

## **Beneficial Effects of Bioactive Compounds in Humans**



# **VALUTAZIONE DEL POTENZIALE EFFETTO VASCULOPROTETTIVO DEL PEPTIDE NSLTP2 DEL FRUMENTO**

**Silvana Hrelia**

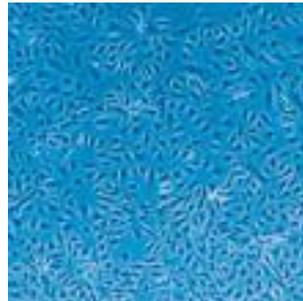
*Alma Mater Studiorum – University of Bologna*

# ENDOTHELIAL DYSFUNCTION

## Healthy Endothelium

- Vasodilatory ( $\uparrow$  NO, PGI<sub>2</sub>)
- $\downarrow$  Oxidative stress, low uric acid
- Anti-coagulant ( $\downarrow$  PAI-1, vWF, P-selectin)
- Anti-inflammatory ( $\downarrow$  sICAM, sVCAM, E-selectin, CRP, TNF- $\alpha$ , IL-6, MCP-1)
- $\uparrow$  Repair (EPCs),  $\downarrow$  Damage (CECs, MPs)

Sena CM, *BBA* 2013; 1832: 2216–2231



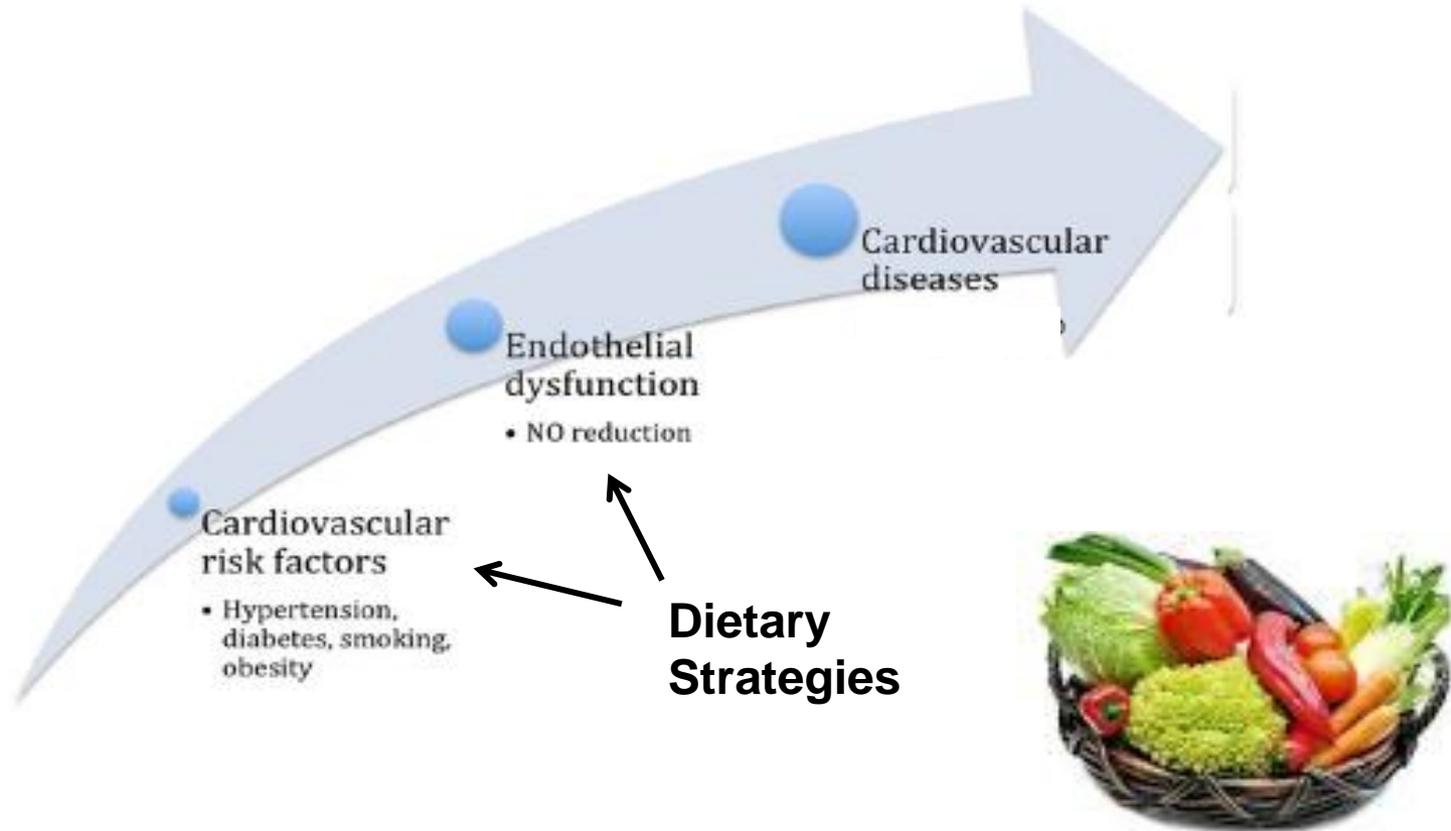
## Dysfunctional Endothelium

- Impaired vasodilation ( $\downarrow$  NO, PGI<sub>2</sub>)
- $\uparrow$  Oxidative stress, uric acid
- Pro-coagulant ( $\uparrow$  PAI-1, vWF, P-selectin)
- Pro-inflammatory ( $\uparrow$  sICAM, sVCAM, E-selectin, CRP, TNF- $\alpha$ , IL-6, MCP-1)
- $\downarrow$  Repair (EPCs),  $\uparrow$  Damage (CECs, MPs)

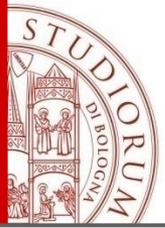
Sena CM, *BBA* 2013; 1832: 2216–2231



# ENDOTHELIAL DYSFUNCTION



Strisciuglio T, *Curr Atheroscler Rep* 2014



# Preventive dietary strategies contribute to the maintenance of vascular function

Dietary consumption of vegetables, fruit, cereals and legumes has been associated with a reduction of risk of development of chronic/degenerative diseases including CVD



