONILAZIONE DELLE PROTEINE

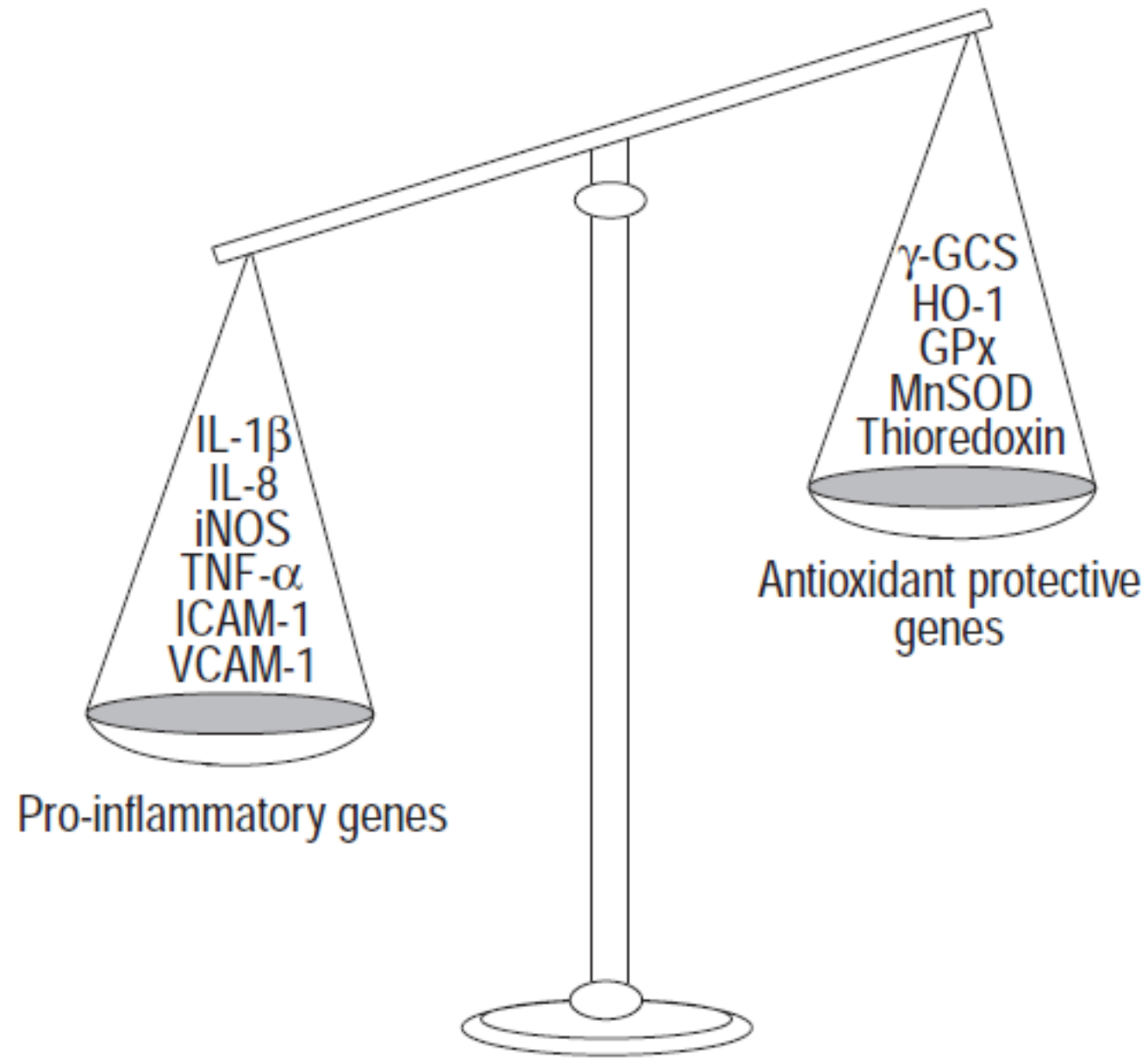


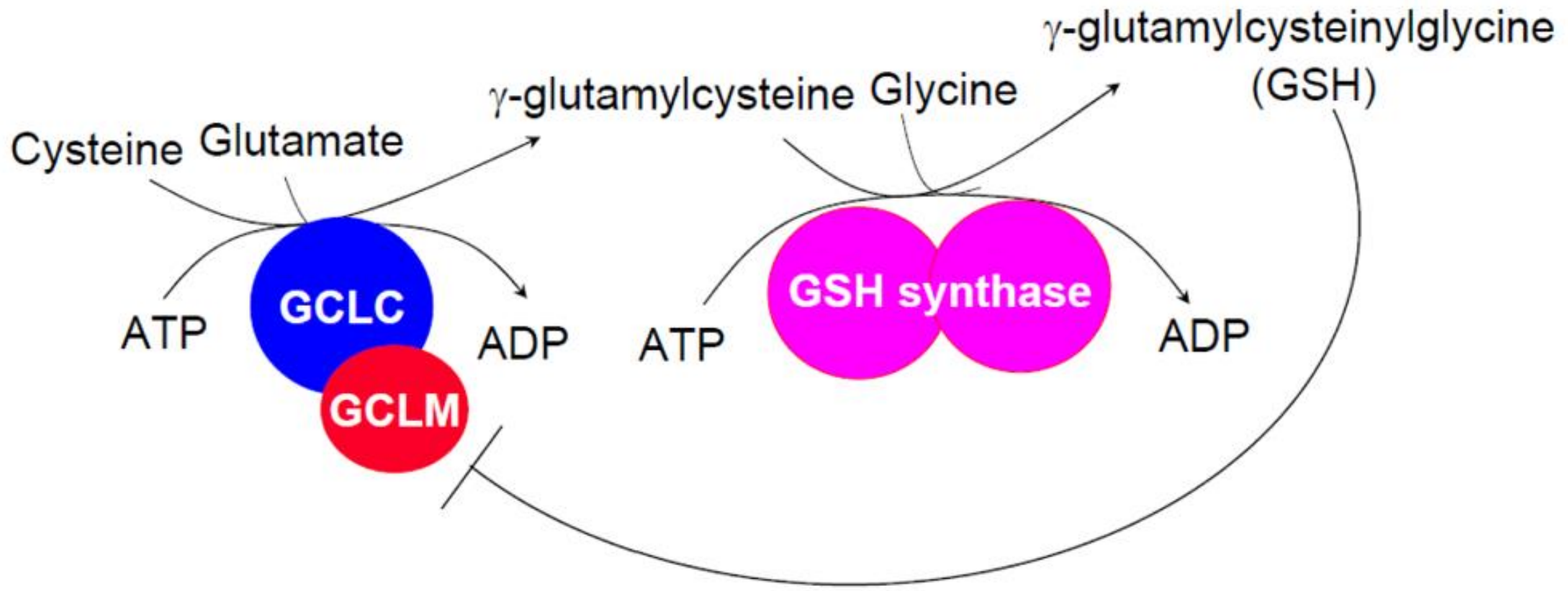
Intracellular redox state (GSH/GSSG) of the cell may play a key role in the regulation and potentiation of the inflammatory responses in lung cells

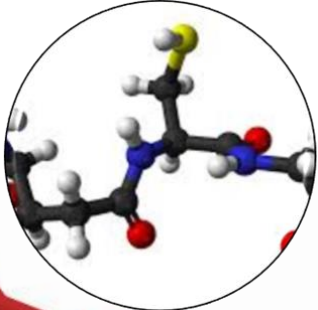
[Shelly C. Lu. *Biochim Biophys Acta* 2013; 1830(5): 3143–3153]

[Rahman I, MacNee W. *Eur Respir J* 2000; 16: 534±554]



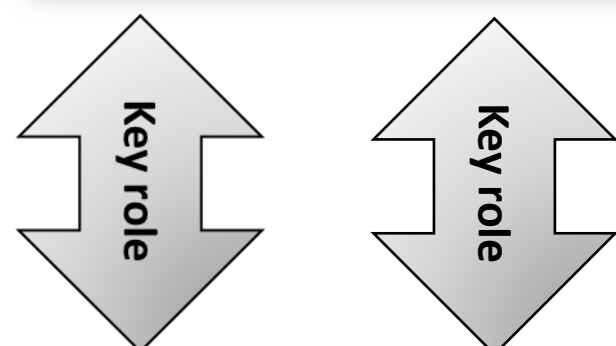
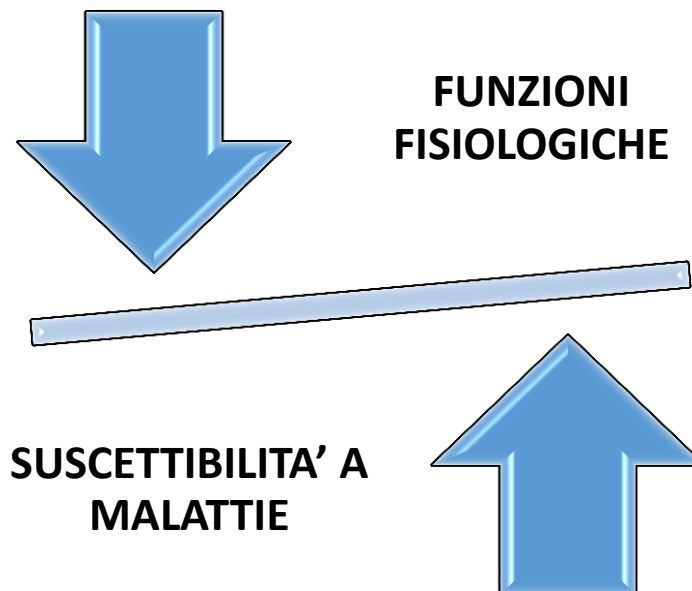




