

Beneficial Effects of Bioactive Compounds in Humans



VALUTAZIONE DEL POTENZIALE EFFETTO VASCULOPROTETTIVO DEL PEPTIDE NSLTP2 DEL FRUMENTO

Silvana Hrelia

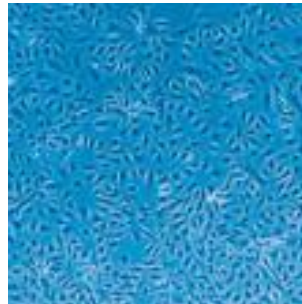
Alma Mater Studiorum – University of Bologna

ENDOTHELIAL DYSFUNCTION

Healthy Endothelium

- Vasodilatory (\uparrow NO, PGI_2)
- \downarrow Oxidative stress, low uric acid
- Anti-coagulant (\downarrow PAI-1, vWF, P-selectin)
- Anti-inflammatory (\downarrow sICAM, sVCAM, E-selectin, CRP, $\text{TNF-}\alpha$, IL-6, MCP-1)
- \uparrow Repair (EPCs), \downarrow Damage (CECs, MPs)

Sena CM, *BBA* 2013; 1832: 2216–2231



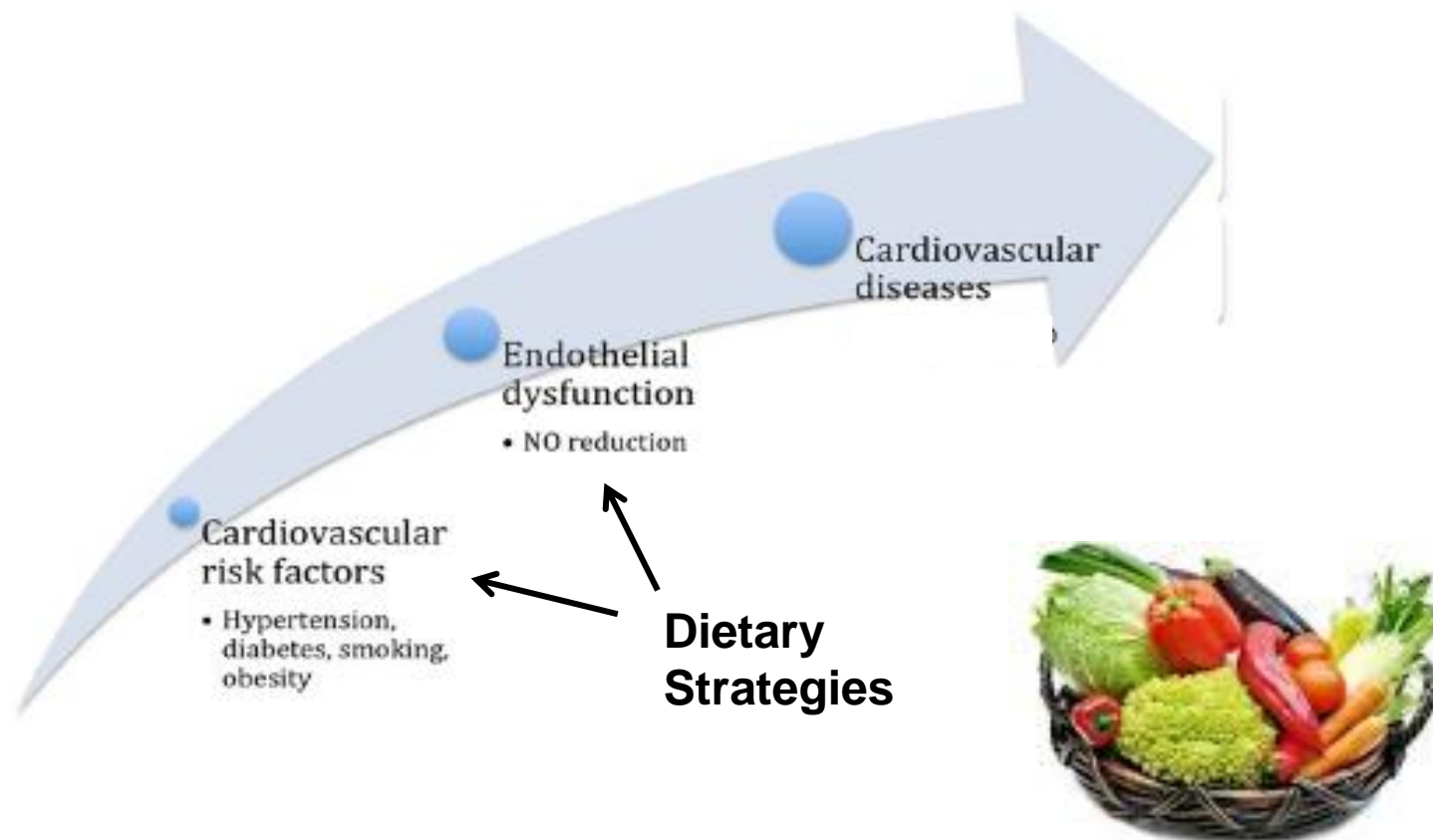
Dysfunctional Endothelium

- Impaired vasodilation (\downarrow NO, PGI_2)
- \uparrow Oxidative stress, uric acid
- Pro-coagulant (\uparrow PAI-1, vWF, P-selectin)
- Pro-inflammatory (\uparrow sICAM, sVCAM, E-selectin, CRP, $\text{TNF-}\alpha$, IL-6, MCP-1)
- \downarrow Repair (EPCs), \uparrow Damage (CECs, MPs)