**Mediterranean Diet**

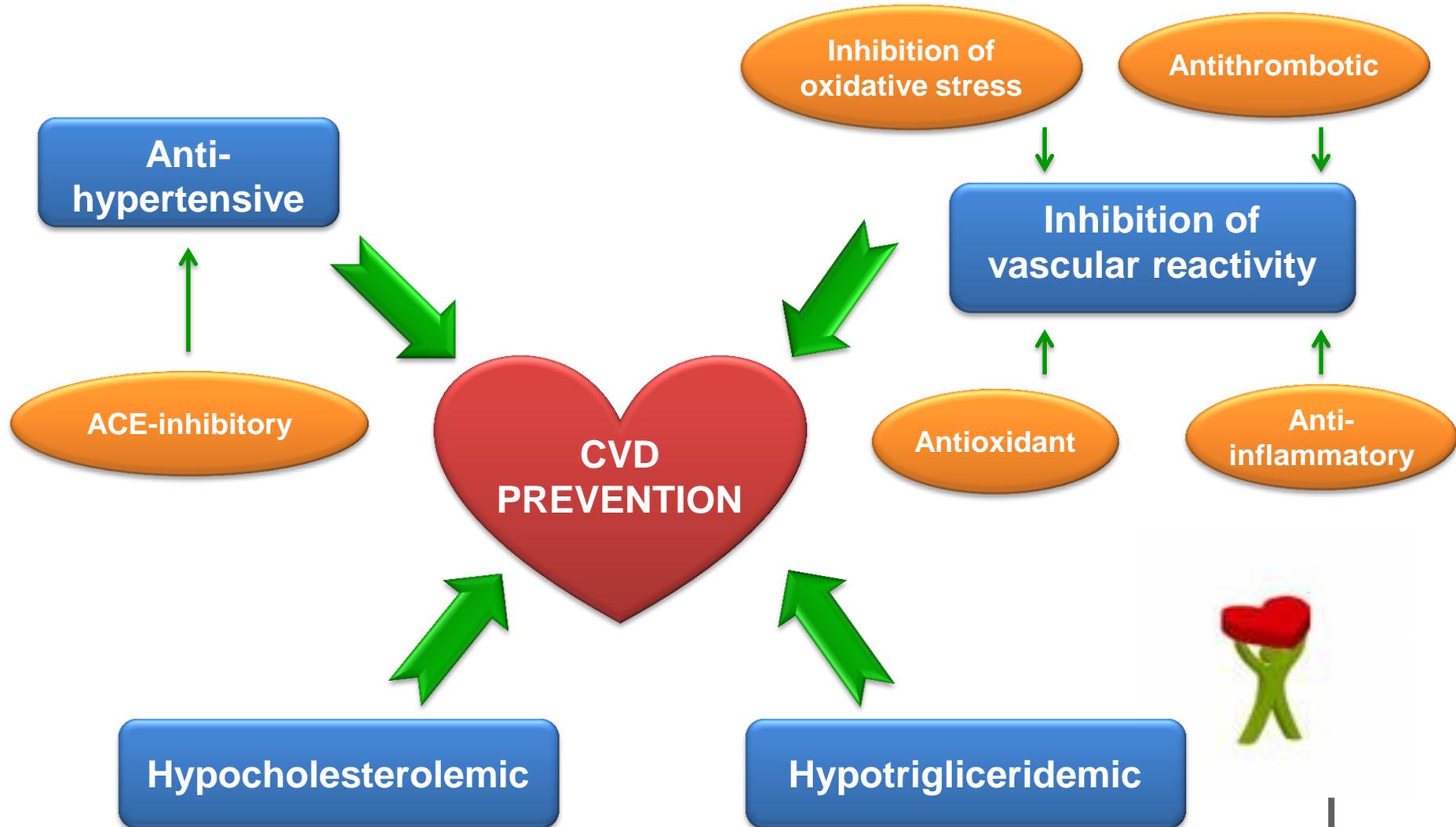


**BIOACTIVE PEPTIDES**



**POLYPHENOLS**

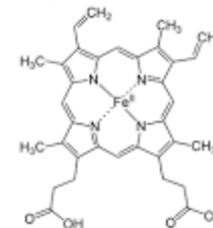
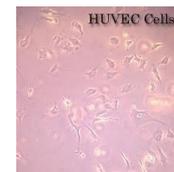
# BIOACTIVE PEPTIDES



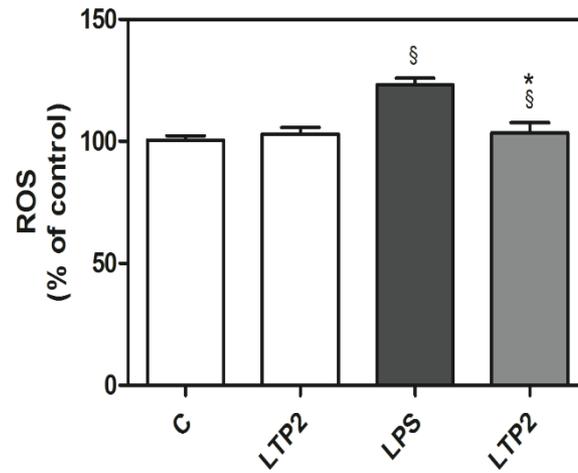
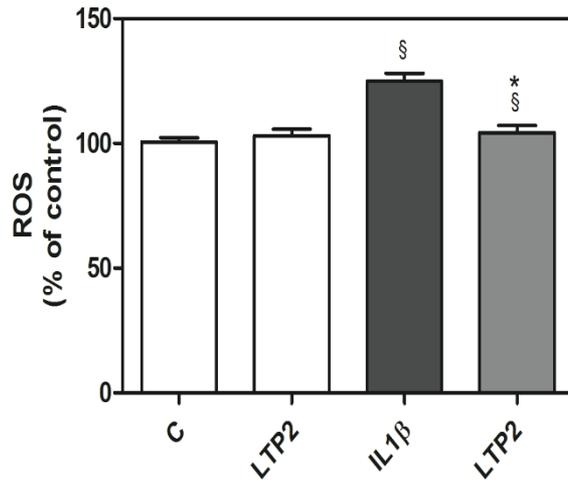
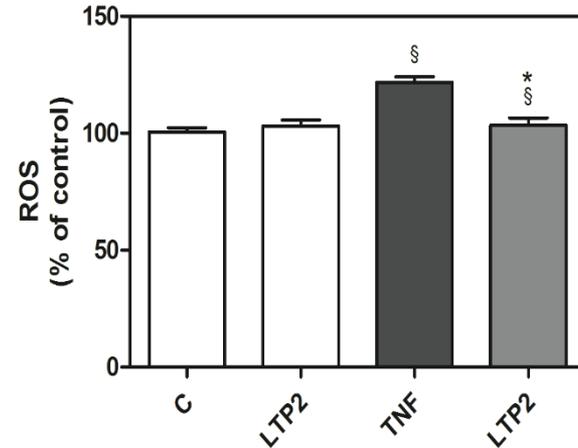
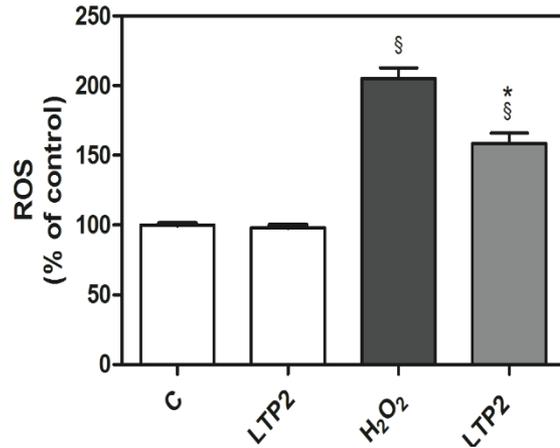
# AIM OF THE STUDY

