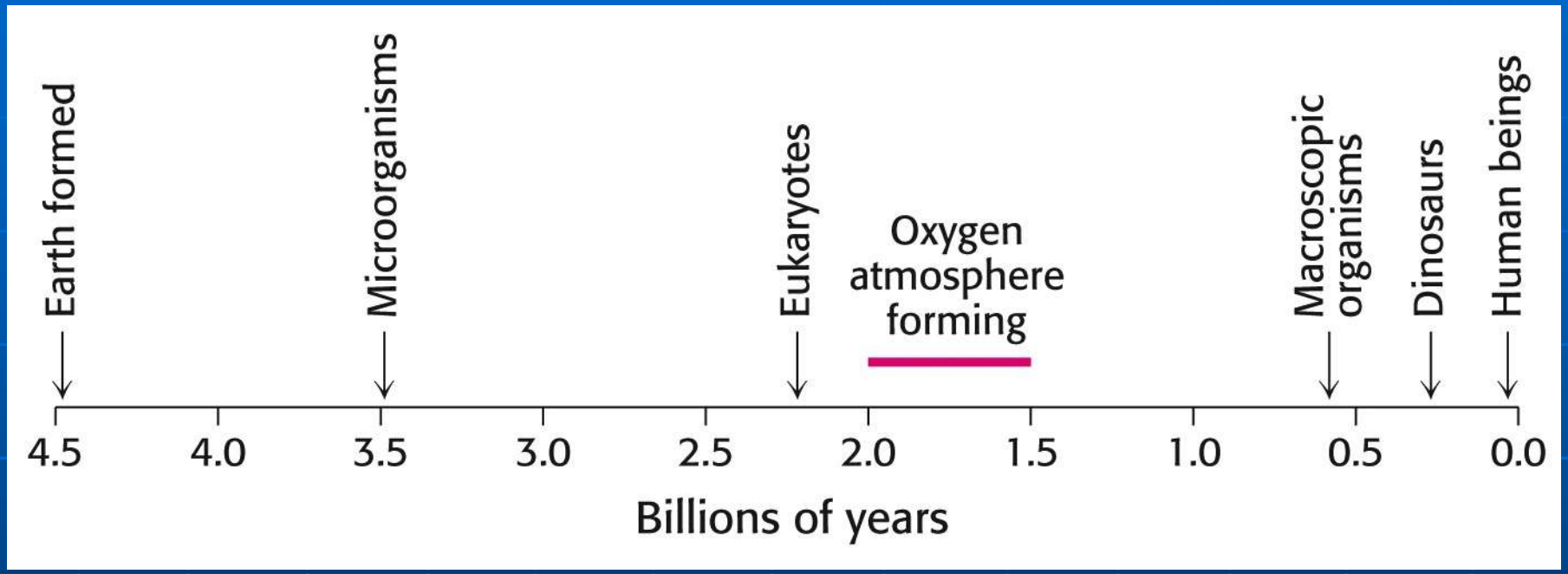

Earth formed
Microorganisms
Eukaryotes
Oxygen atmosphere forming
Macroscopic organisms
Dinosaurs
Human beings
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Billions of years

Oxidative stress
And reactive oxygen species (ROS)

The biological mechanism of oxygen reduction, the transfer of four electrons to the O_2 molecule, can directly or indirectly produce partially reduced forms of oxygen or chemical species with high reactivity and oxidizing ability, collectively referred to as ***"reactive oxygen species" ("reactive oxygen species", ROS).***

Some of these species are free radicals, molecules with one or more orbitals containing an unpaired electron, **as** $\cdot O_2^-$ ***superoxide radical*, or as** $\cdot OH$ ***hydroxyl radical***;

Other as H_2O_2 ***hydrogen peroxide***, or as $^1O_2$ ***singlet oxygen***, are not radical but are ROS highly reactive chemical species capable of generating radicals or to participate in radical chain reactions.

The first three ROS listed above are intermediate in the ***univalent oxygen reduction*** process.

Mitochondrial respiration generates ROS and radicals

In the process of O_2 reduction univalent superoxide radical is produced by one electron reduction, H_2O_2 by two electrons, the hydroxyl radical by three electrons.

$$O_2 + 1\ e^- \rightarrow \cdot O_2^-$$

$$\cdot O_2^- + 1\ e^- + 2\ H^+ \rightarrow H_2O_2$$

$$H_2O_2 + 1e^- + H^+ \rightarrow H_2O + \cdot OH$$

$$\cdot OH + 1e^- + H^+ \rightarrow H_2O$$

The hydroxyl radical can be formed also from H_2O_2 by Fenton chemistry reaction:

$$H_2O_2 + Fe^{2+}\ (o\ Cu^+) \rightarrow Fe^{3+}\ (o\ Cu^{2+}) + \cdot OH + OH^-$$

Another free radical, produced by the normal metabolism of several animal tissues, is *nitric oxide* $NO^\cdot$, which readily reacts with the superoxide radical, producing *peroxynitrite* $OONO^-$, also included among the ROS.

Nitric oxide NO•

Nitric oxide has several functions in human body. Among these the relaxing effect on the smooth muscles of the vessels, which produces vasodilatation and consequent hypotension, can be of crucial importance for the control of blood pressure.

The reaction of $NO^{\cdot}$ with $^{\cdot}O_2^-$, reducing the concentration of the radical vasodilatator, may have a pathogenic role in the establishment of arterial hypertension. Moreover, the product of the above reaction -*the peroxynitrite*- might oxidize thiol residues essential for the enzymatic activity and/or modify -hydroxylation, nitration- of amino acids involved in the molecular mechanisms of signal transmission.

The negative effects of radicals and ROS

The production of ROS as intermediates or products of normal aerobic metabolism is relatively high: it was estimated that approximately 2% of the oxygen we breathe in generates superoxide radical and other ROS, without considering other pathological events (e.g. infections, inflammatory reactions, etc.).

The superoxide radical is itself little toxic, but can generate hydrogen peroxide, hydroxyl radical and peroxynitrite. Even hydrogen peroxide, up to concentrations in the order of micromolar, has poor ability as pro-oxidant; but it can directly attack some enzymes when it reaches concentrations above 50 μM .