Sena CM, *BBA* 2013; 1832: 2216–2231



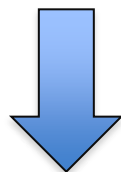
ENDOTHELIAL DYSFUNCTION





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

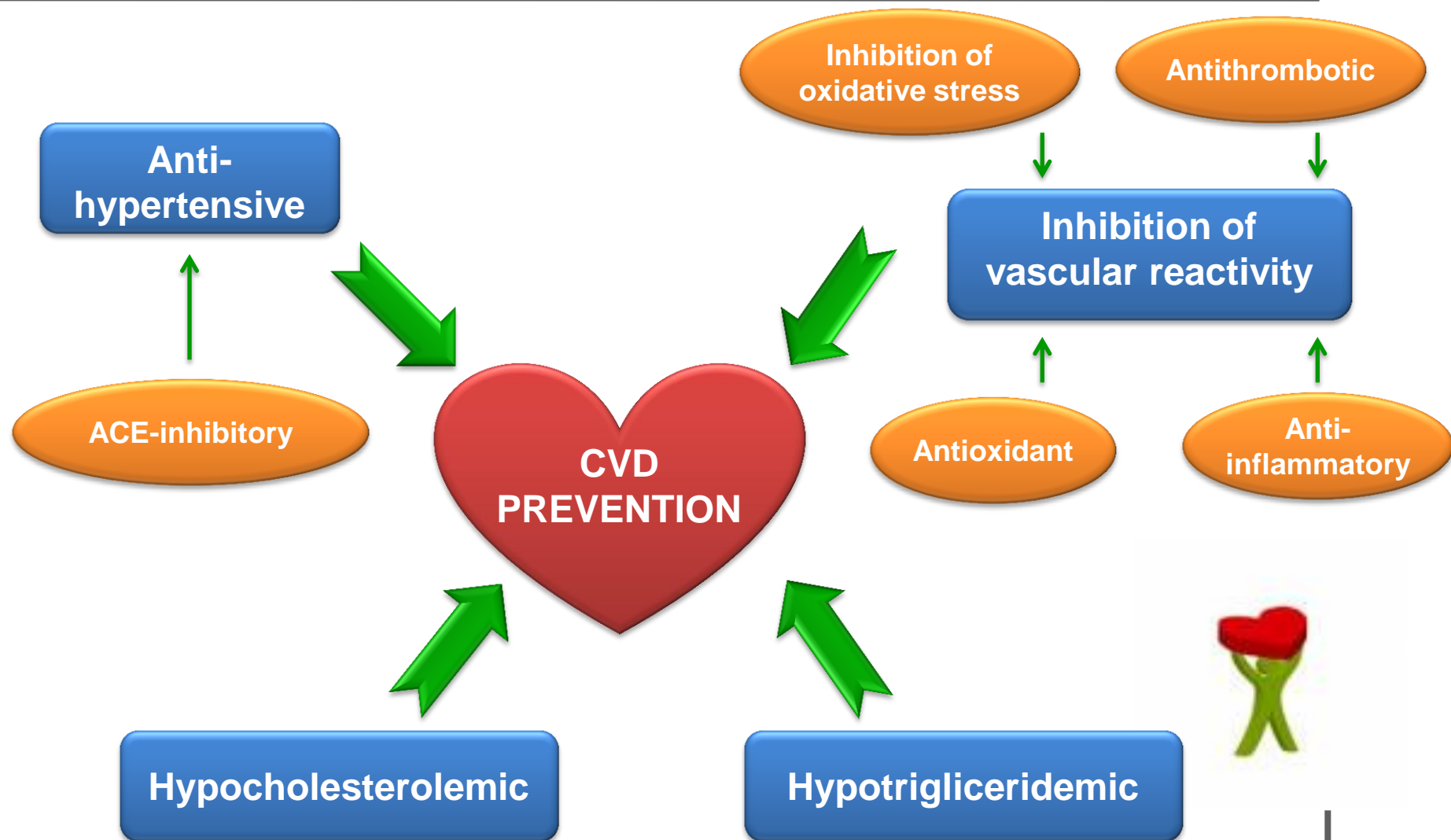


BIOACTIVE PEPTIDES



POLYPHENOLS

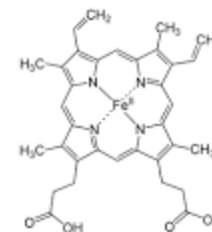
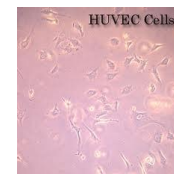
BIOACTIVE PEPTIDES