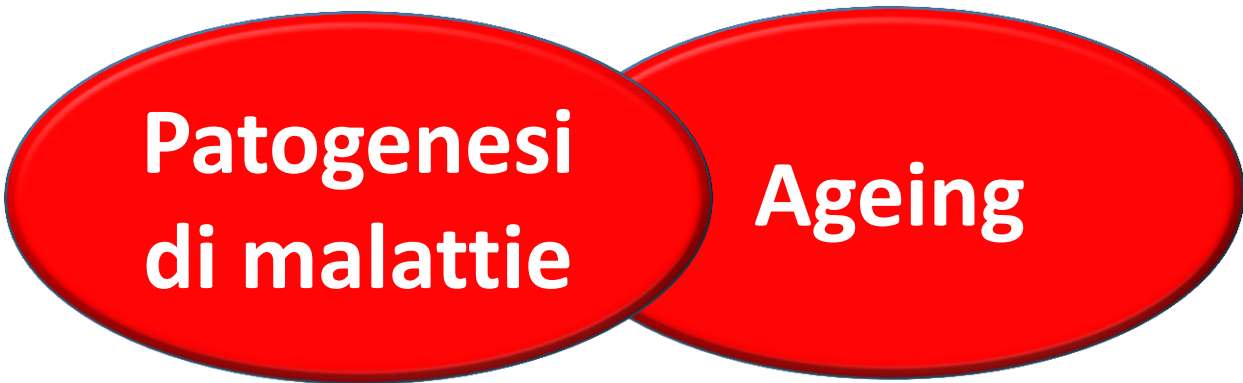


DECREASE

## Stress Ossidativo



- Alzheimer
- Parkinson
- Malattie epatiche
- Fibrosi cistica
- kwashiorkor
- Anemia falciforme
- AIDS
- HIV
- Cancro
- Cardiovascolari
- Diabete







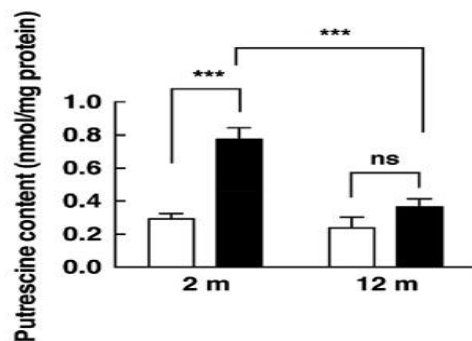
## Aggravation of brain infarction through an increase in acrolein production and a decrease in glutathione with aging



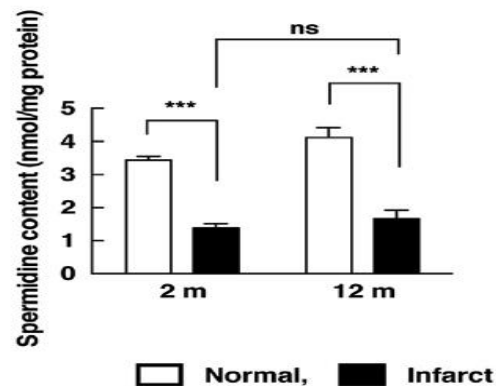
Takeshi Uemura <sup>a</sup>, Kenta Watanabe <sup>b</sup>, Misaki Ishibashi <sup>b</sup>, Ryotaro Saiki <sup>a</sup>, Kyoshiro Kuni <sup>b</sup>, Kazuhiro Nishimura <sup>b</sup>, Toshihiko Toida <sup>b</sup>, Keiko Kashiwagi <sup>c</sup>, Kazuei Igarashi <sup>a, b, \*</sup>

### A. Polyamine contents

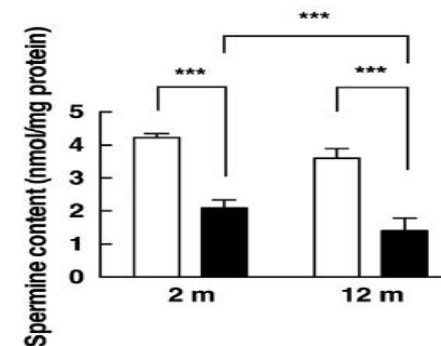
#### Putrescine



#### Spermidine

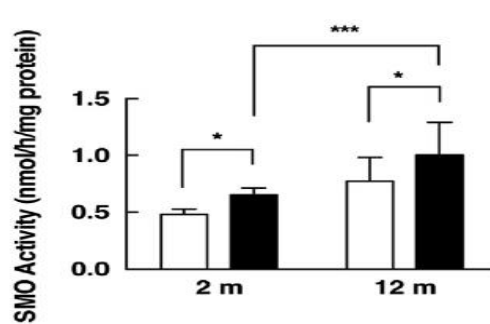


#### Spermine

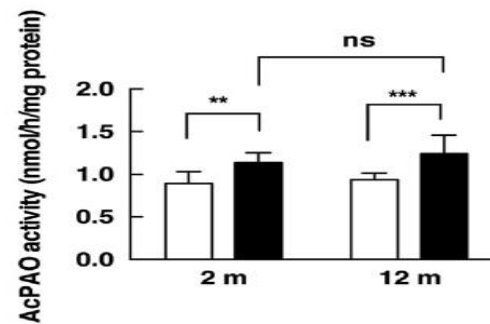


### B. Polyamine metabolizing enzyme activities

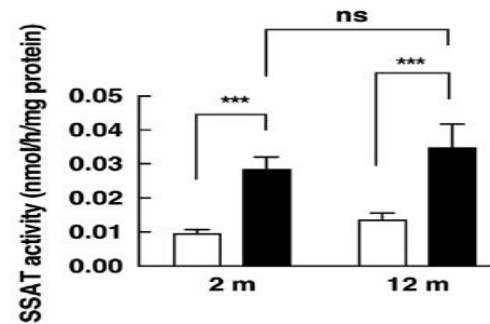
#### SMO



#### AcPAO



#### SSAT



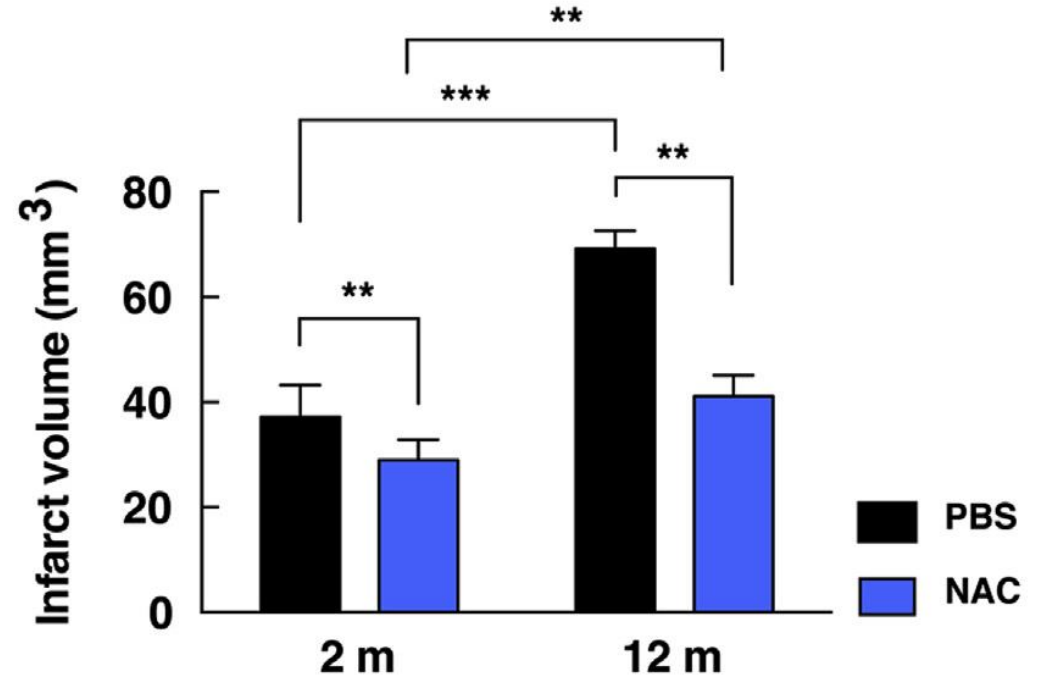
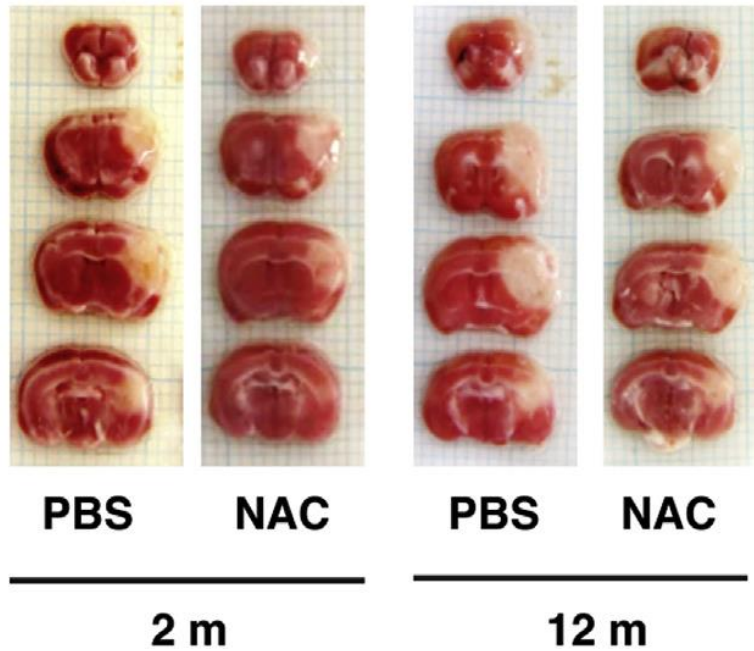