## ***Investigation of biological activities of selected bioactive peptide (nsLTP2), isolated in wheat, which may contribute to vascular endothelium health protection***

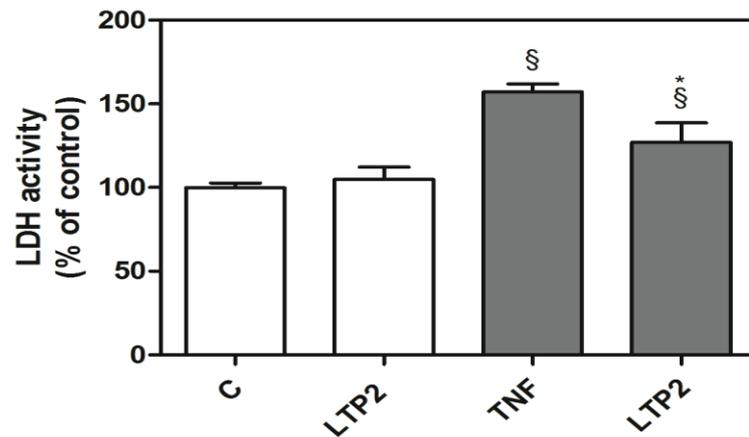
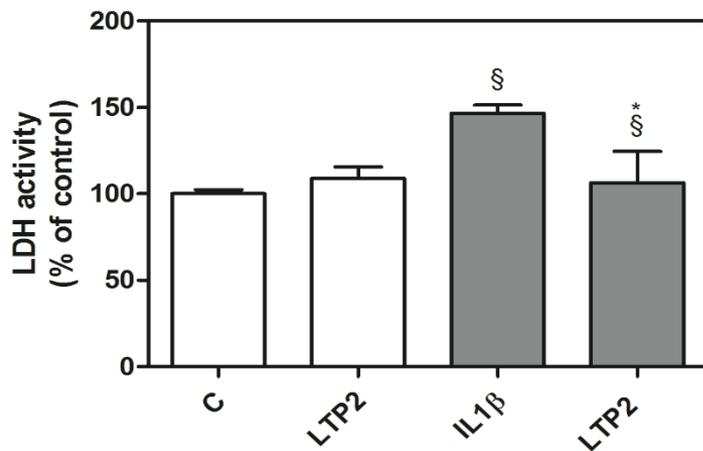
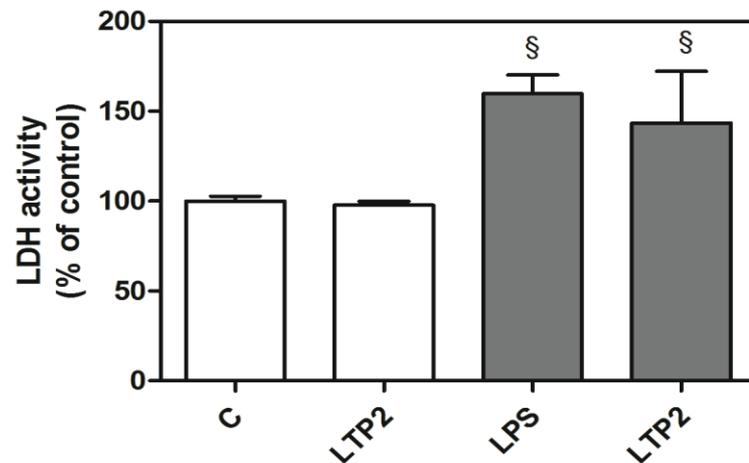
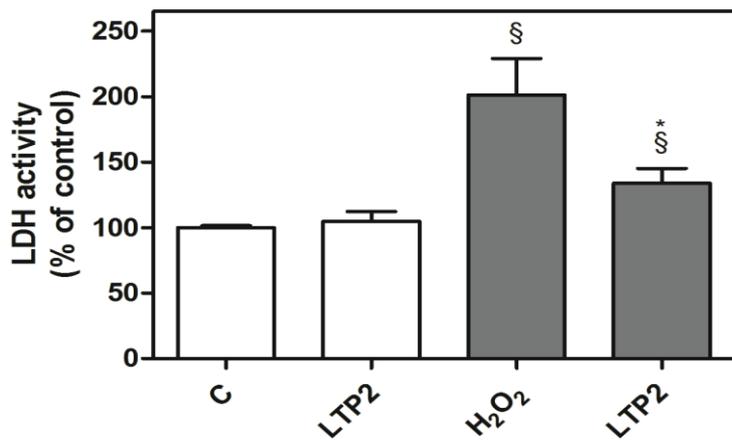
- HUVECs treated with oxidative ( $H_2O_2$ ) and inflammatory (TNF- $\alpha$ , IL-1 $\beta$ , LPS) agents, as a model system
- Evaluation of nsLTP2 ability to influence intracellular ROS level and to prevent cell damage
- Analysis of nsLTP2 capacity to modulate specific marker of inflammatory processes, such as Adhesion Molecules (ICAM-1, VCAM-1, E-selectin)
- Analysis of nsLTP2 ability to modulate cellular regulator of inflammation (HO-1)