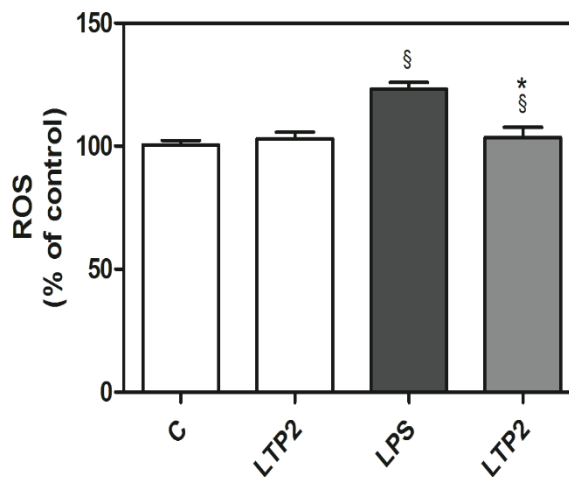
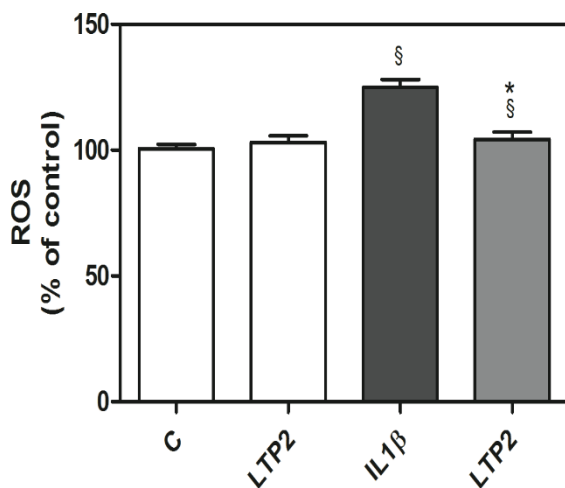
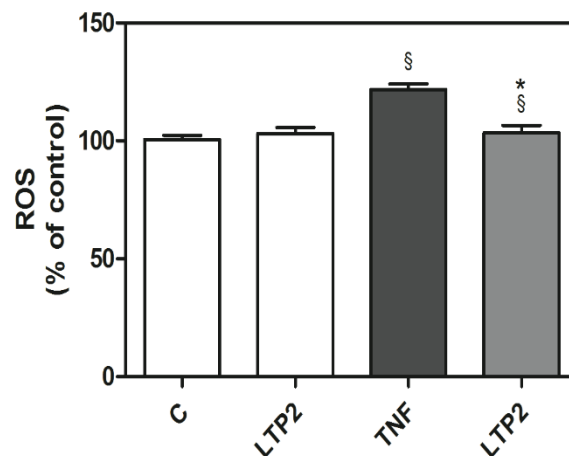
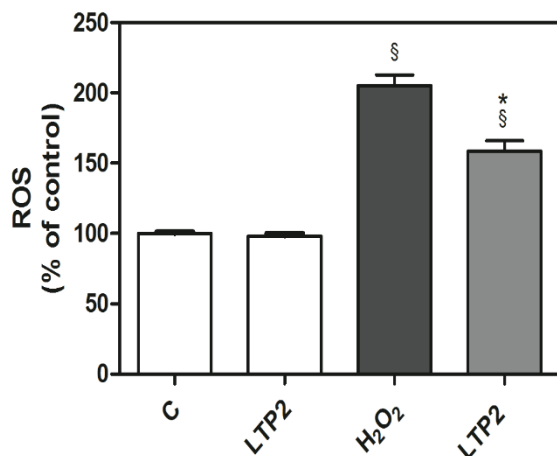
AIM OF THE STUDY

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

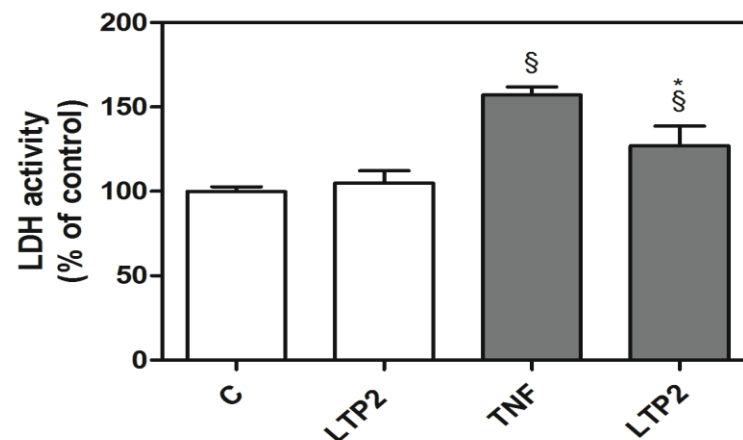
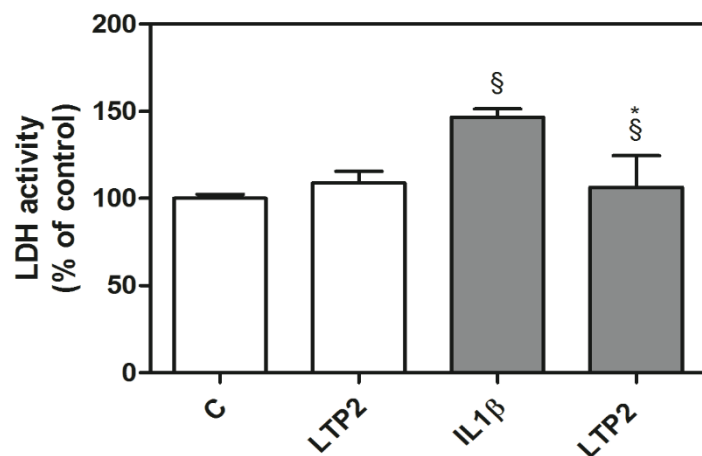
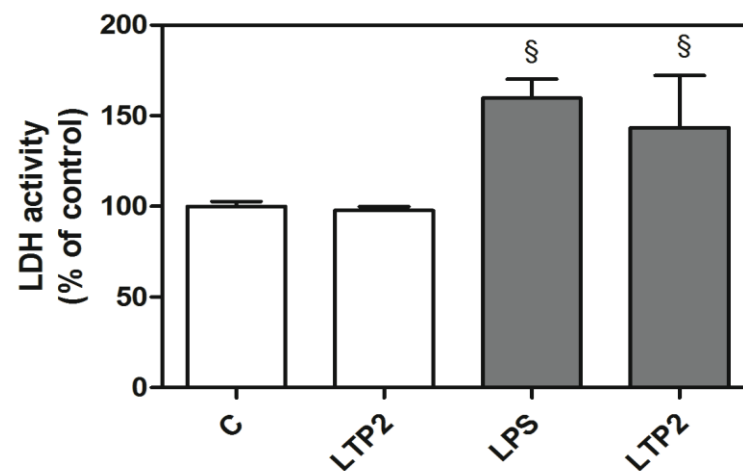
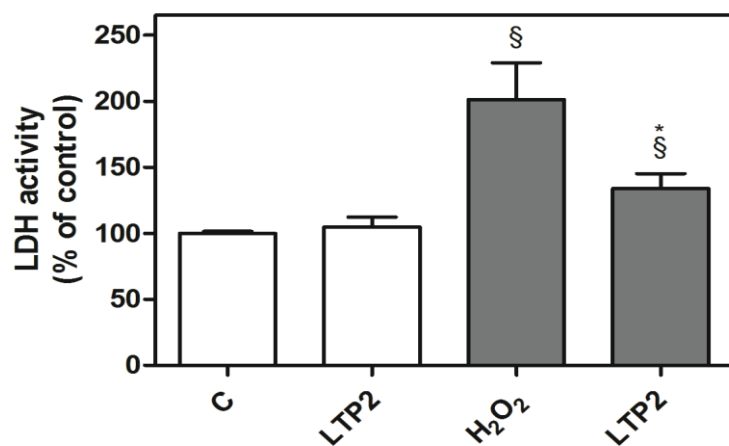
- HUVECs treated with oxidative (H_2O_2) and inflammatory (TNF- α , IL-1 β , LPS) agents, as a model system
- Evaluation of nsLTP2 ability to influence intracellular ROS level and to prevent cell damage
- Analysis of nsLTP2 capacity to modulate specific marker of inflammatory processes, such as Adhesion 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