Aggravation of brain infarction through an increase in acrolein production and a decrease in glutathione with aging



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**C. Effect of NAC on infarct volume**





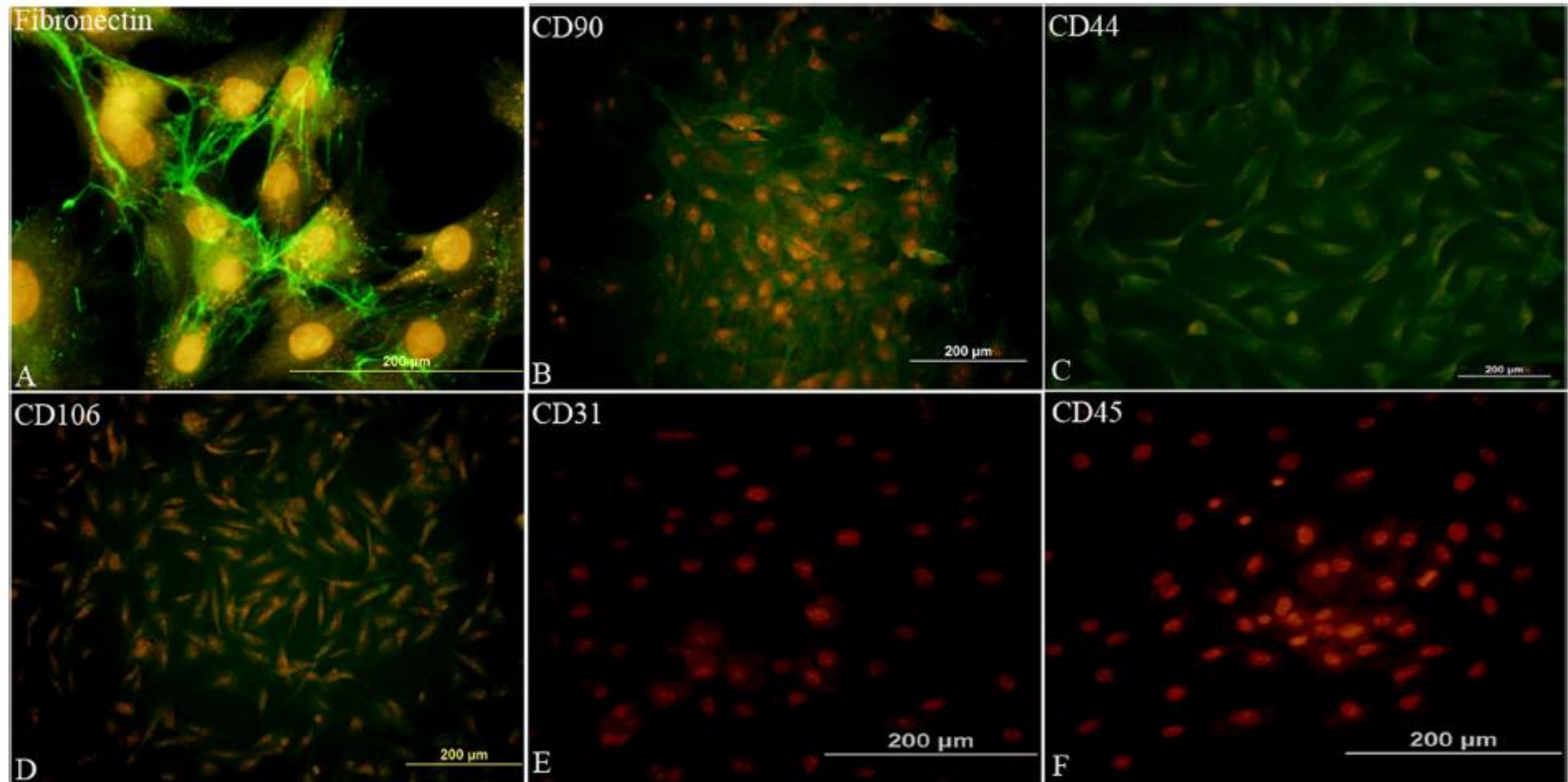


Aggravation of brain infarction through an increase in acrolein production and a decrease in glutathione with aging



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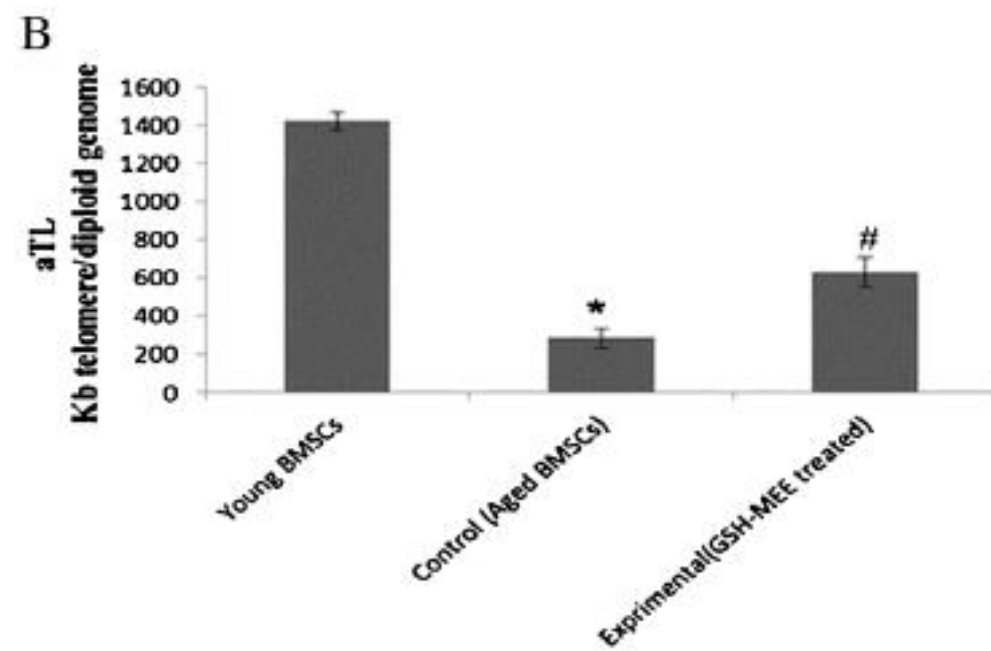
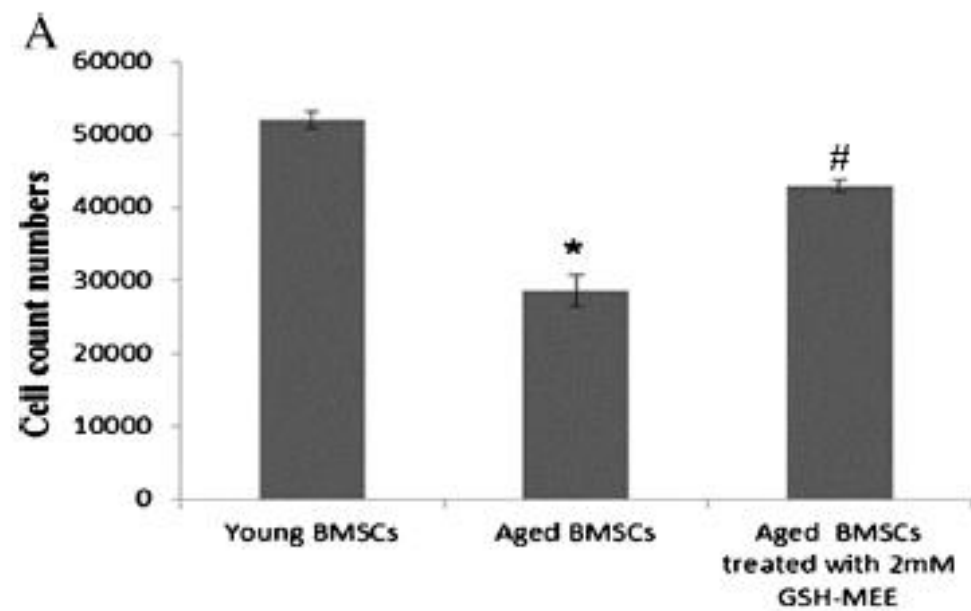
- Acroleina è maggiormente coinvolta nel danno ai tessuti in diverse malattie (ictus, insufficienza renale, Alzheimer, etc.)
  - L'aggravarsi dell'ictus in età avanzata è dovuto ad aumento di acroleina e diminuzione di GSH
  - GSH è coinvolto nella riduzione della porzione di cervello infartuato
  - N-acetylcysteine è un potente *scavenger* di acroleina più dei ROS