# Antioxidant activity of nsLTP2



# Cytoprotective activity of nsLTP2

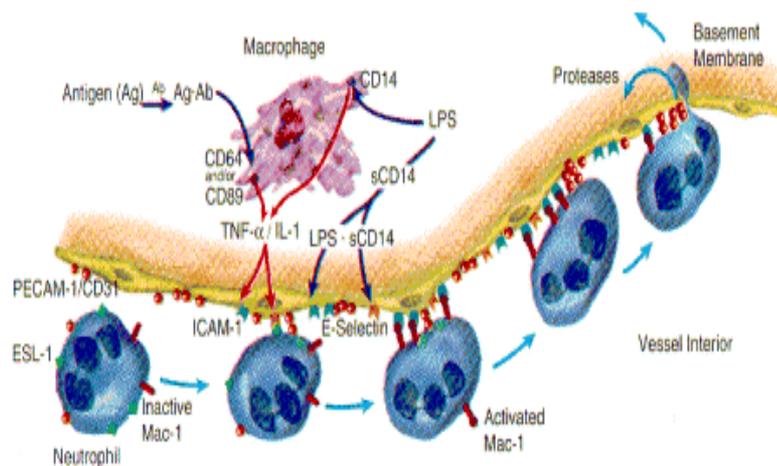


# Anti-inflammatory effects of nsLTP2

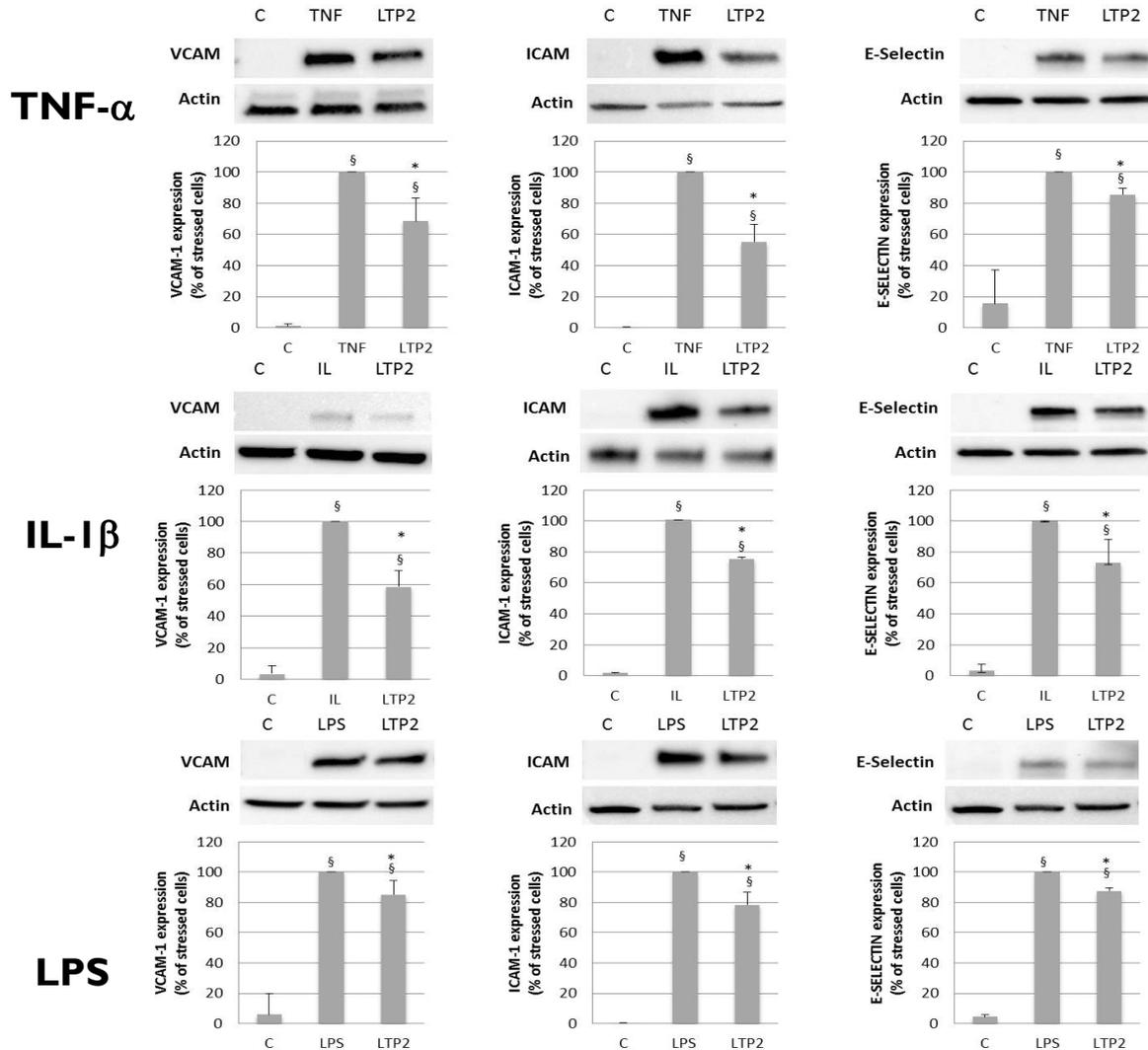
Inflammatory activation of the endothelium triggers the production or up-regulation of several **cell adhesion molecules**.