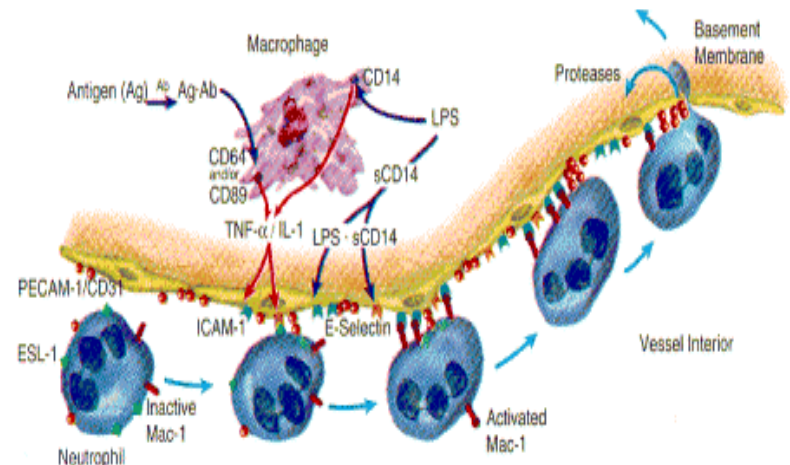


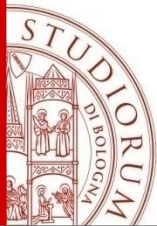
Anti-inflammatory effects of nsLTP2

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

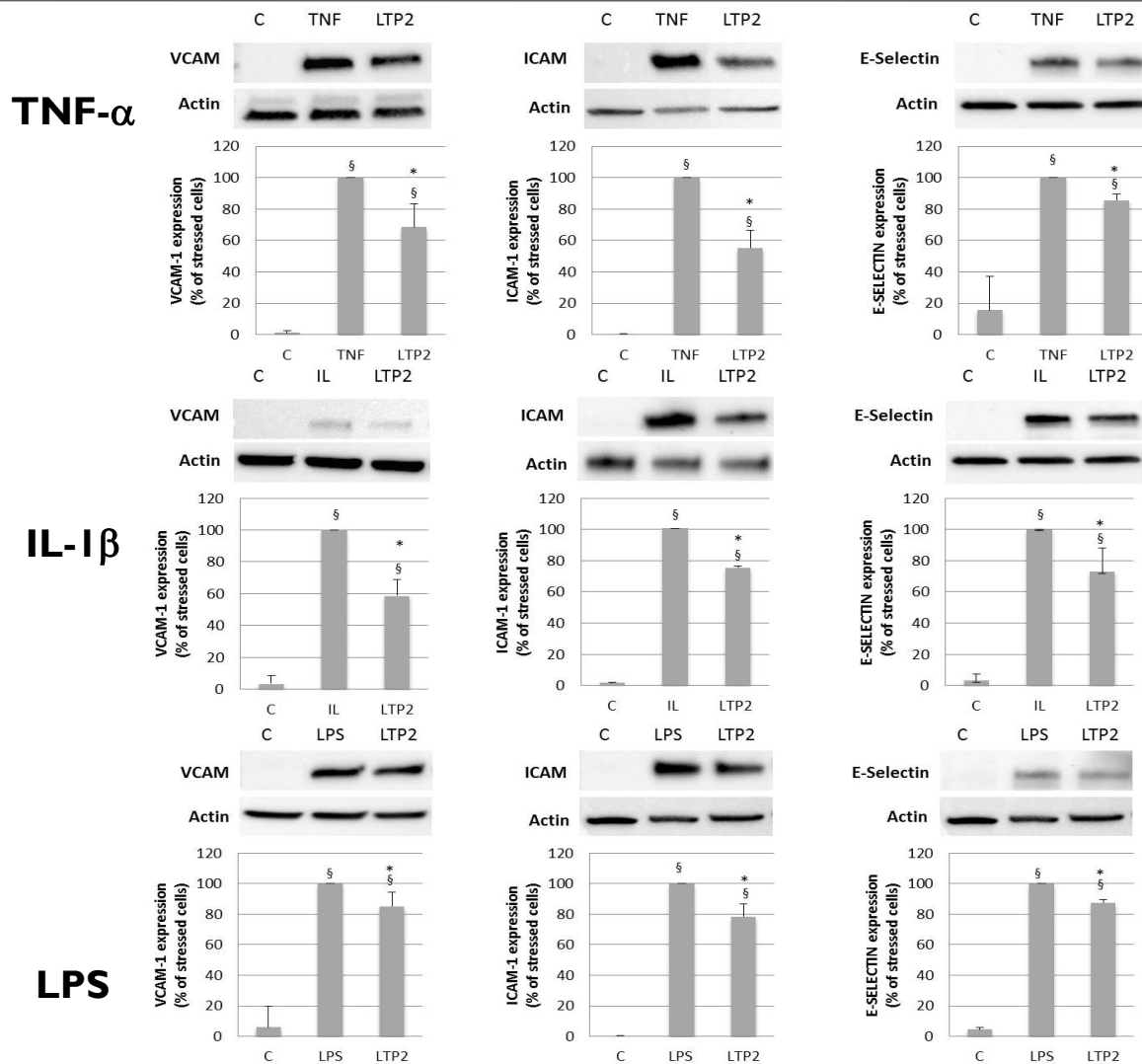
Since stimulated endothelial cells have an indispensable role in leukocyte recruitment producing inflammatory mediators and expressing adhesion molecules involved in cardiovascular disease (Makó *et al.*, Cytometry A. 2010), the effect of nsLTP2 has been assessed under resting and pro-inflammatory conditions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