The biological toxic effect of $^{\cdot}O_2^-$ and H_2O_2 is mainly due to the $^{\cdot}OH$ generation. The *hydroxyl radical* is also produced by ionizing radiation, both of natural origin (radon, cosmic radiation) or artificial (X-rays, γ-radiation, etc.), which decompose the water molecule (radiolysis) generating $^{\cdot}OH$. This radical is extremely reactive and can trigger chain reactions, causing serious damage to biological membranes (lipid peroxidation), protein and DNA.

Defense mechanisms against radicals and ROS

To protect against the risk of biomolecules damage due to the effect of radicals and ROS, aerobic organisms have developed several lines of defense:

1. Enzymes able to convert and inactivate some radicals and/or reactive oxygen species;

2. Proteins that by direct binding (chelators) of iron and copper, control the concentration of free metal ions, and thus the Fenton reaction;

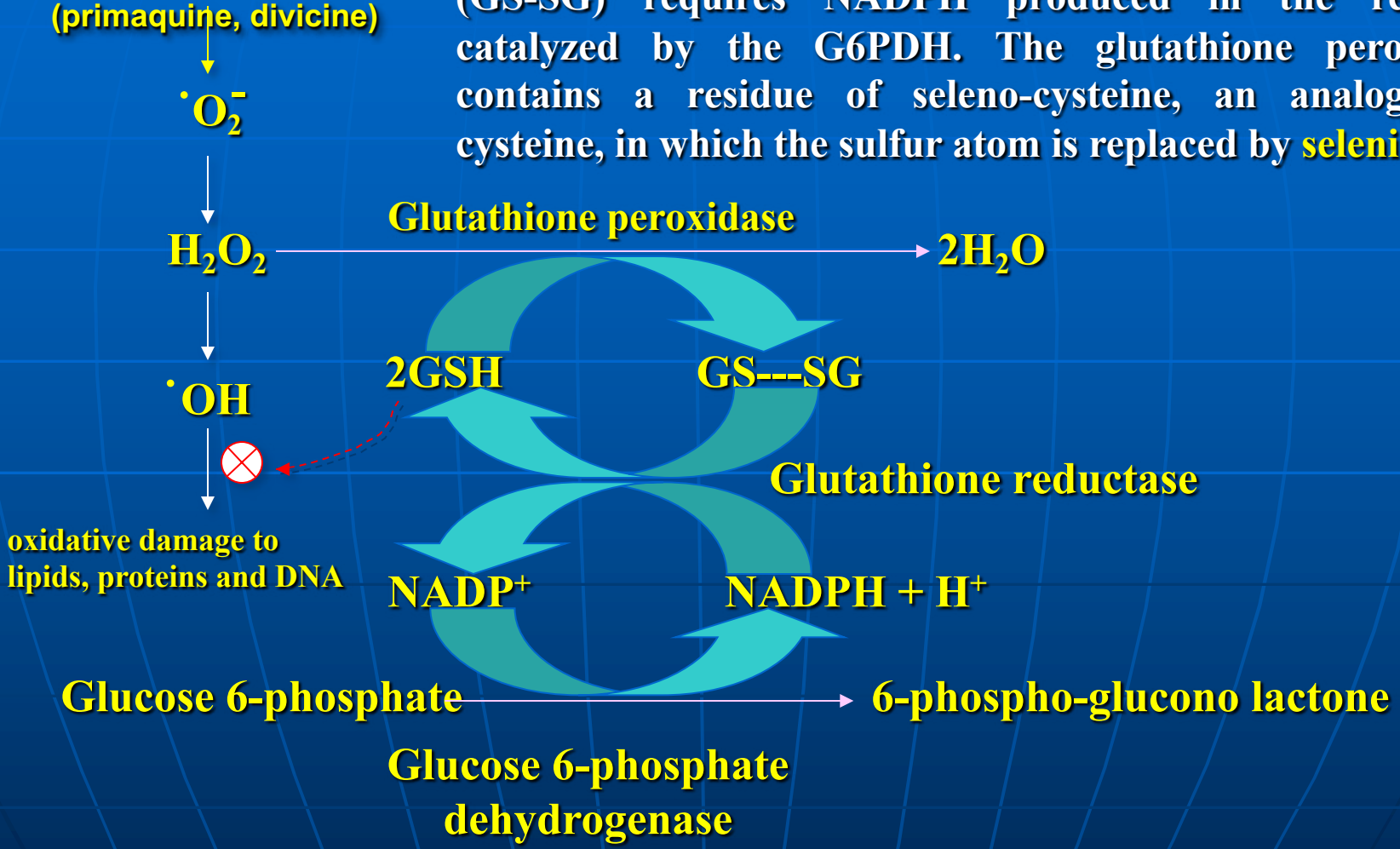
3. Non-protein antioxidants, able to remove already formed ROS and to slow or block the propagation of radical species (Chain Reactions).

Role of NADPH and glutathione in protecting cells against reactive oxygen species (ROS) .

mitochondrial respiration, respiratory burst, ionizing radiation, freezing-thawing, herbicides, drugs

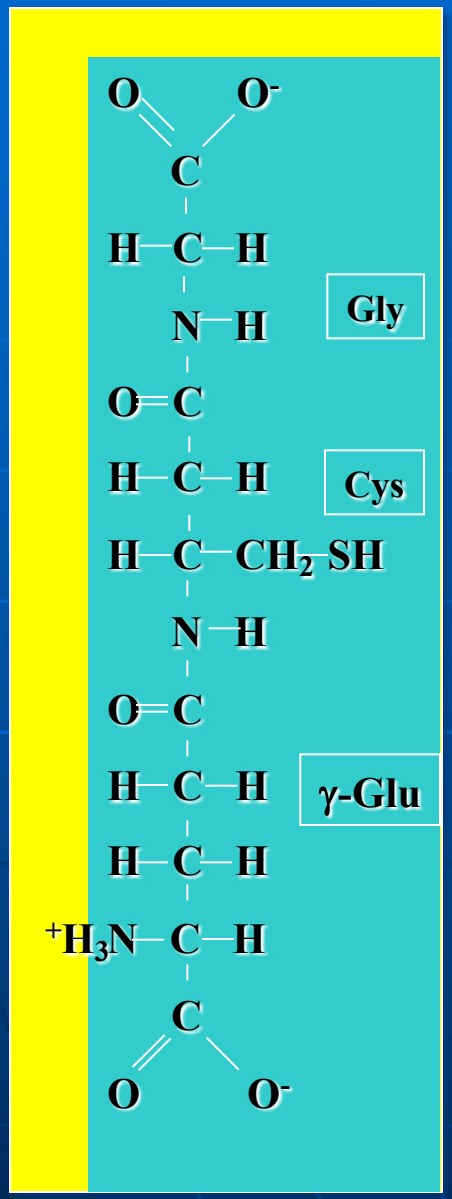
(primaquine, divicine)