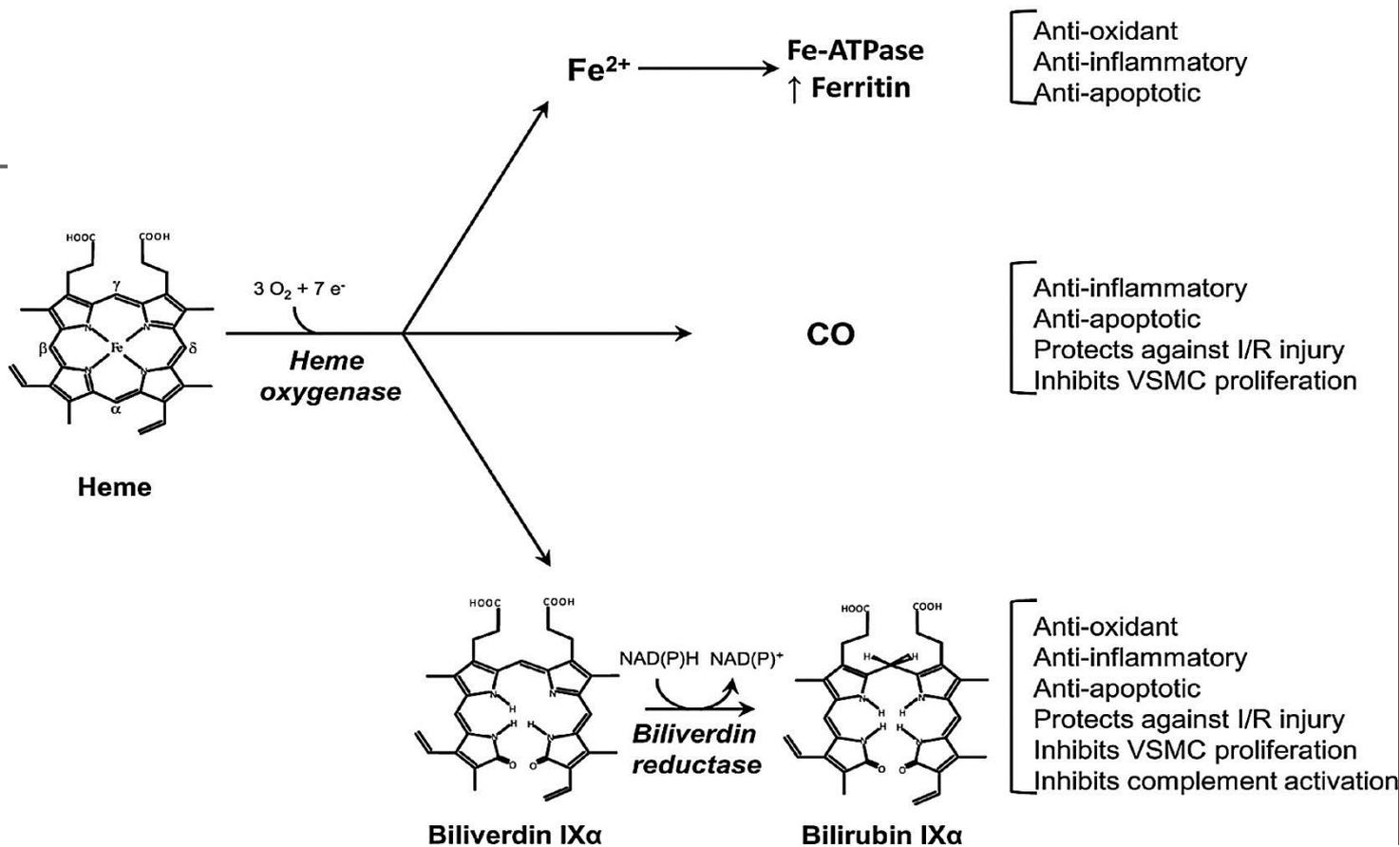
Since stimulated endothelial cells have an indispensable role in leukocyte recruitment producing inflammatory mediators and expressing adhesion molecules involved in cardiovascular disease (Makó *et al.*, Cytometry A. 2010), the effect of nsLTP2 has been assessed under resting and pro-inflammatory conditions on the expression of:

- **Vascular cell adhesion molecule-1 (VCAM-1)**
- **Intracellular adhesion molecule-1 (ICAM-1)**
- **E-selectin**