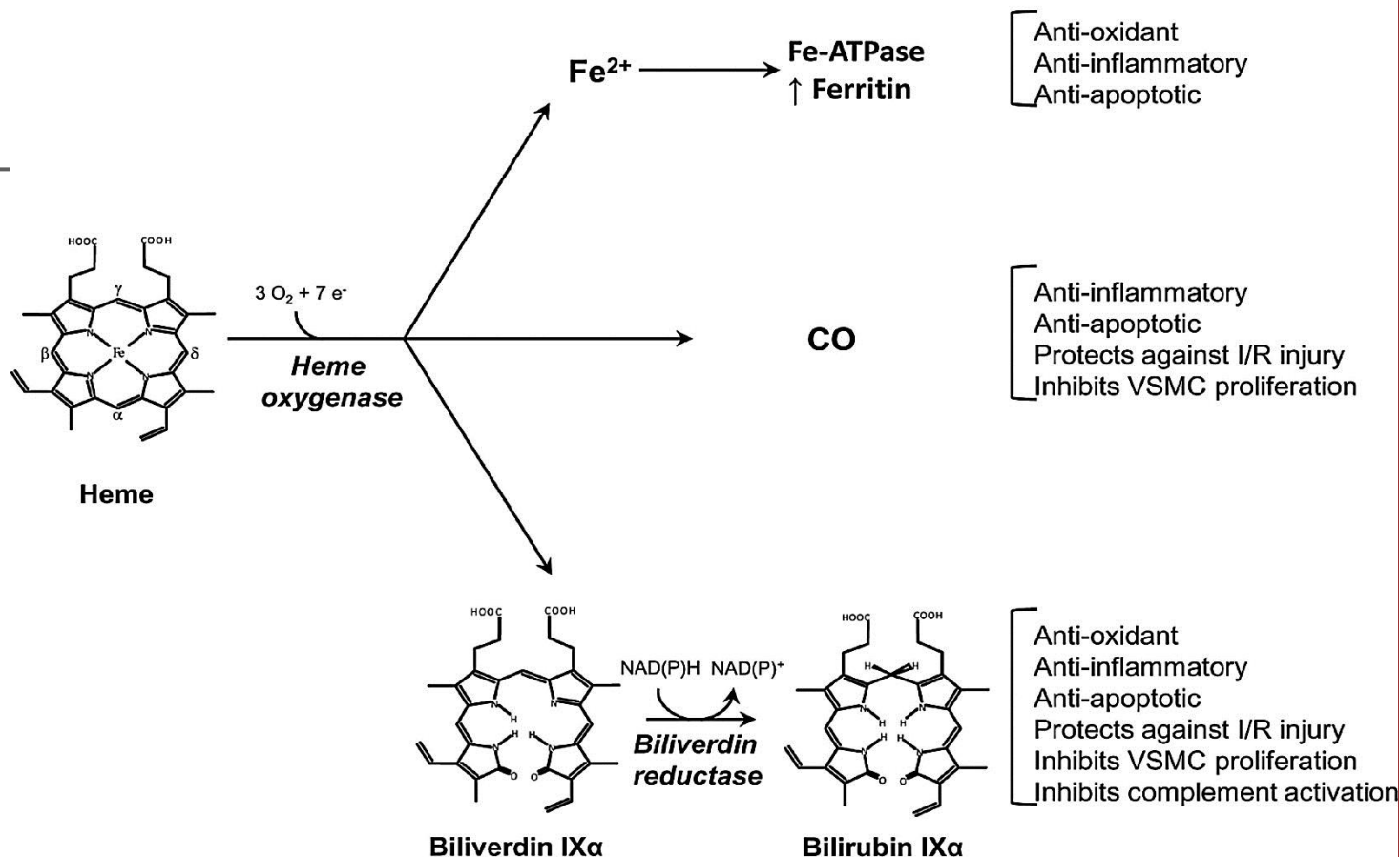
- **Vascular cell adhesion molecule-1 (VCAM-1)**
- **Intracellular adhesion molecule-1 (ICAM-1)**
- **E-selectin**





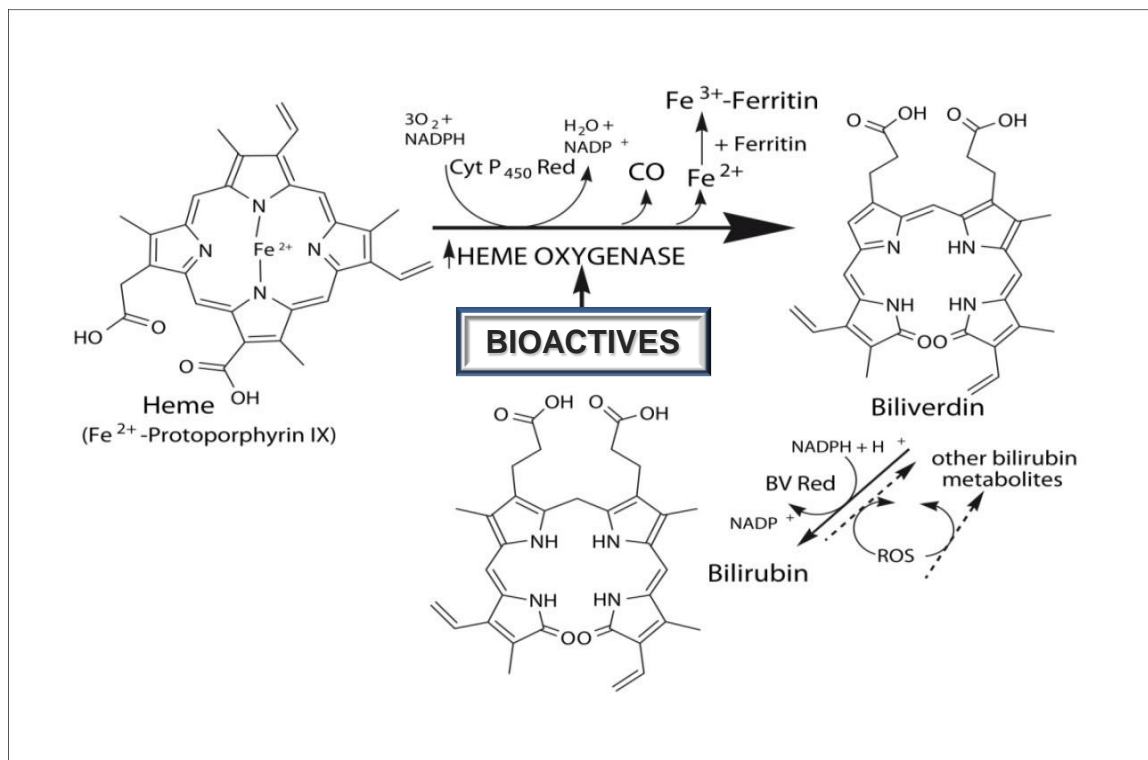
Anti-inflammatory activity of nsLTP2: effects on adhesion molecules