Reduced glutathione (GSH) protects cell oxidation by degrading the hydrogen peroxide and the free hydroxyl radical. The regeneration of GSH from its oxidized form (GS-SG) requires NADPH produced in the reaction catalyzed by the G6PDH. The glutathione peroxidase contains a residue of seleno-cysteine, an analogue of cysteine, in which the sulfur atom is replaced by selenium.



GLUTATHIONE REDUCED

Tripeptide: γ-glutamilcisteynilglycine



Other enzyme systems with ANTIOXIDANT ACTION

The *superoxide dismutase* catalyzes the dismutation of two molecules of superoxide radical (one is oxidized and the other reduced) to oxygen and hydrogen peroxide:

$$2 \bullet O_2^- + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2$$

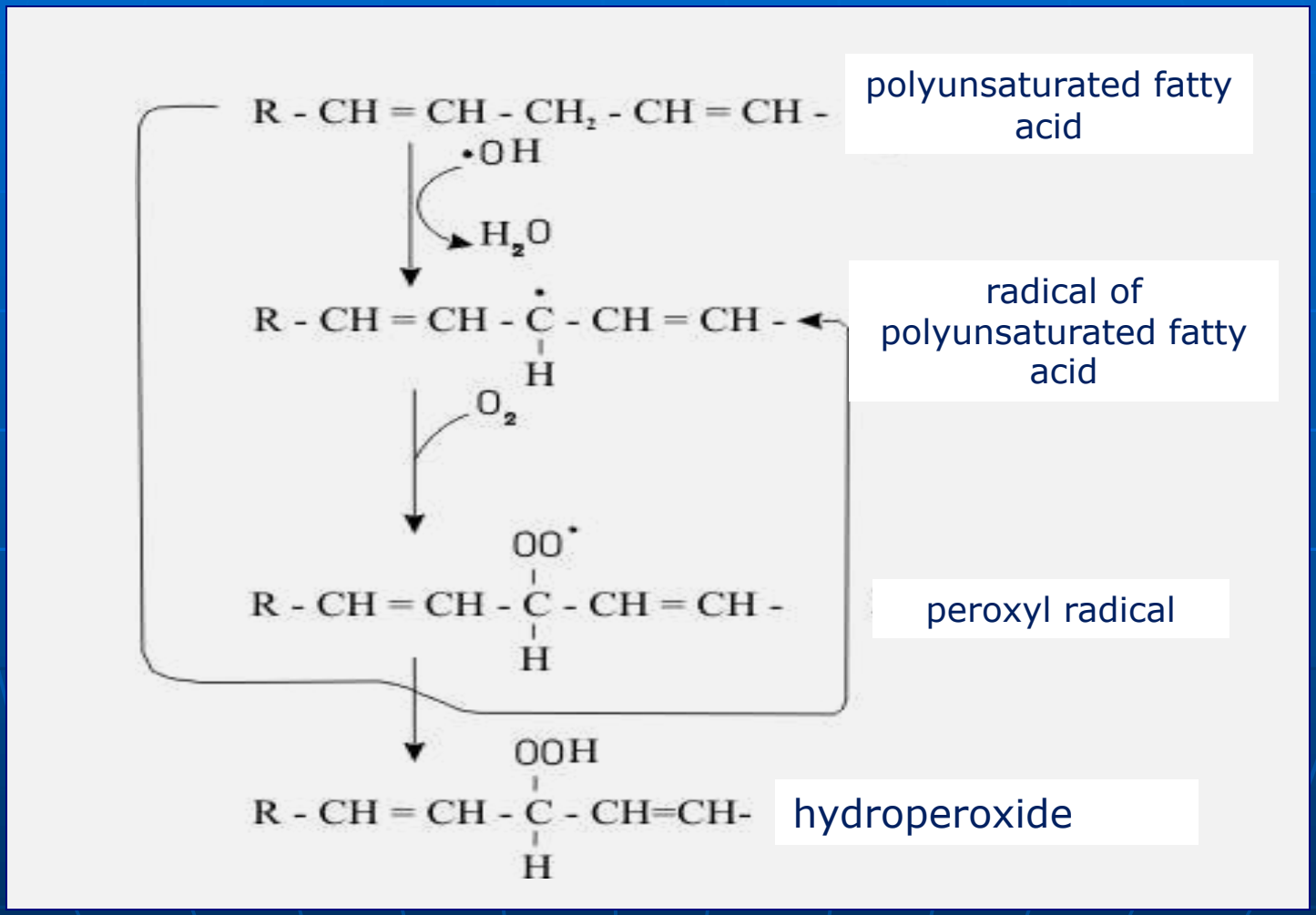
SOD Cu Zn cytosol
SOD Mn mitochondria

$$H_2O_2 \xrightarrow{\text{catalase}} H_2O + 1/2O_2$$

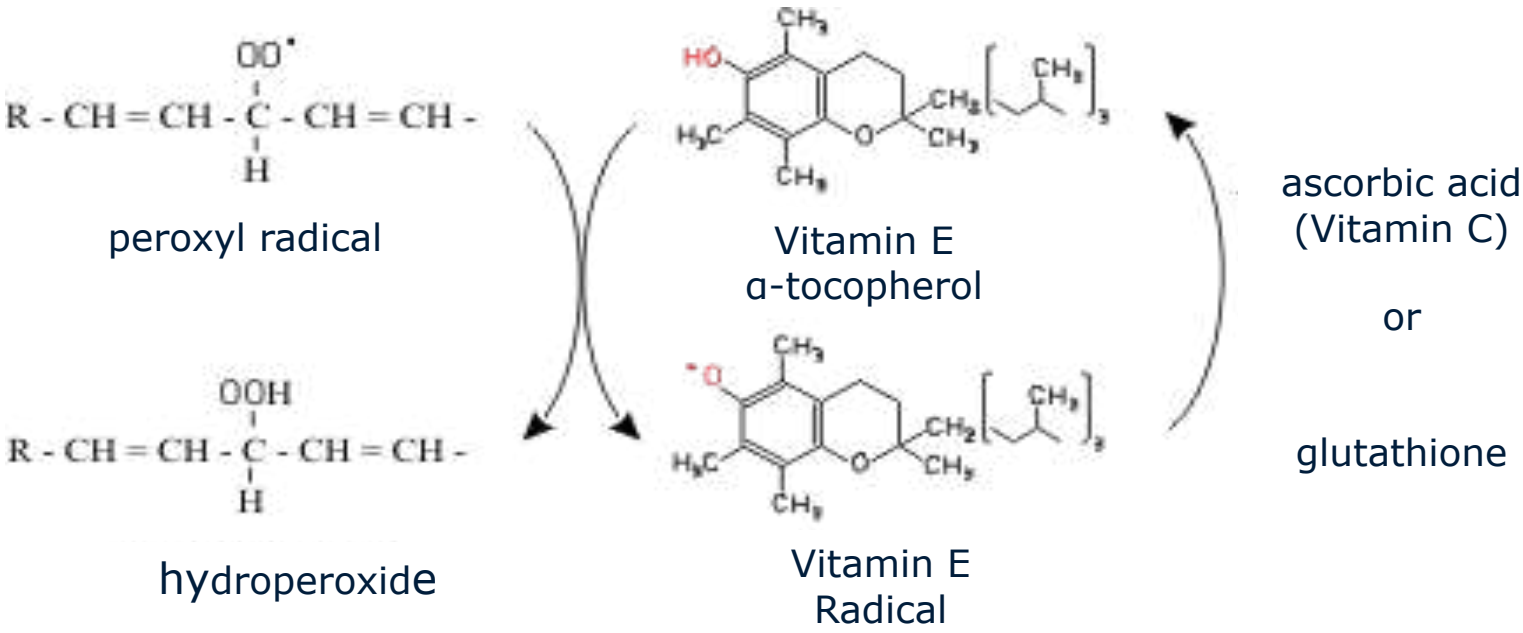
$$NADPH + H^+ \qquad NADP^+$$

Lipid peroxidation

(chain reaction)



Vitamin E



Vitamin E is an important antioxidant vitamin, the content of tocopherols should be in proportion to the amount of polyunsaturated fatty acids, very easily oxidized.

Vitamin E may exert antioxidant activity when the ratio tocopherols (mg %): polyunsaturated fatty acids (g %) is close to 1 or at least higher than 0.8

The biochemical effects peroxidized lipids

Peroxidized lipids result from the oxidative degradation of polyunsaturated fatty acids (PUFAs) in cell membranes, which can occur due to exposure to reactive oxygen species (ROS) or free radicals. Here's a breakdown of the biochemical effects of peroxidized lipids:

1. Membrane Damage: Peroxidized lipids can disrupt the integrity and fluidity of cell membranes. This can compromise the structural and functional integrity of cells and organelles, leading to cellular dysfunction.

2. Inflammation: Peroxidized lipids serve as pro-inflammatory mediators by activating inflammatory signaling pathways. They can stimulate the production of pro-inflammatory cytokines and chemokines, exacerbating the inflammatory response.

3. Oxidative Stress: Peroxidized lipids contribute to oxidative stress by generating reactive oxygen species (ROS) through lipid peroxidation reactions. ROS can further damage cellular components such as proteins, nucleic acids, and lipids, leading to cellular dysfunction and cell death.

4. Cell Signaling: Peroxidized lipids can modulate cell signaling pathways by altering the activity of enzymes and transcription factors involved in cellular processes such as proliferation, differentiation, and apoptosis.