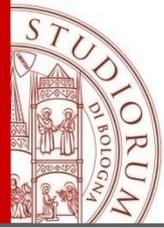


# Anti-inflammatory activity of nsLTP2: effects on adhesion molecules

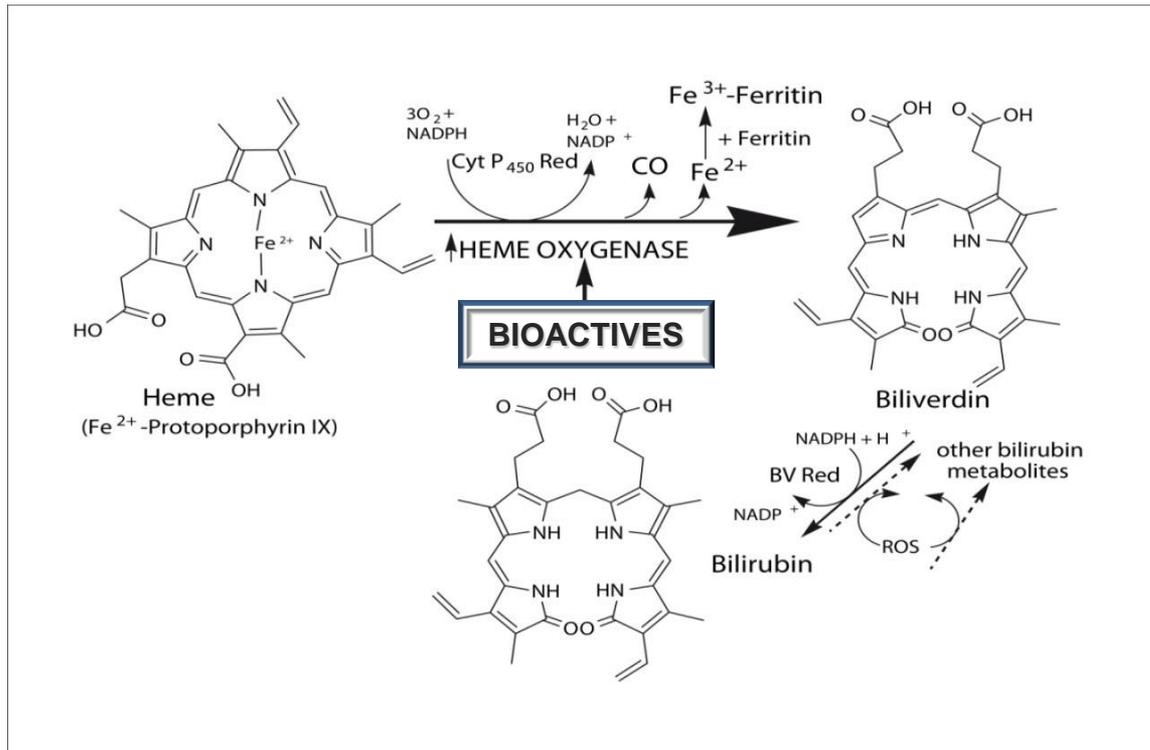




**Heme oxygenase-mediated degradation of heme.** In endothelial cells free heme is cytotoxic and potentiates toxic effects of  $\text{H}_2\text{O}_2$ . The interaction of HO with heme generates equimolar amounts of carbon monoxide (CO), biliverdin, and free iron ( $\text{Fe}^{2+}$ ). Biliverdin reductase subsequently catalyzes the conversion of biliverdin to bilirubin. The products of heme degradation exert a variety of effects on endothelial and vascular smooth muscle cells, which are protective against atherosclerosis.

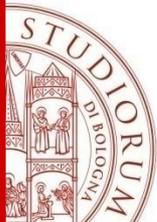


# Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1

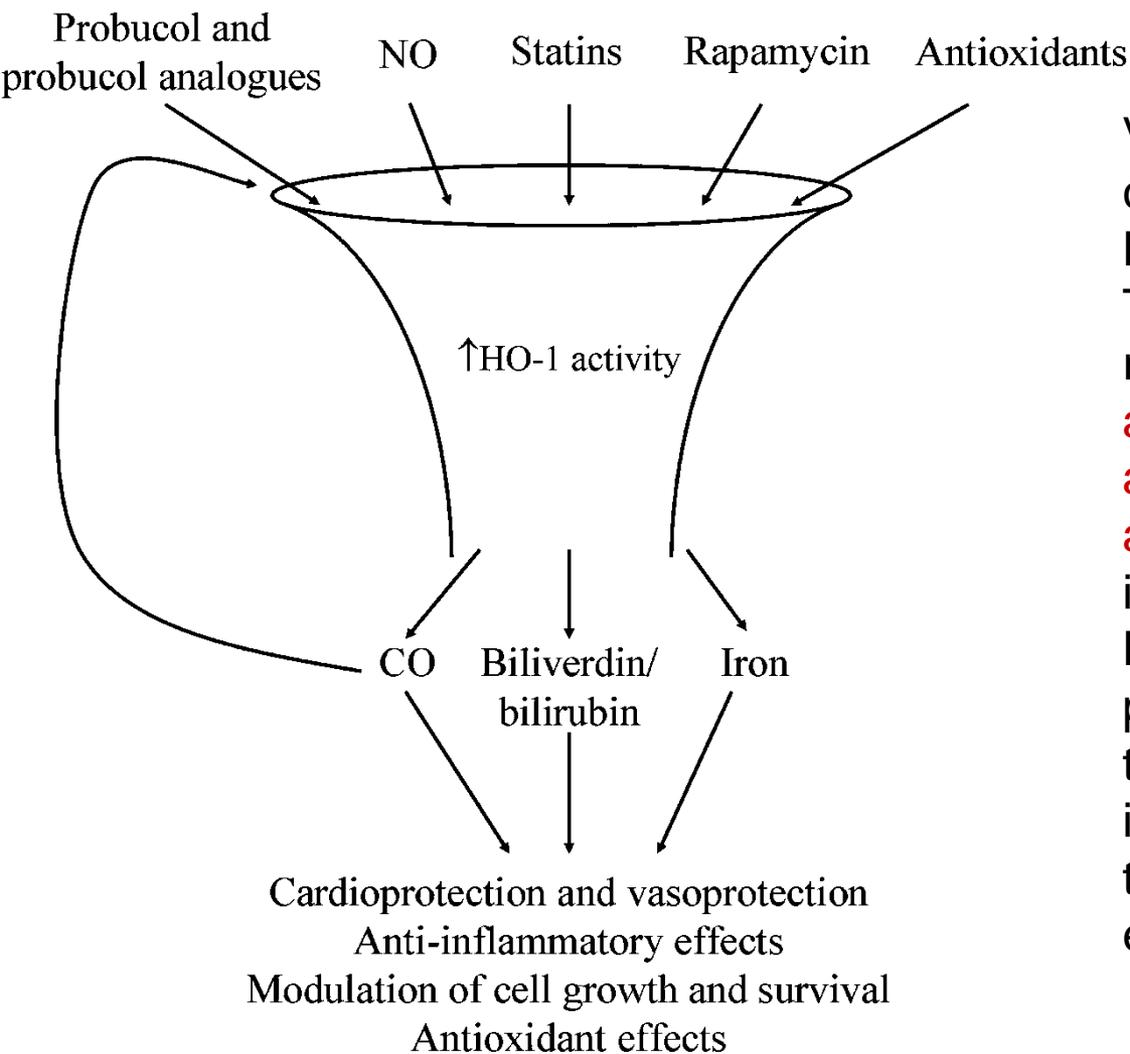


HO-1 exerts pleiotropic effects on the vascular endothelium, including antiapoptotic, anti-inflammatory and antioxidant actions [Calay and Mason, Antioxidants & Redox Signaling, 2014].

Some common **dietary phytochemicals** are known HO-1 inducers [Haines et al., Acta Physiol, 2012], as well as drugs currently used in therapy [Calay and Mason, Antioxidants & Redox Signaling 2014].