Heme oxygenase-mediated degradation of heme. In endothelial cells free heme is cytotoxic and potentiates toxic effects of H_2O_2 . The interaction of HO with heme generates equimolar amounts of carbon monoxide (CO), biliverdin, and free iron (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.

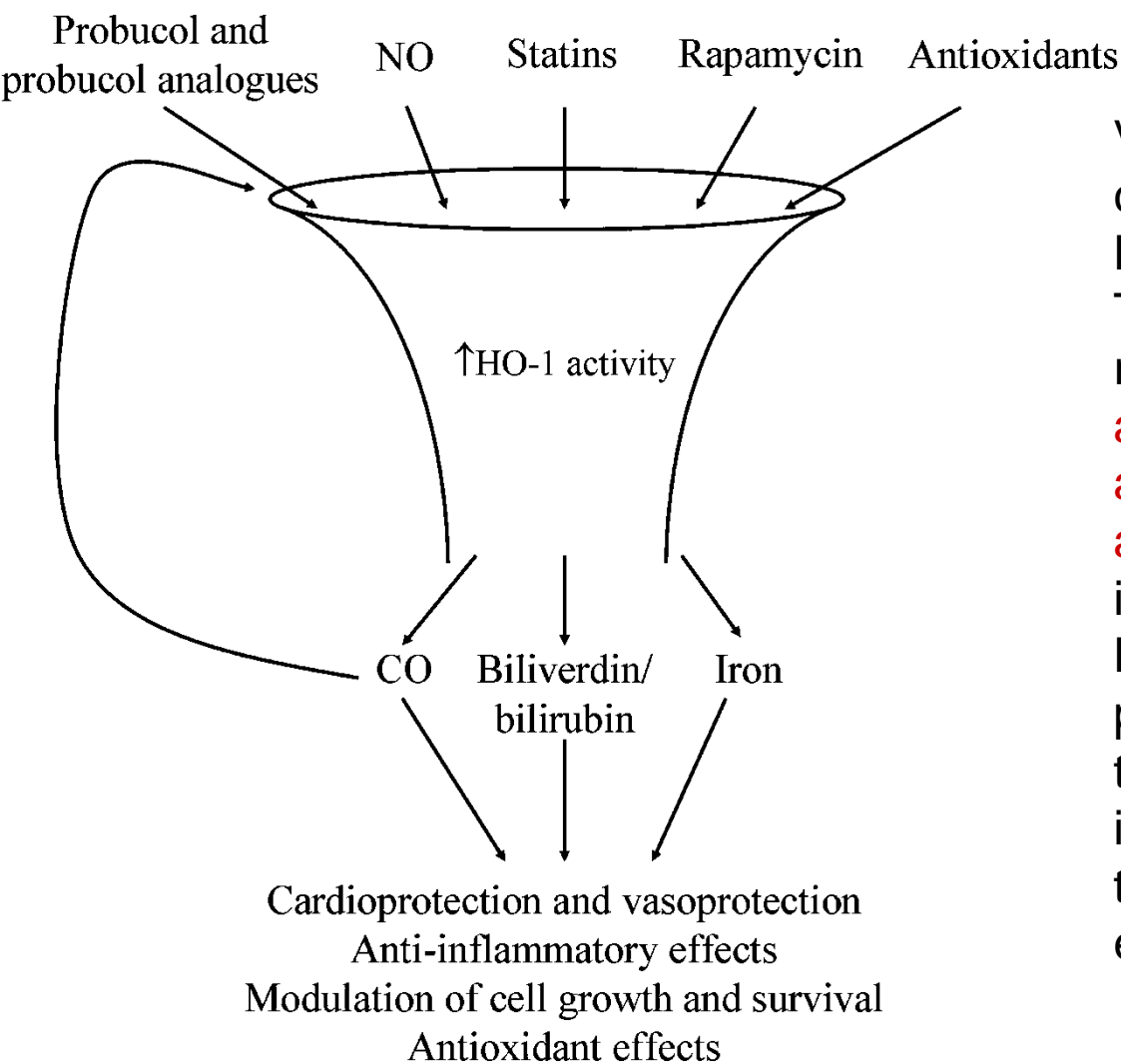
Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1