5. Formation of Reactive Aldehydes: During lipid peroxidation, reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) are generated as by-products. These reactive aldehydes can covalently modify proteins and nucleic acids, leading to cellular dysfunction and contributing to the pathogenesis of various diseases.

Overall, peroxidized lipids play a central role in mediating oxidative stress, inflammation, and cellular damage, and their accumulation has been implicated in the pathogenesis of numerous diseases, including cardiovascular disease, neurodegenerative disorders, and cancer.

Minerals

The minerals represent a relatively small part of the human body weight.

However, they are involved in the formation of tissues and are critical for many biological functions and for growth.

Their bioavailability depends on different biological variables, such as genetic factors, sex, age, presence of some pathologies, the state of nutrition, the amount and biodiversity of gut microflora, physical activity, stress, etc.

Minerals

The minerals with important biological role for all organisms are :

Na and K, Mg, Ca, Zn, Mn, Fe, Se, Co, Cu

- The mineral salts (1.5-2%) consisting of magnesium and potassium phosphate , calcium salts, Fe, S, Cu, Zn, etc., are present in the outer part of the caryopsis of cereals.

CLASSIFICATION of NUTRIENT MINERALS

- **Macronutrients**

(elements present in moderate amount in the organism)

P, Mg, Ca, S, Na, K, Cl, with a daily requirement in the order of grams or tenths of grams

Some macronutrient, such as Na, K, Ca, are relatively abundant in the body (>> 1 g / Kg) and play an essential role for their ionic properties and/or the ability to give more or less stable interactions in different biological contexts

✓Micronutrients or oligo-elements

(Elements present at low concentration in the body)

Mn, Fe, Co, Cu, Zn, with a daily requirement in the order of milligrams or micrograms.

the micronutrients are present in organisms (≤ 1 g/kg) at low or very low concentration (≤ 1 mg/Kg). Their importance are linked to their redox properties or functions (i.e. Lewis acid or structural functions). These elements often are in complex with protein structures.

ABSORBED AND UNABSORBED MINERALS

- an absorbed nutrient passes through the intestinal mucosa, enters in the blood stream and then it can be excreted in the urine or in the bile within one or two days.