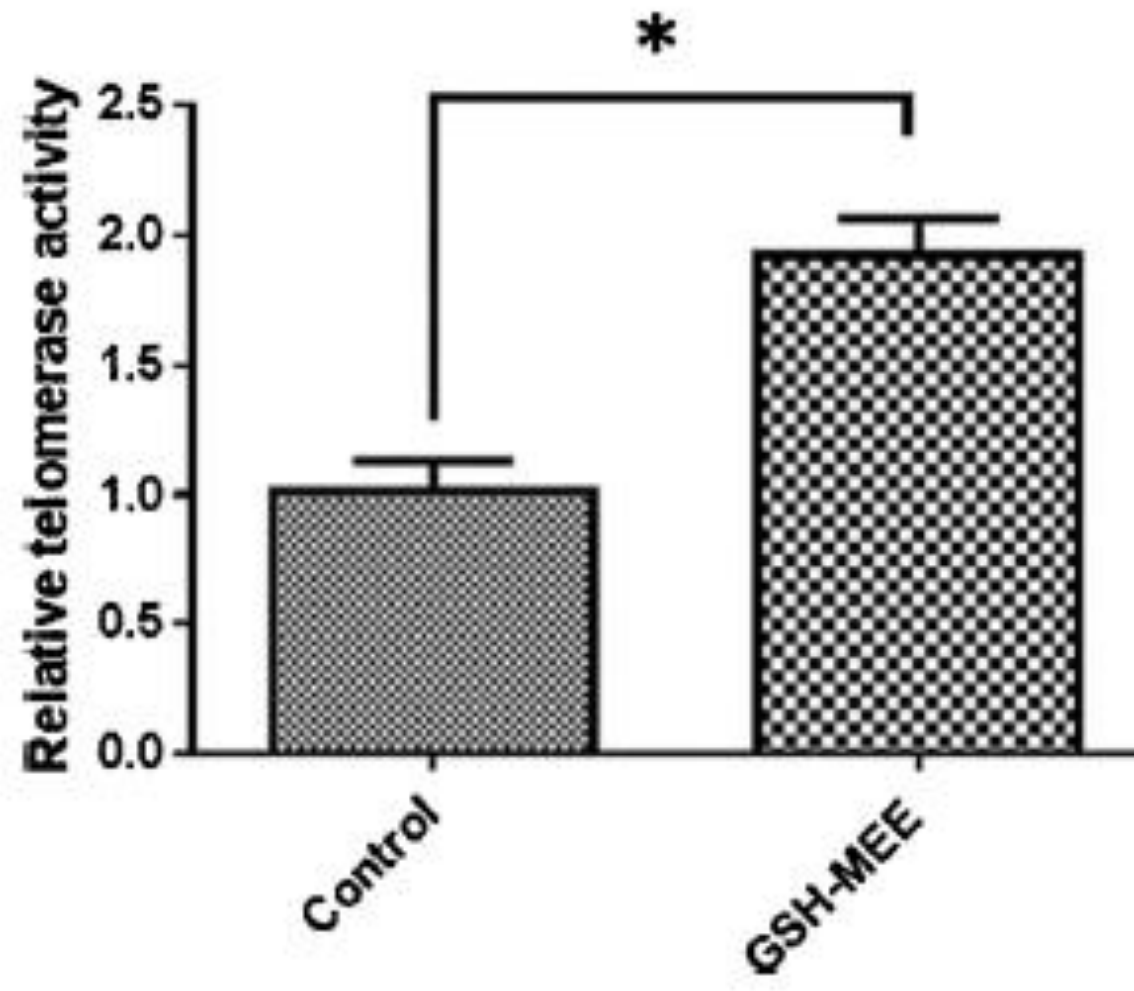


**Figure 1.** Represents the immunostaining of BMSCs at the third passage of (A) Fibronectin, (B) CD90, (C) CD44, (D) CD106, (E) CD31, and (F) CD45, respectively. The primary antibodies for these markers were labeled BMSCs and incubated with secondary antibodies conjugated with

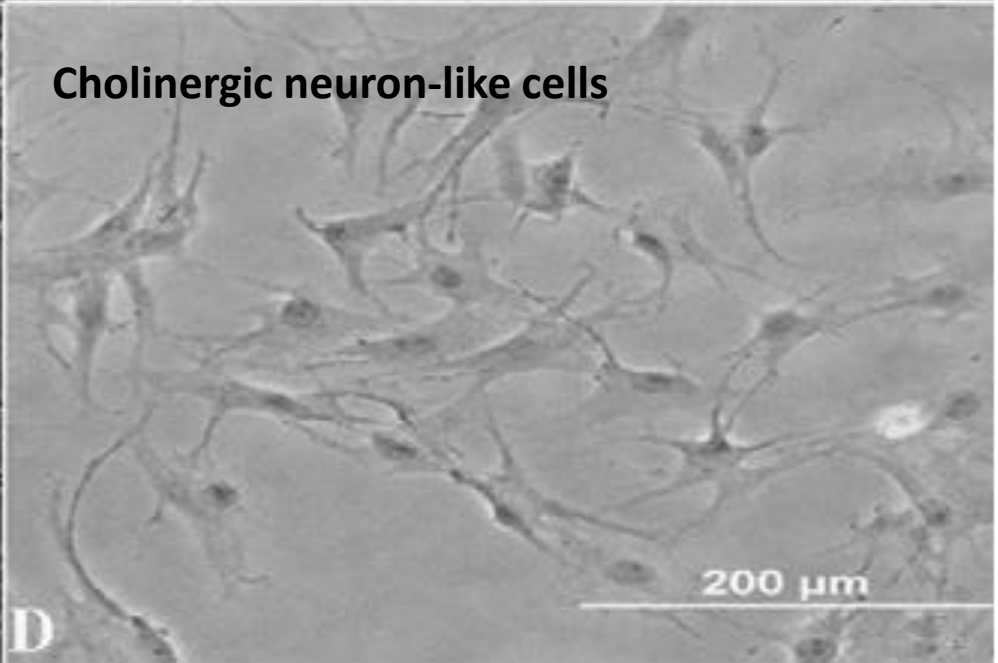
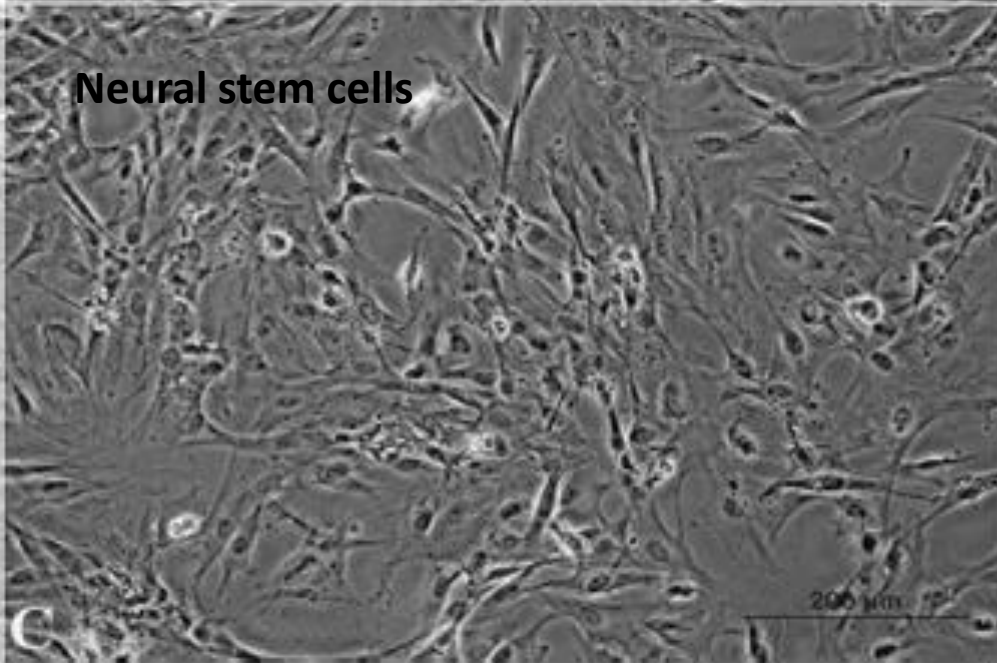
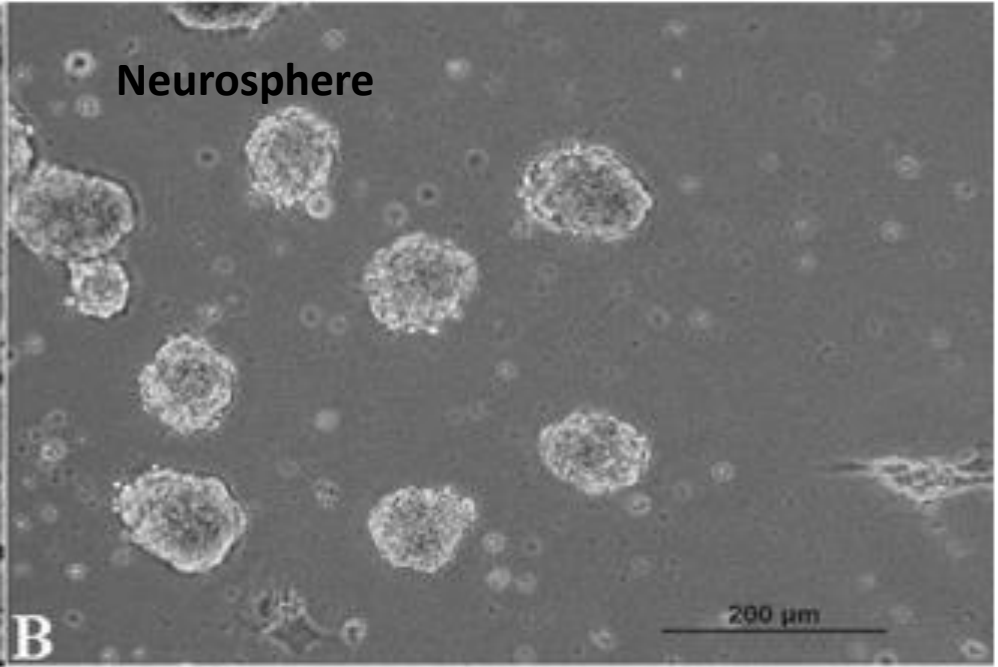
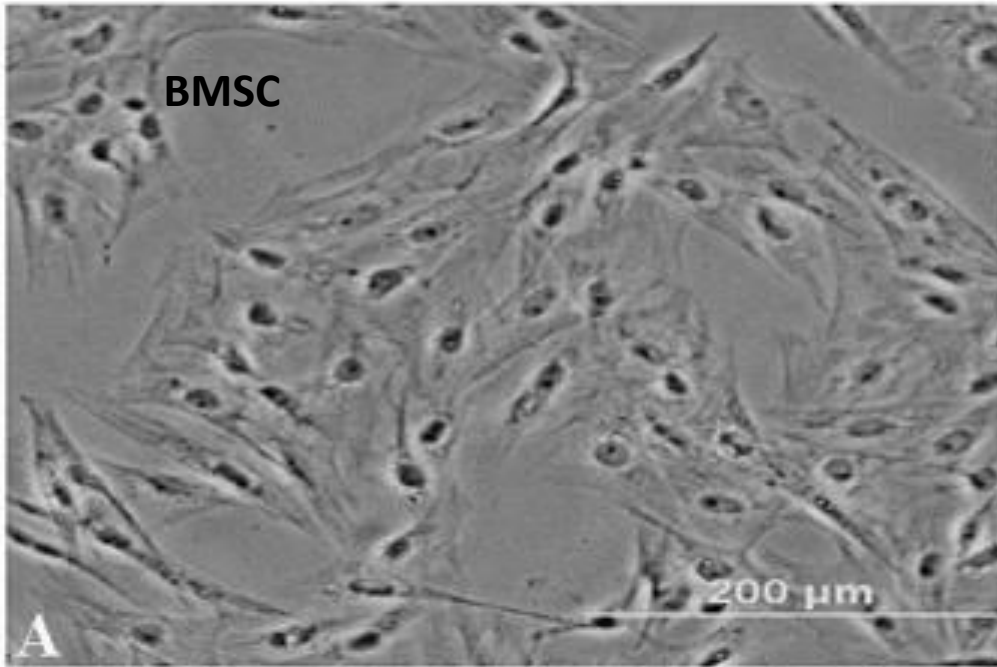
FITC. Cells with green staining cytoplasm were positive. The nuclei were stained with propidium iodide; these are illustrated in *yellow* or *orange*. (scale bar = 200  $\mu\text{m}$ , all).