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

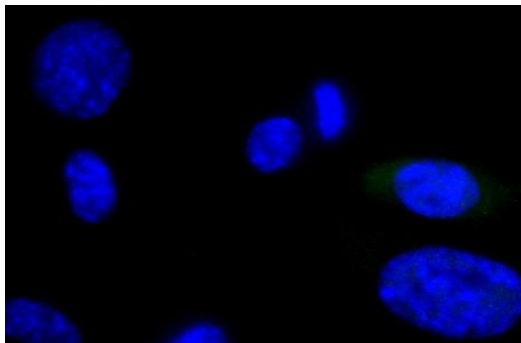
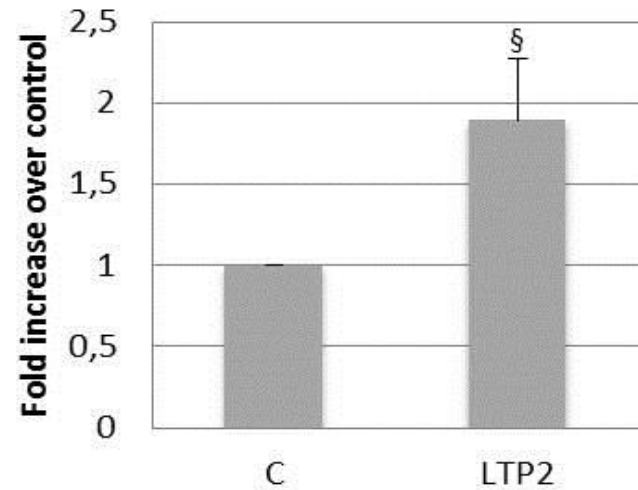
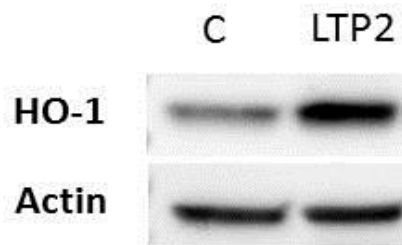
Some common **dietary phytochemicals** are known HO-1 inducers [Haines et al., Acta Physiol, 2012], as well as drugs currently used in therapy [Calay and Mason, Antioxidants & Redox Signaling 2014].

Haeme oxygenase-1 as a therapeutic funnel

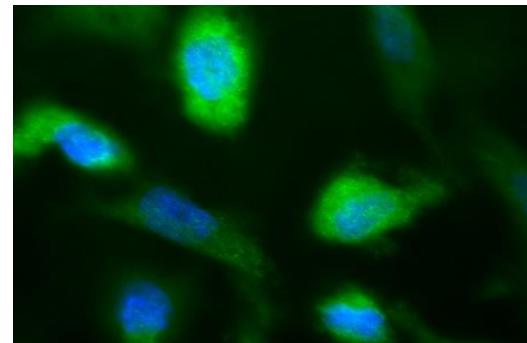


Various classes of therapeutic compounds induce HO-1 expression and activity. The products of this enzymatic reaction then mediate the **antiinflammatory, antioxidant, and/or growth-modulating activities** associated with increased HO-1 expression. Some of the products, such as CO, themselves increase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