- An unabsorbed nutrient doesn't enter the enterocytes, doesn't pass the intestinal mucosa and cannot be excreted.

- A complete elimination of a substance not absorbed can occur also after twelve days in humans.

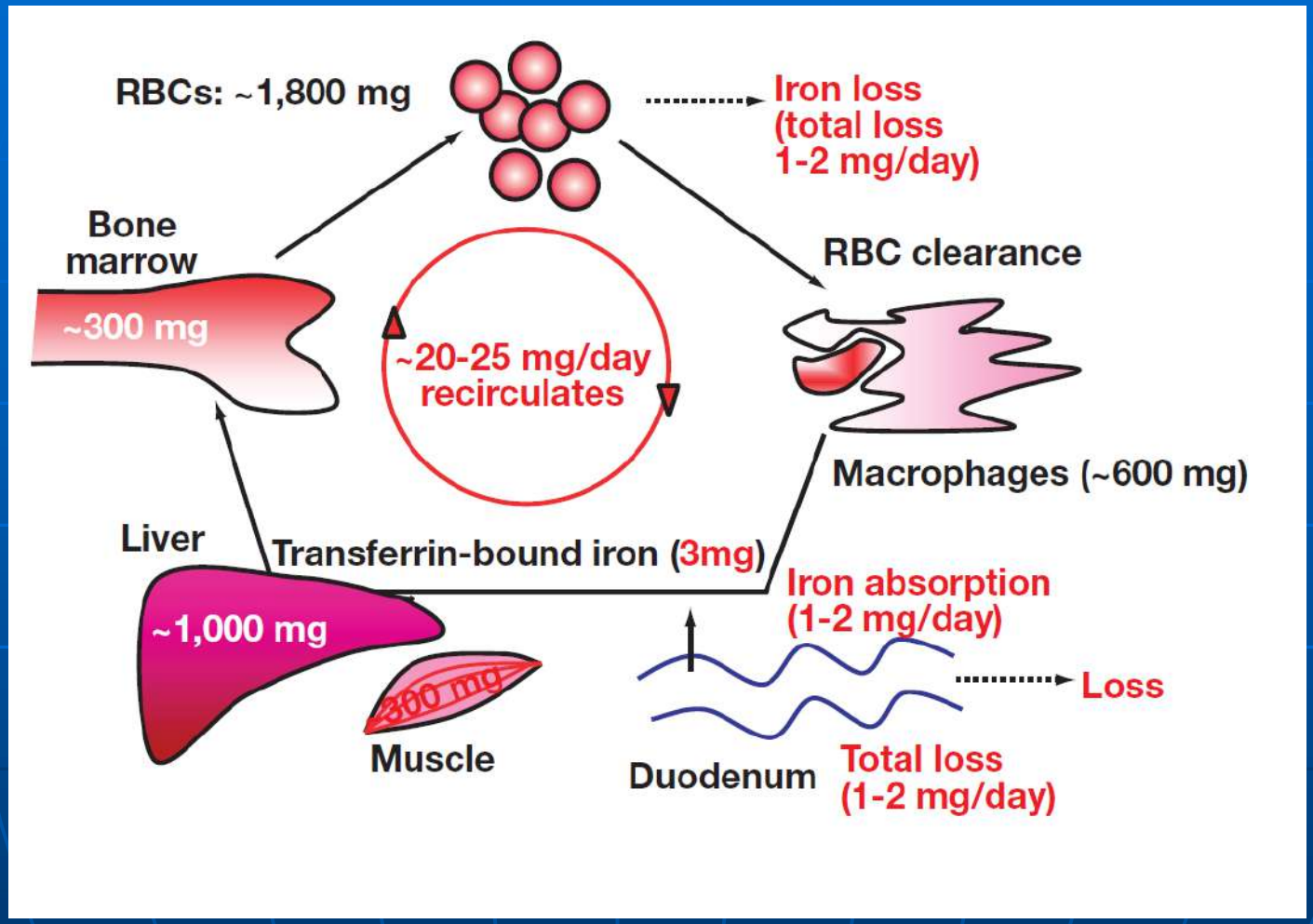
- A nutrient that enters in the enterocytes, and is there temporarily stored to be released again in the intestinal lumen, is not to be considered a nutrient absorbed.