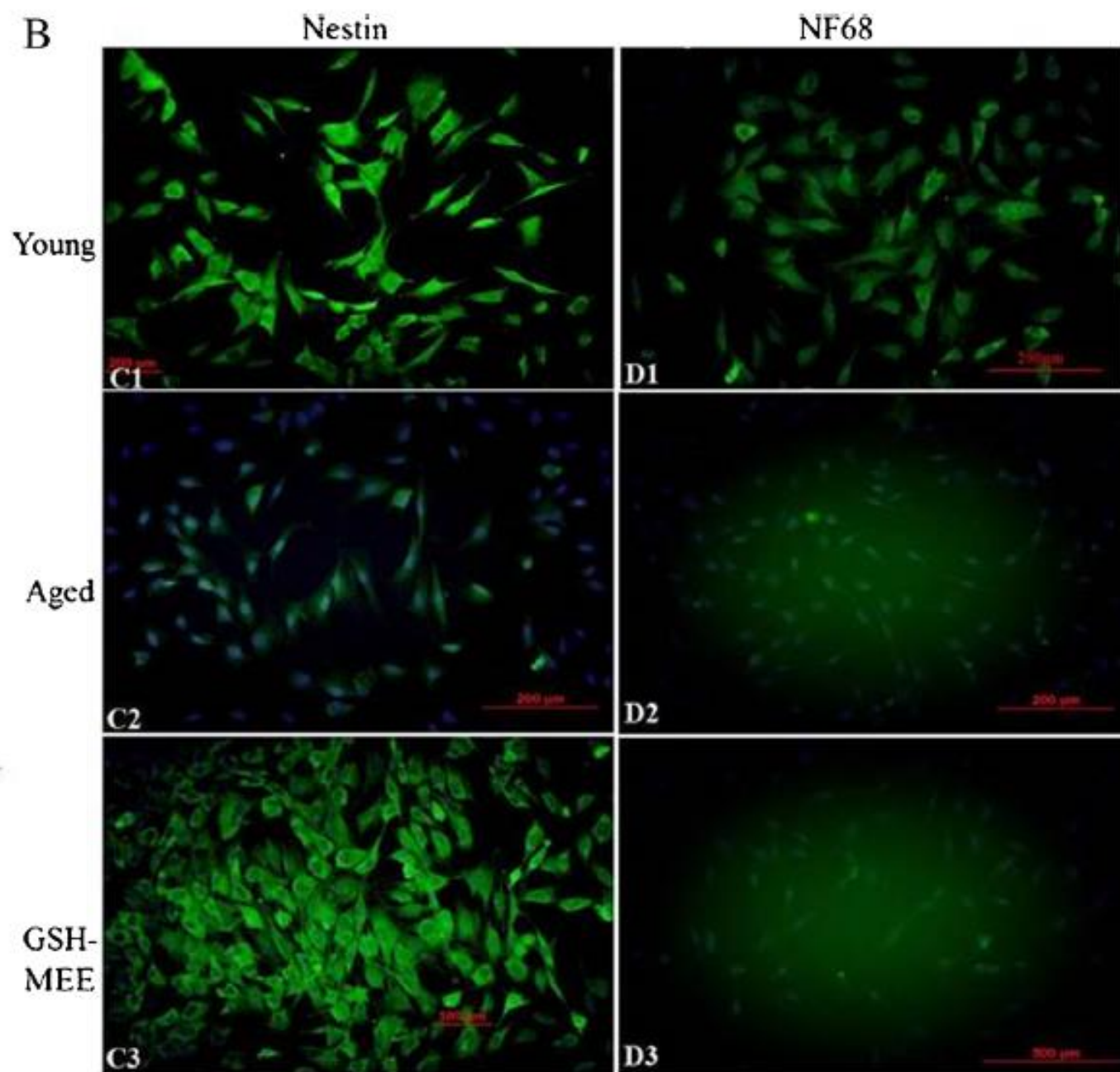
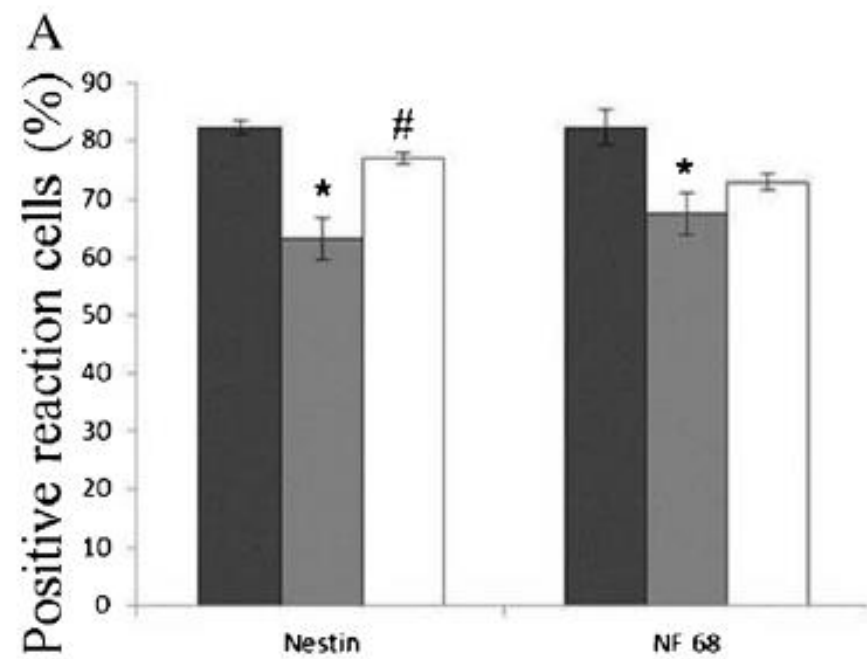