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



Control

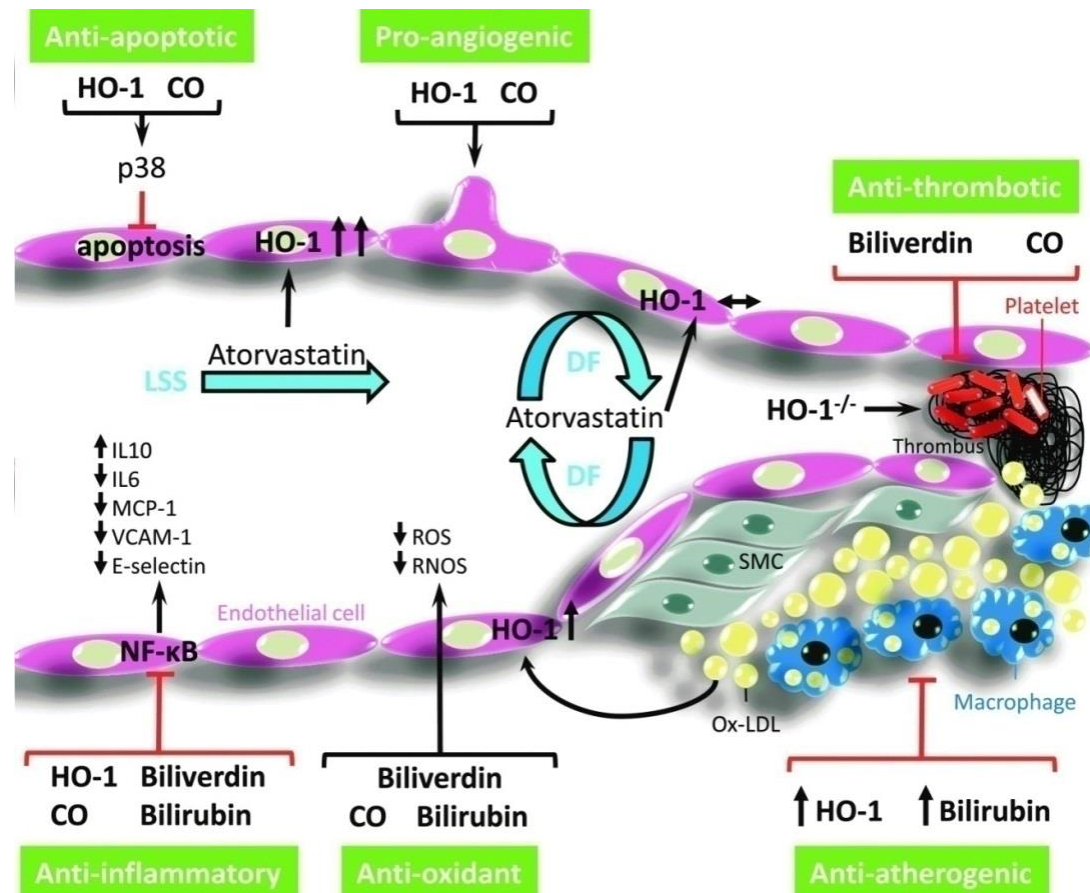


nsLTP2

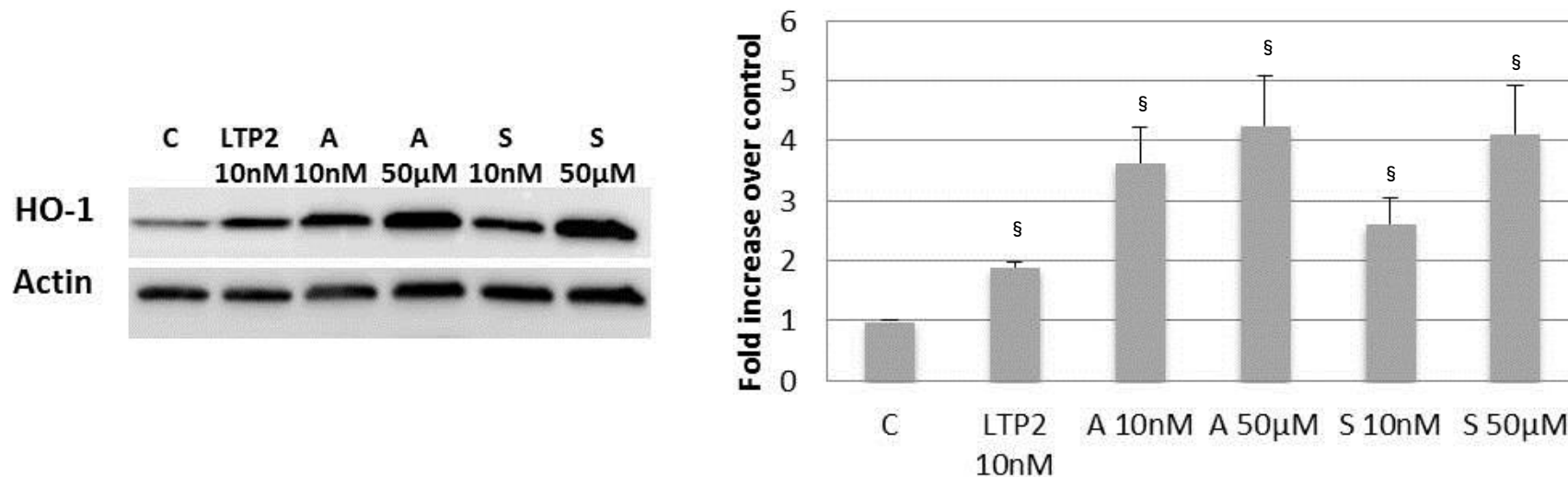
Anti-inflammatory effects of nsLTP2 and the role of Haeme oxygenase-1

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

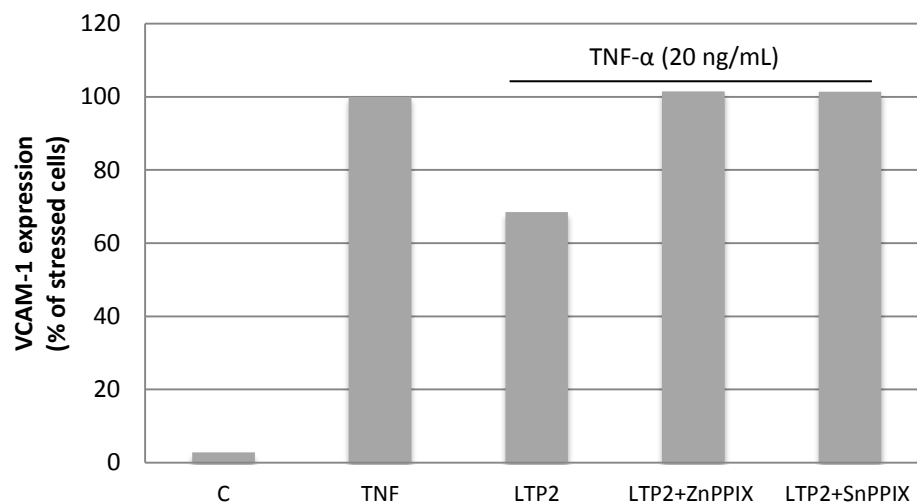
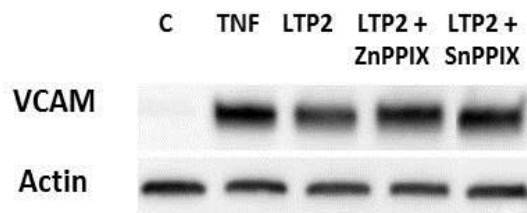


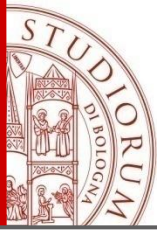
Effect of nsLTP2 (LTP2), Atorvastatin and Simvastatin on HO-1 protein expression level
 HUVECs were treated with 10 nM nsLTP2 (LTP2), 10 nM - 50 μM Atorvastatin (A) or 10 nM - 50 μM Simvastatin (S) for 24 h. Cell lysates were subjected to SDS-PAGE and Western blotting analysis of HO-1 protein expression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

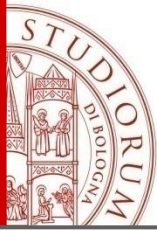
In order to examine whether up-regulation of HO-1 induced by nsLTP2 is correlated to the down-regulation of adhesion molecule expression (i.e. VCAM) induced by TNF- α , experiments were conducted using two HO-1 inhibitors: Zinc Protoporphyrin IX (ZnPPIX) and Tin Protoporphyrin IX (SnPPIX).





Conclusion

- nsLTP2 exerts **antioxidant and cytoprotective effects** on HUVECs stressed by different oxidative (H_2O_2) and inflammatory agents (TNF- α , IL1- β , LPS)
- nsLTP2 is able to **increase HO-1 expression**
- nsLTP2 **decreases the expression of VCAM, ICAM and E-Selectin** induced by inflammatory agents (TNF- α , IL1- β , 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**