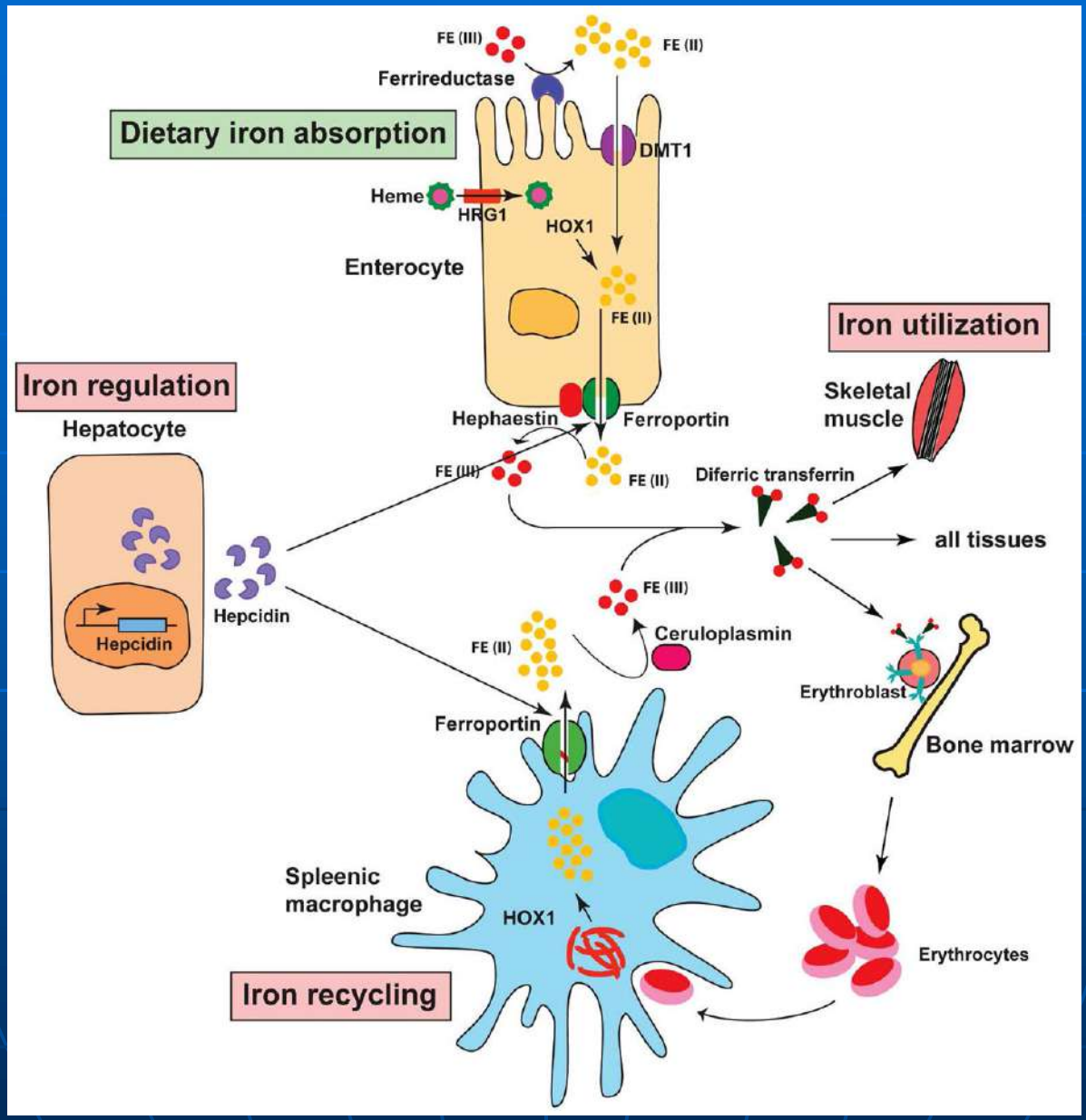
100 g	Sodium (mg)	Potassium (mg)	Calcium (mg)	Magnesium (mg)	Iron (mg)
Barley	3	160	16	37	2.0
Corn meal	1	120	6	106	1.8
Oat flakes	2	340	53	145	3.6
Spaghetti	5	160	23	35	1.2
Rice (hard polished)	6	110	24	28	0.8
Rye (dark flour)	2	439	23	83	2.6
Soy (flour)	-	*1160*	*199*	*240*	*8.4*
Corn (wholemeal flour)	2	290	41	113	3.3
Corn (clear chiara)	2	150	16	25	0.8
Bread (white)	540	100	10	26	1.7
Bread (wholemeal)	540	220	23	93	2.5
rusks	250	150	130	37	0.6

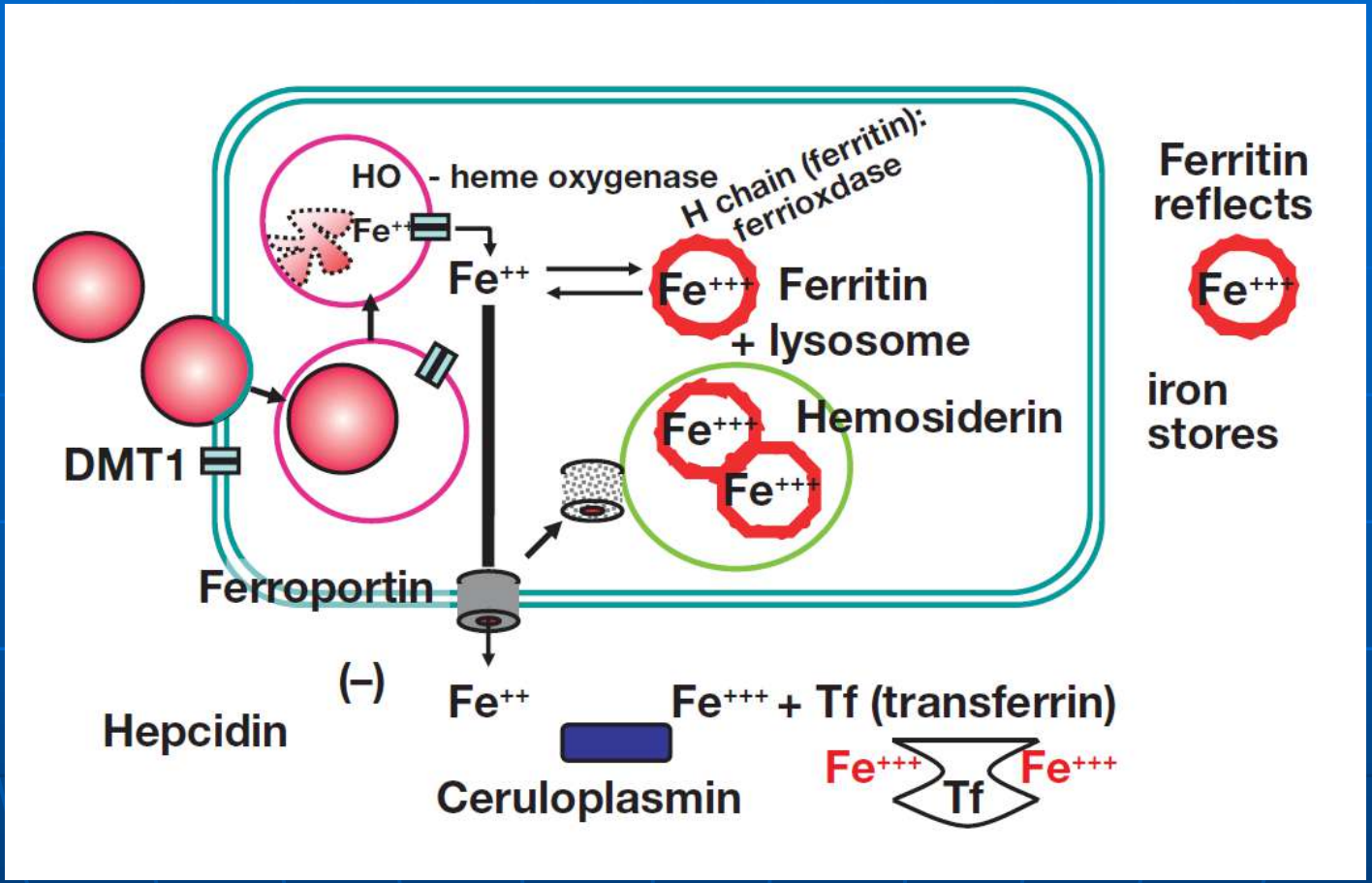
IRON ABSORPTION,TRANSPORT AND EXCRETION

Iron is contained in a wide variety of food.