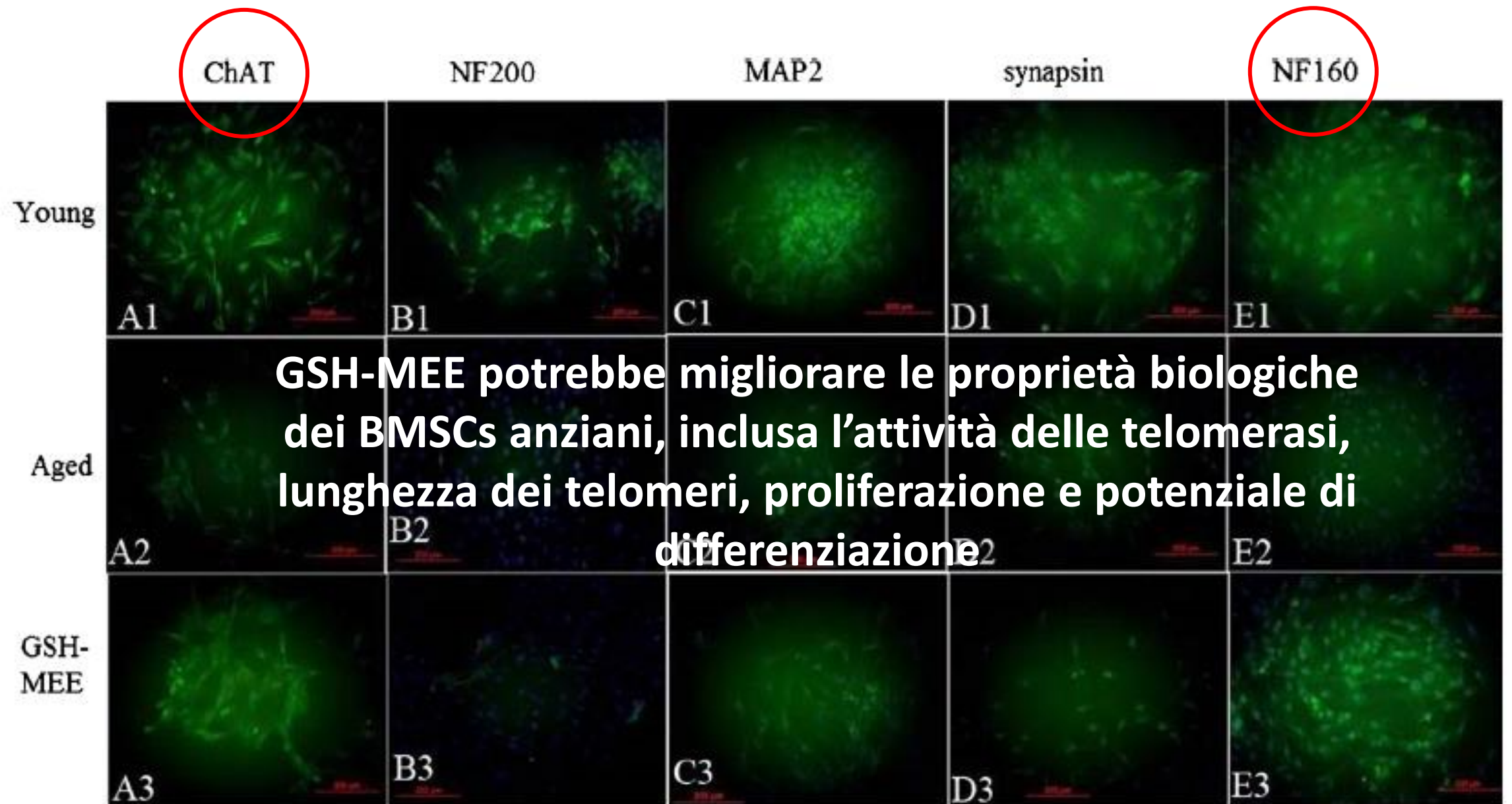










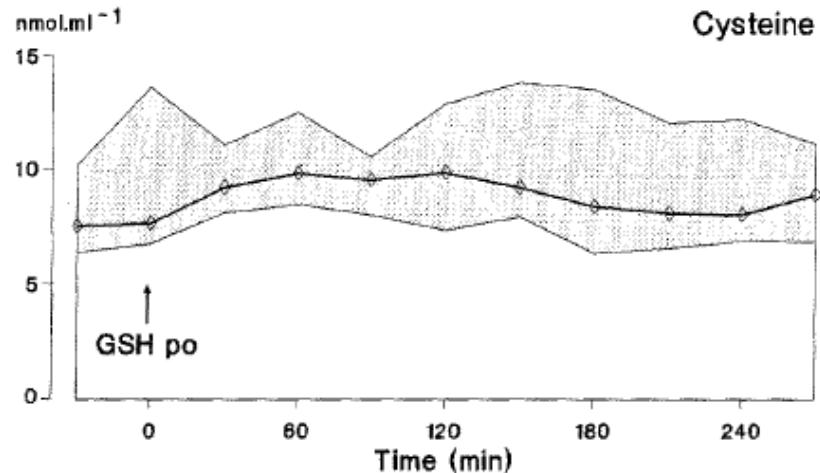
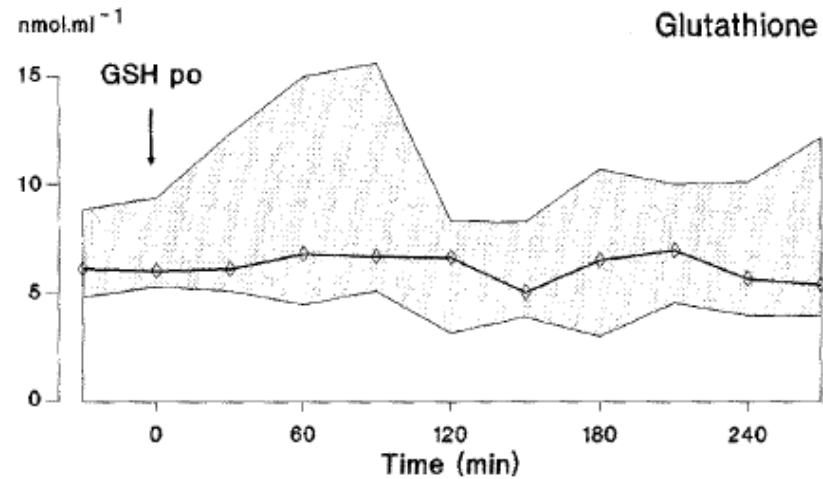


# The systemic availability of oral glutathione

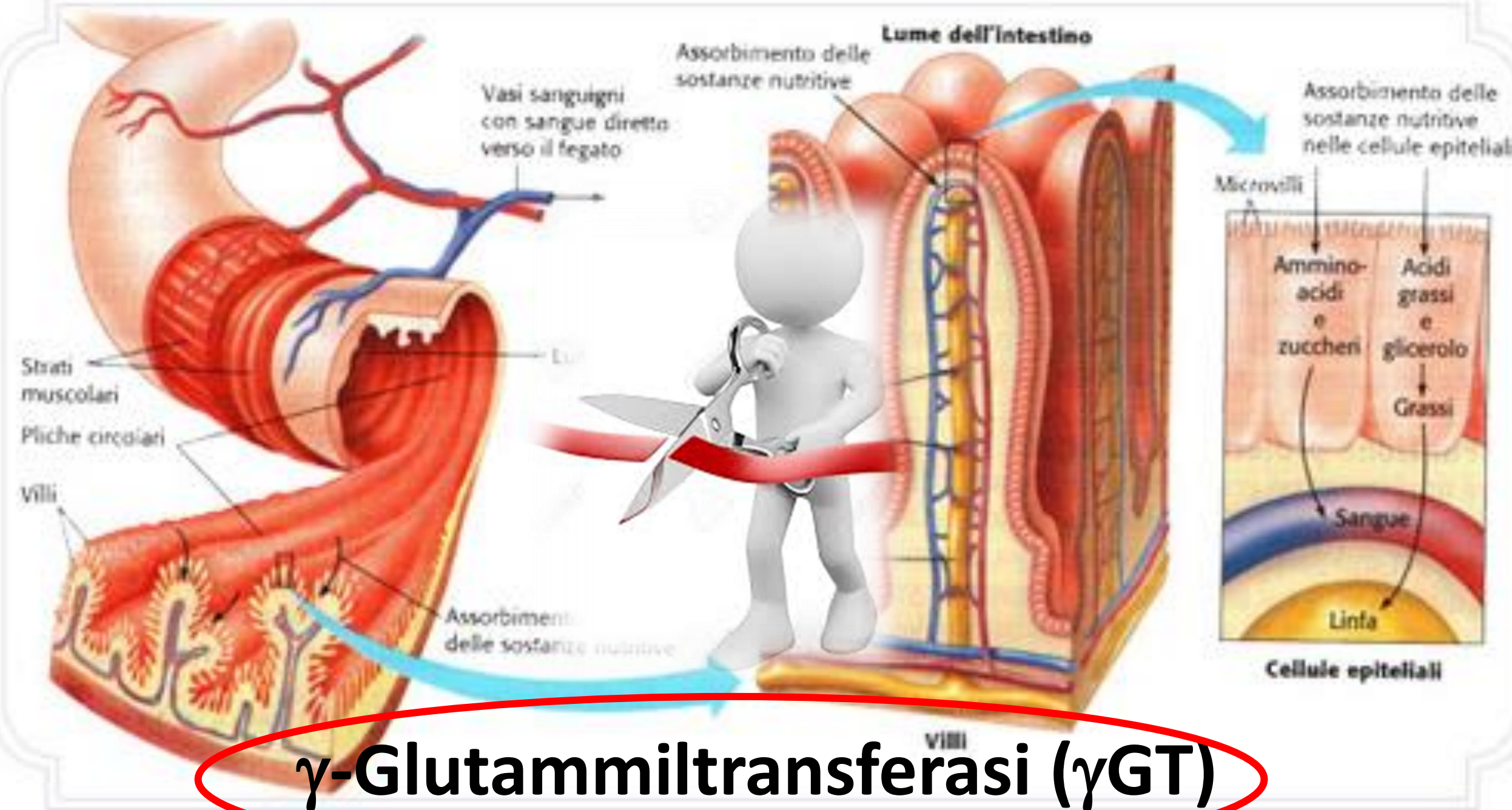
A. Witschi, S. Reddy, B. Stofer, and B. H. Lauterburg