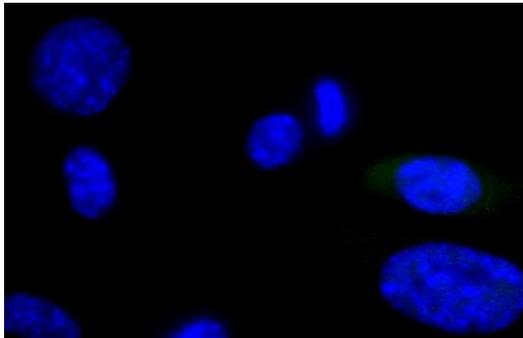
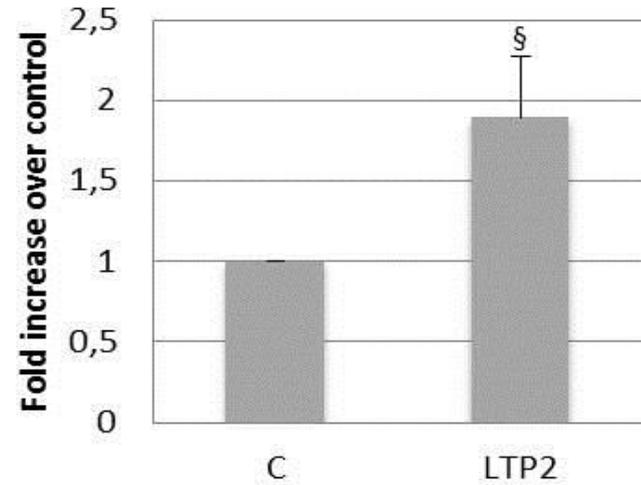
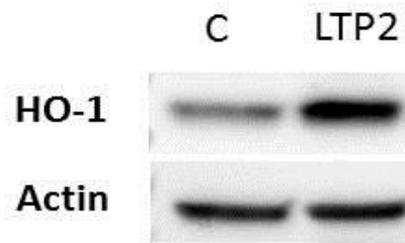


# Haeme oxygenase-1 as a therapeutic funnel

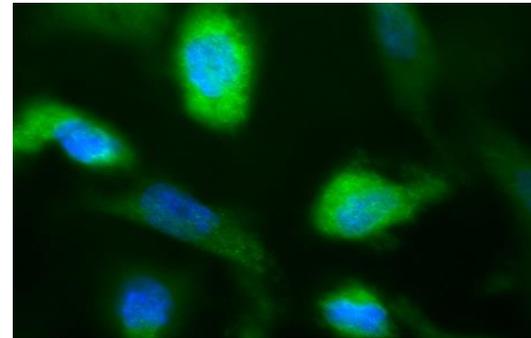


Various classes of therapeutic compounds induce HO-1 expression and activity. The products of this enzymatic reaction then mediate the **antiinflammatory, antioxidant, and/or growth-modulating activities** associated with increased HO-1 expression. Some of the products, such as CO, themselves increase HO-1 expression, thereby amplifying the biologic effect.

# Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1



Control

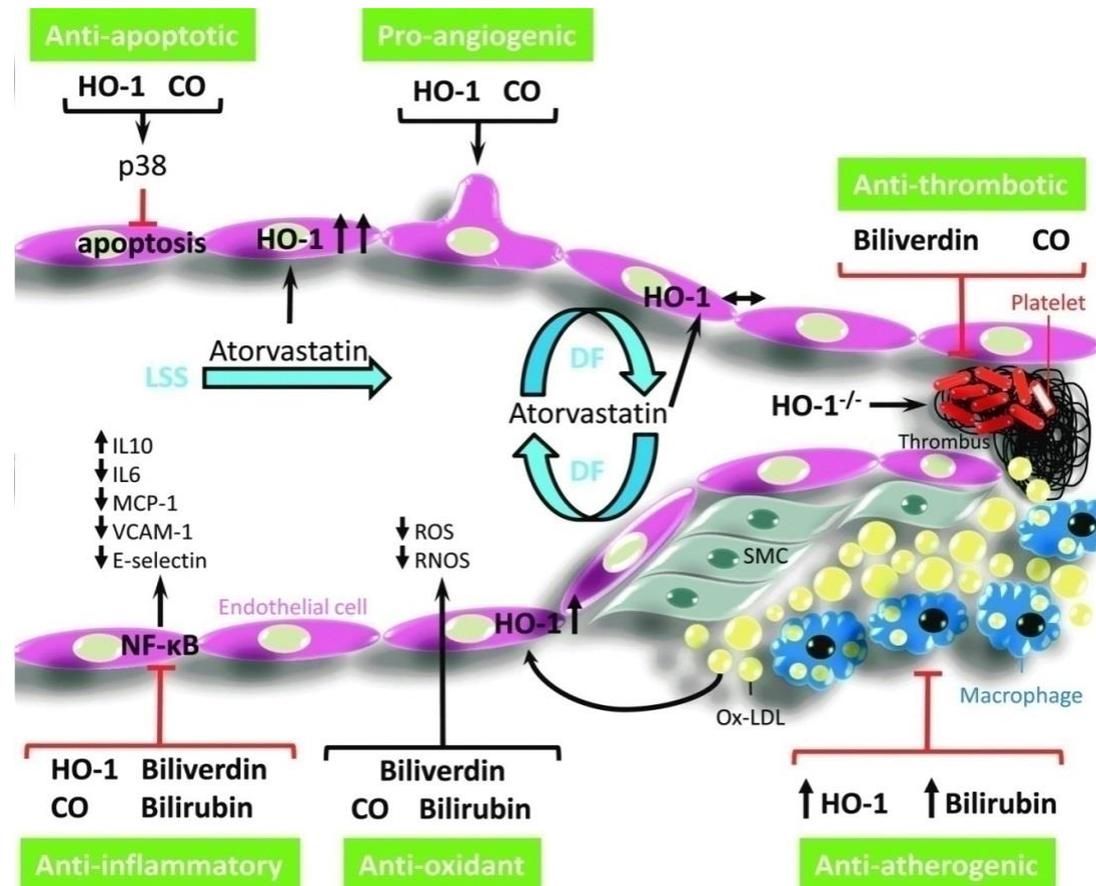


nsLTP2

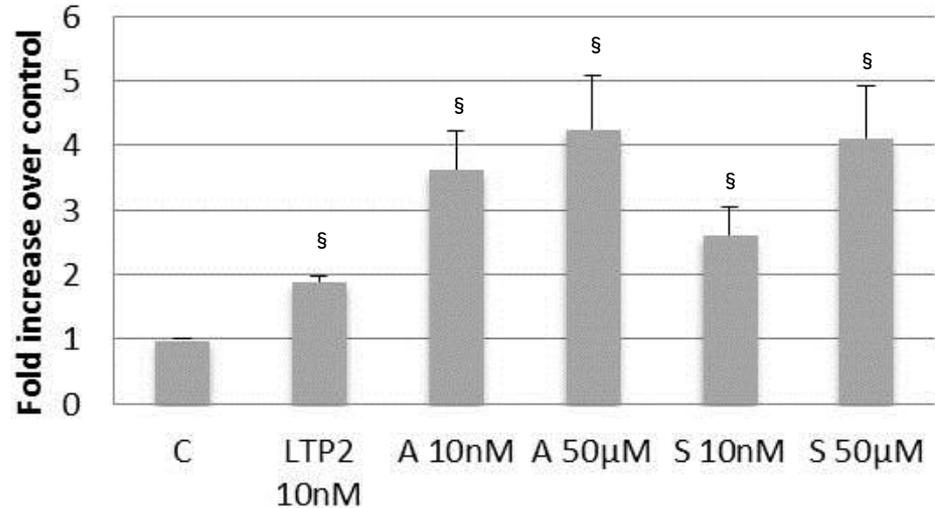
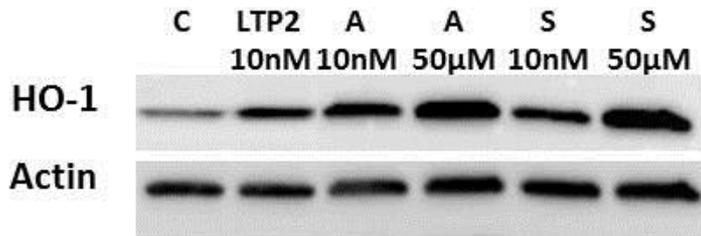
# Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1

It has been suggested that statins, in addition to their well-known biological effect of HMG-CoA reductase inhibitors, are able to increase HO-1 expression.

The statin-mediated induction of HO-1 is supposed to be a significant contributor to the pleiotropic effects of statins on the vascular endothelium, including antiapoptotic, anti-inflammatory and antioxidant actions



# Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1

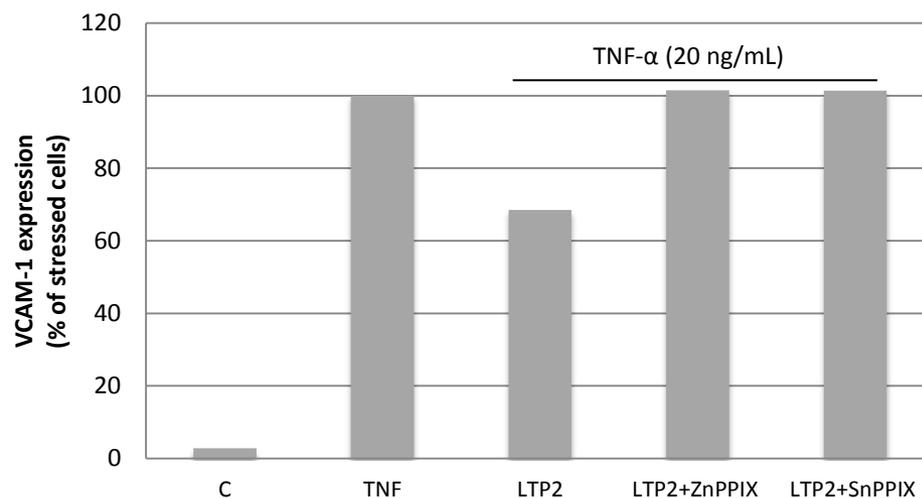
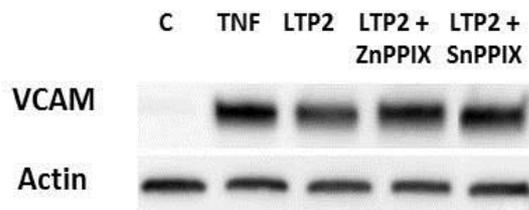


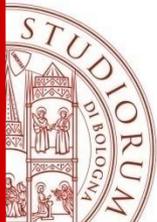
**Effect of nsLTP2 (LTP2), Atorvastatin and Simvastatin on HO-1 protein expression level**  
 HUVECs were treated with 10 nM nsLTP2 (LTP2), 10 nM - 50 μM Atorvastatin (A) or 10 nM - 50 μM Simvastatin (S) for 24 h. Cell lysates were subjected to SDS-PAGE and Western blotting analysis of HO-1 protein expression levels. Data are expressed as fold increase over control and represent means  $\pm$  SD of three independent experiments. <sup>s</sup>  $p < 0.05$ , compared with control HUVECs.

# Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1

In recent studies, **HO-1** has been shown to ameliorate inflammation, in part **through its ability to inhibit expression of endothelial adhesion molecules.**

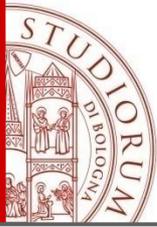
In order to examine whether up-regulation of HO-1 induced by nsLTP2 is correlated to the down-regulation of adhesion molecule expression (i.e. VCAM) induced by TNF- $\alpha$ , experiments were conducted using two HO-1 inhibitors: Zinc Protoporphyrin IX (ZnPPiX) and Tin Protoporphyrin IX (SnPPiX).





# Conclusion

- nsLTP2 exerts **antioxidant and cytoprotective effects** on HUVECs stressed by different oxidative ( $H_2O_2$ ) and inflammatory agents (TNF- $\alpha$ , IL1- $\beta$ , LPS)
- nsLTP2 is able to **increase HO-1 expression**
- nsLTP2 **decreases the expression of VCAM, ICAM and E-Selectin** induced by inflammatory agents (TNF- $\alpha$ , IL1- $\beta$ , LPS)
- the induced **expression of HO-1** may contribute to the underlying mechanism of the anti-inflammatory activity exerted nsLTP2



# POTENTIAL PITFALLS

- The notion of targeting HO-1 to achieve pharmacologic and therapeutic benefit is becoming widely accepted.
- As research continues to uncover positive actions of HO-1 on the maintenance of normal physiology and repair of pathology, increasing numbers of potential applications are likely to be discovered.
- **nsLTP2 as a HO-1 inducer might represent a potential tool to protect the vascular system against various stressors preventing several pathological conditions**



**Thanks for  
your attention**