It is an essential component of blood and an oligoelement needed to prevent anemia.


RBCs: ~1,800 mg
Iron loss
(total loss
1-2 mg/day)
Bone
marrow
~300 mg
~20-25 mg/day
recirculates
RBC clearance
Macrophages (~600 mg)
Liver
Transferrin-bound iron (3mg)
~1,000 mg
300 mg
Muscle
Iron absorption
(1-2 mg/day)
Loss
Duodenum
Total loss
(1-2 mg/day)


Dietary iron absorption
FE (III)
Ferrireductase
FE (II)
DMT1
Heme
HRG1
HOX1
Enterocyte
FE (II)
Iron utilization
Iron regulation
Hepatocyte
Hephaestin
Ferroportin
Skeletal muscle
FE (III)
FE (II)
Diferric transferrin
all tissues
Hepcidin
Hepcidin
FE (III)
Ceruloplasmin
FE (II)
Erythroblast
Ferroportin
Bone marrow
Spleenic macrophage
HOX1
Erythrocytes
Iron recycling


HO - heme oxygenase
H chain (ferritin): ferrioxdase
Fe++
Fe++
Fe+++ Ferritin
+ lysosome
Fe+++ Hemosiderin
Fe+++
DMT1
Ferroportin
(–)
Hepcidin
Fe++
Fe+++ + Tf (transferrin)
Fe+++ Tf Fe+++
Ceruloplasmin
Ferritin reflects
Fe+++
iron stores

Iron is an atypical nutrient:

- ✓ the iron deficiency is not associated with particularly clear or devastating symptoms;
- ✓ It is stored in large quantities in the human body linked to a protein called ferritin;
- ✓the iron is present as free-form only in very low concentrations in the body. The iron is not present in free form because it is mainly bound to proteins. In addition, Fe^{3+} (ferric) is practically insoluble and the ferrous iron Fe^{2+} can be toxic to cells.

Iron is an essential nutrient for all organisms because it is present in various proteins and enzymes with fundamental biological functions;

oxygen transport (hemoglobin and myoglobin), electron transport chain (cytochromes, NAD dependent dehydrogenase, flavoenzyme)

~ 7% of the iron in the human body is present as a form bound to metalloenzymes.

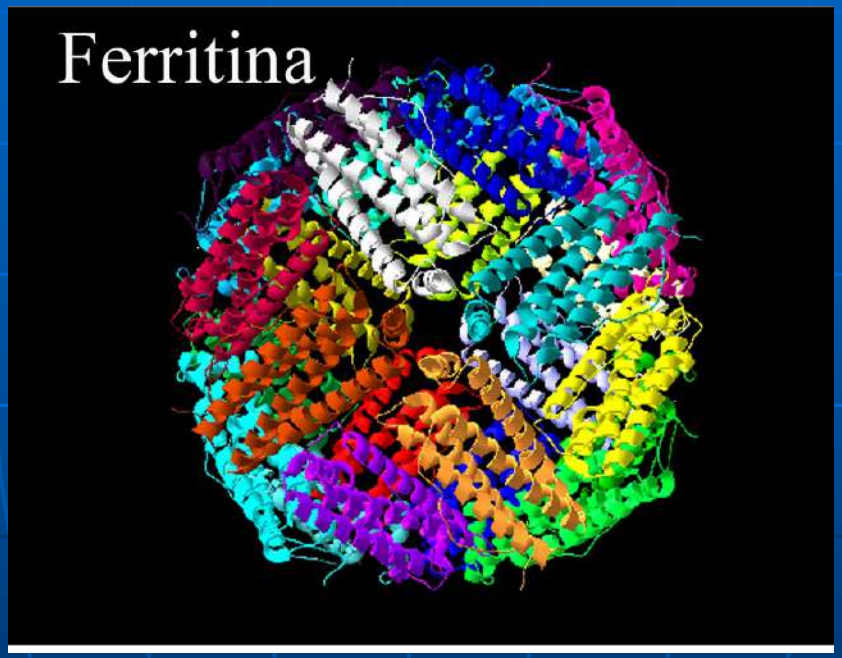
Metalloenzymes are classified into heme-iron or

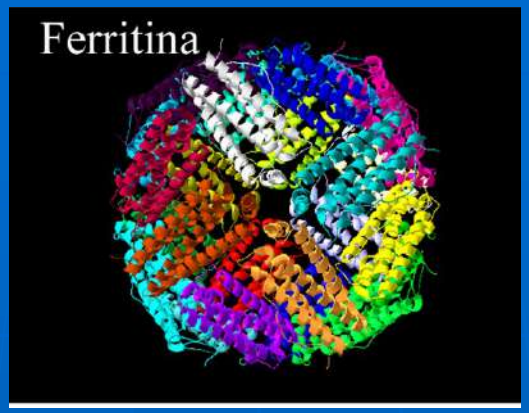
non- heme iron enzymes

Non-heme iron enzymes	FUNCTIONS
Succinate dehydrogenase	Krebs Cycle
Aconitase	Krebs Cycle
ribonucleotide reductase	Reduction of dNTP in the DNTP for DNA the synthesis of DNA
xanthine dehydrogenase	Purine catabolism
adrenodoxin	synthesis of steroid hormones
Δ^9-desaturase	Synthesis of unsaturated fatty acids
NADH dehydrogenase	Respiratory chain
Coenzyme Q reductase	Respiratory chain
Lipoxygenase	Synthesis of leukotrienes and eicosanoids

Ferritin

The iron bound to ferritin is between 5 and 30%.