European Journal of **Clinical  
Pharmacology**

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Plasma concentrations of unbound glutathione (top) and unbound cysteine (bottom) in seven healthy volunteers after the oral administration of 0.15 mmol/kg/~ glutathione.



**$\gamma$ -Glutamyltransferasi ( $\gamma$ GT)**

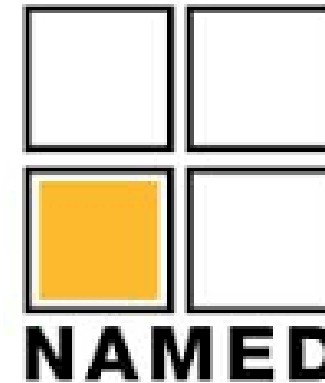




**It is needed to design antioxidant therapeutic strategies for the treatment of various conditions**

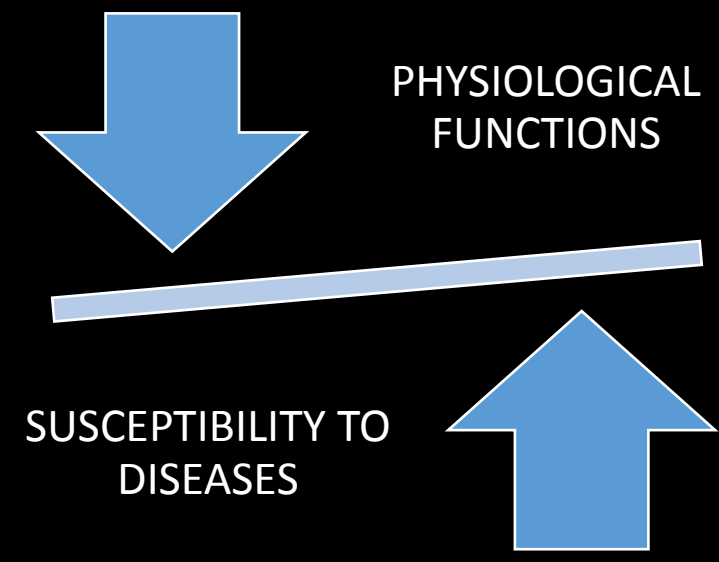
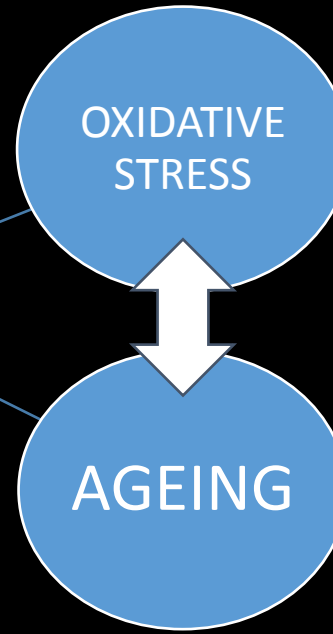
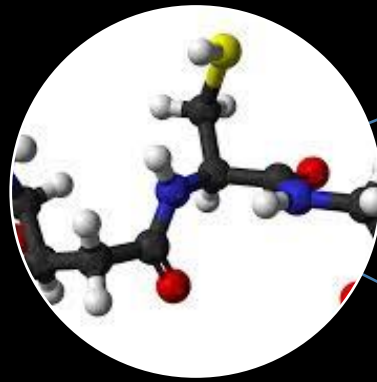


# LABORATORIO DI FARMACOBIOCHIMICA NUTRIZIONE E NUTRACEUTICA



# COLLABORARE E INNOVARE

# GLUTATHIONE



**Aim:** to evaluate improvement of GSH systemic availability testing an **orobuccal fast-slow** release formulation tablet

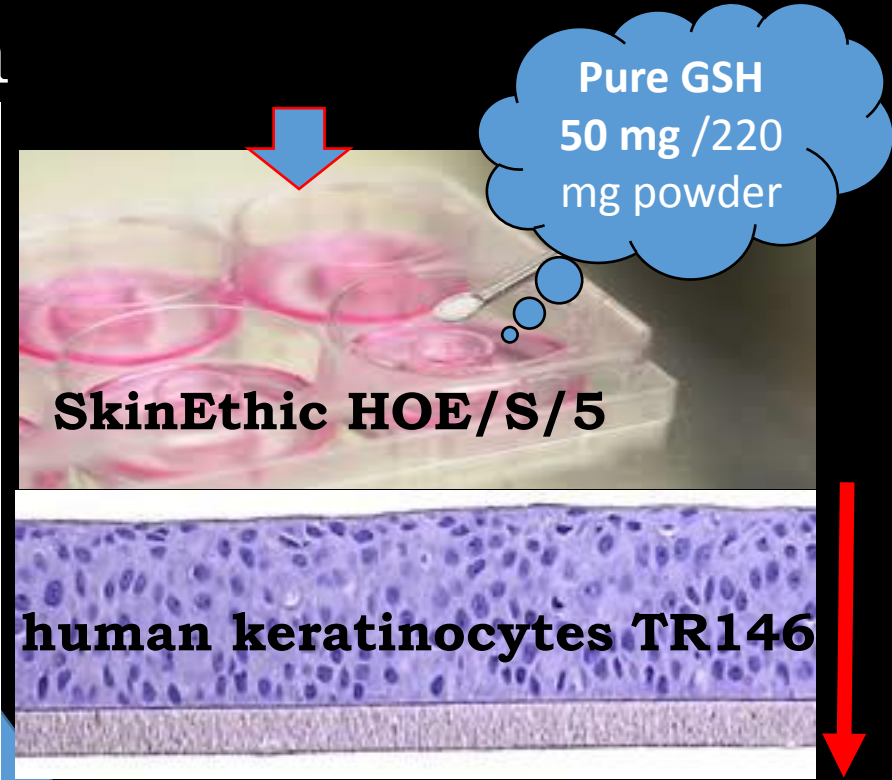
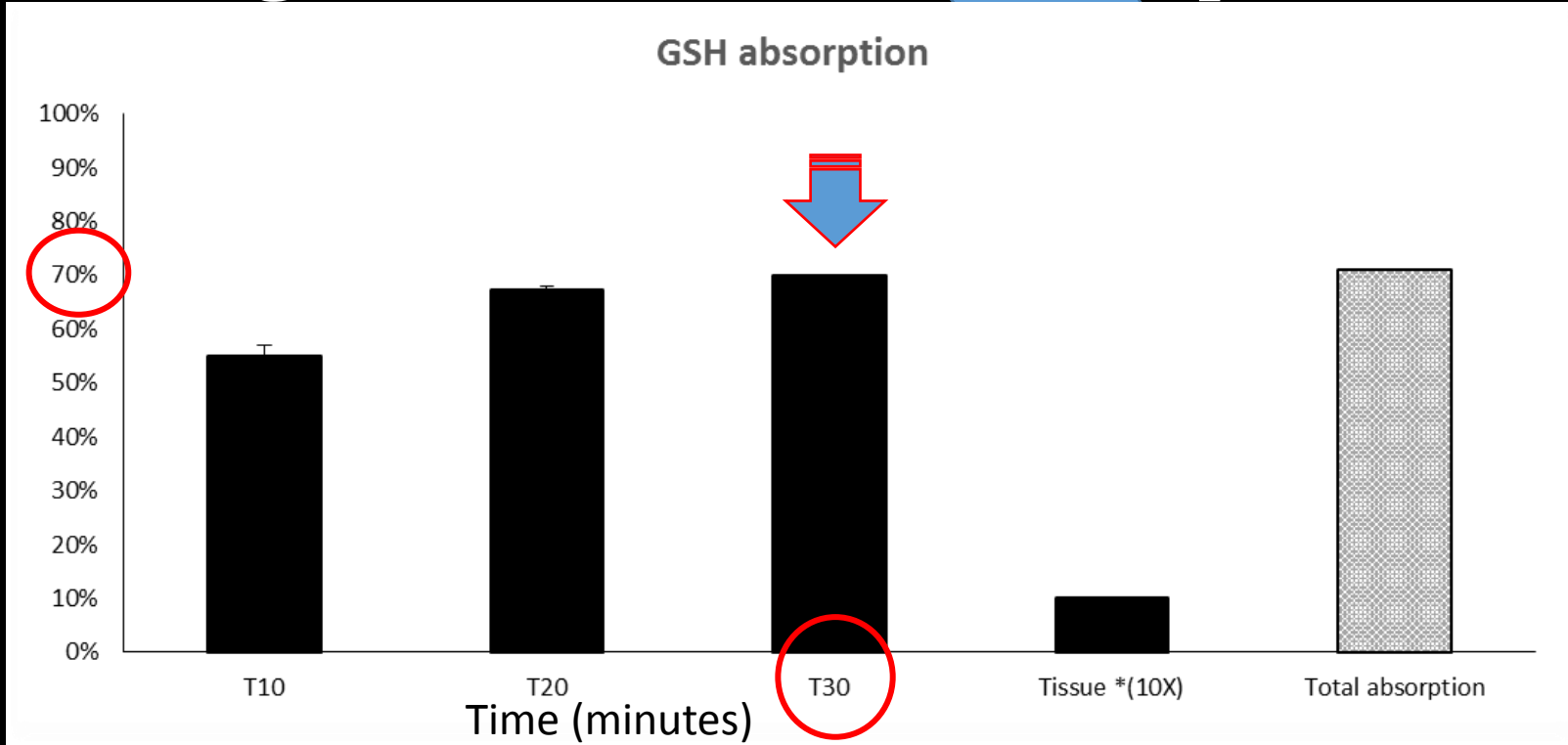
dissolving and releasing immediately GSH upon contact with the oral mucosa bypassing the intestinal degradation



differentiated release of selected ingredients



# *In vitro*: orobuccal fast-slow GSH release formulation through reconstructed oral epithelium



Glutathione dose by HPLC

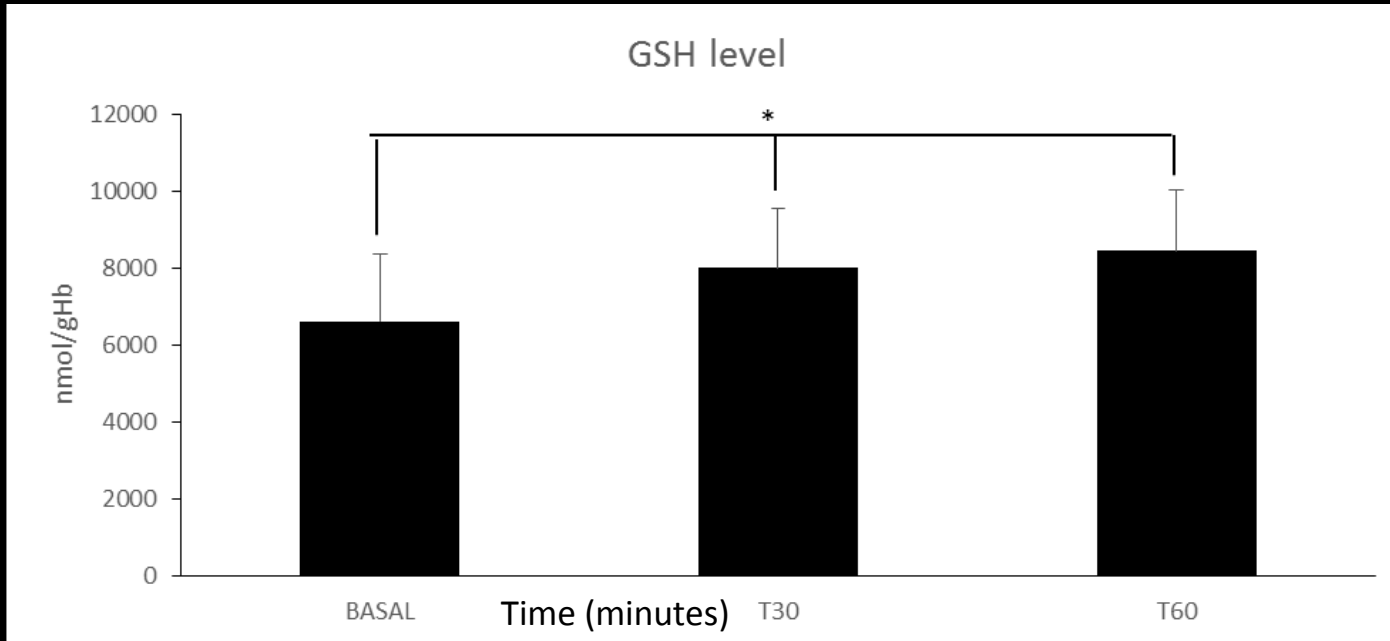
## MTT toxicity test

in vitro data obtained from the MTT irritation test performed on the HOE tissue for the evaluation of tissue viability

	OD 540	TISSUE VIABILITY	CLASSIFICATION
UNTREATED TISSUE	1.486	100%	
TREATED TISSUE	1.476	99.33%	NI

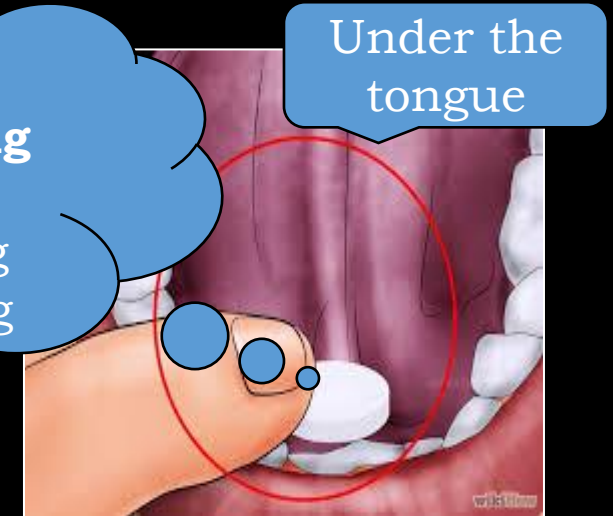
to carry the glutathione for  
dependent absorption through  
the oral mucosa

# **In vivo:** GSH systemic bioavailability using an optimized orobuccal fast-slow release formulation tablet



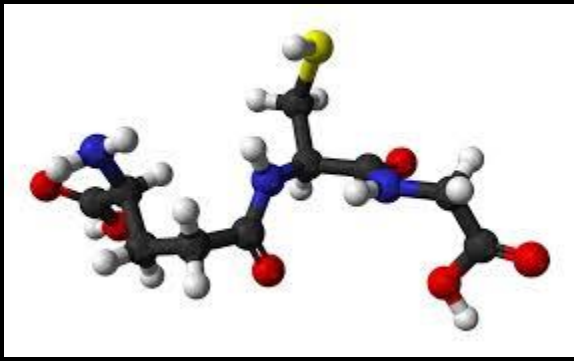
15 healthy volunteers (f, m), w  $60 \pm 5$  Kg, 20-40 year-old

1 tablet:  
**Pure GSH 250 mg**  
L-cystine 50 mg  
Vitamin C 40 mg  
Selenium 55 mcg



alkylating agent N-ethylmaleimide (NEM) [Giustarini et al. *Nature Protocols* 2013; 8(9): 1660–1669]

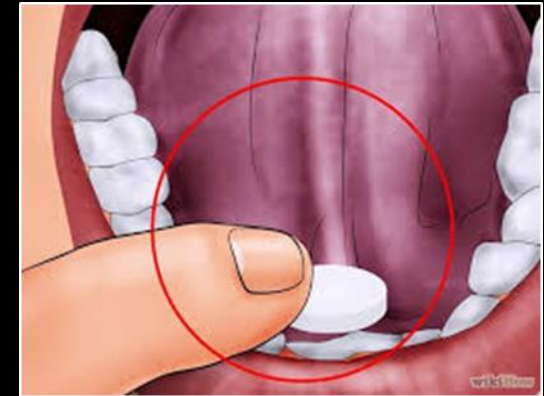
fast absorption rate of GSH through the *in vivo* oral mucosa



✓the intake of GSH, formulated through optimized orobuccal fast-slow release tablets, gave positive results in raising GSH blood concentration



✓probably going to strengthen all *in vivo* by-products and processes that involve this important tripeptide



Orobuccal fast-slow GSH release tablet-**Glutaredox**<sup>®</sup>- is a new, innovative, efficient and functional dosage form



Hindawi Publishing Corporation  
Oxidative Medicine and Cellular Longevity  
Article ID 594191

*Research Article*

## **Bioavailability Study of an Innovative Orobuccal Formulation of Glutathione**

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Angela Michelotti,<sup>2</sup> Mariaelena Carrabetta,<sup>1</sup> Antonio Seneci,<sup>1</sup> Manuela Verri,<sup>3</sup>  
Maurizia Dossena,<sup>3</sup> and Fulvio Marzatico<sup>1</sup>





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Laura Cattaneo  
Alice Ascani  
Alberto Zurma

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*Memory of  
Professor Fulvio Marzatico*





Thank you for attention

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