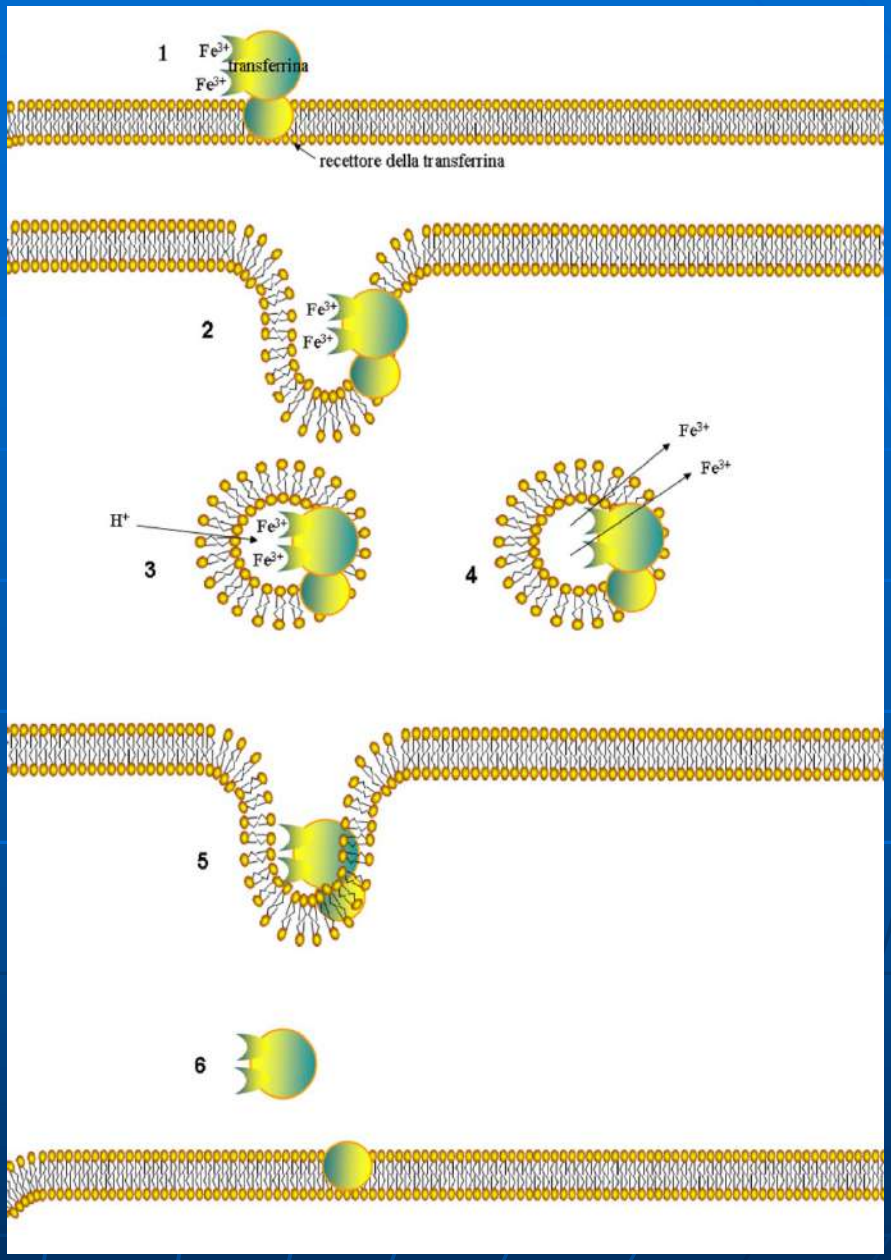


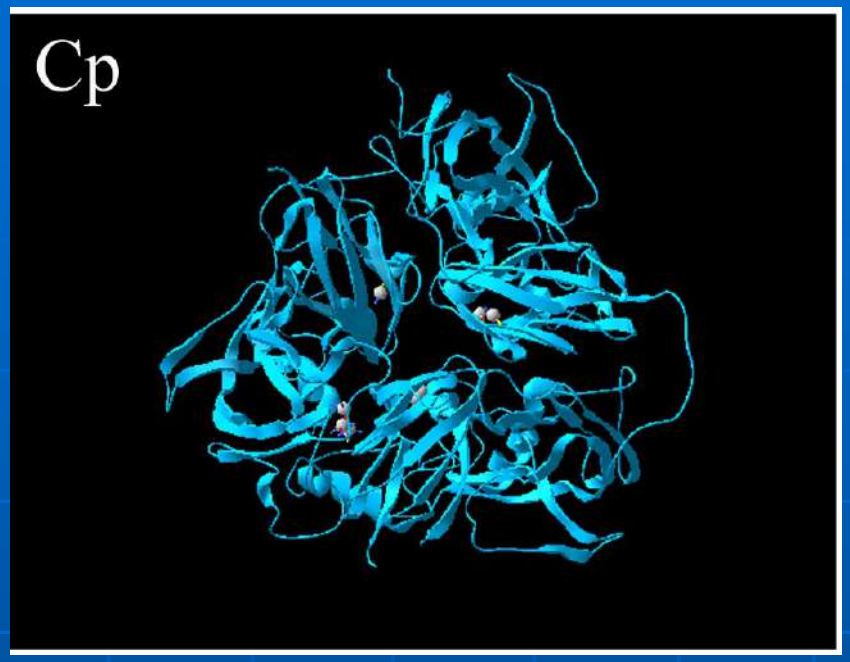
Ferritin has 24 subunits, each of which can bind up to 4,500 atoms of iron in the form of ferric hydroxyphosphate. Iron homeostasis is not guaranteed by systems that modulate the amount of excreted metal, as is the case for the Na and K, but through the variation of the quantity present in the form of ferritin deposits. Hemosiderin is another protein with iron storage function.

Iron Transport: transferrin

transferrin (74 kDa) is a iron-transport protein that bound two metal atoms in oxidized form with carbonate.

The transferrin binds and transports Fe^{3+} to membrane cellular receptors. After the binding, a small region of the plasma membrane invaginates to form a new intracellular membrane vesicle clathrin-coated. Clathrin is removed to form endosomes contain an ATP-dependent proton pump that generate a significant H+ concentration gradien to pH values between 5 and 6 (12.2). Transferrin releases iron at the pH lowered to 5.0 or below.





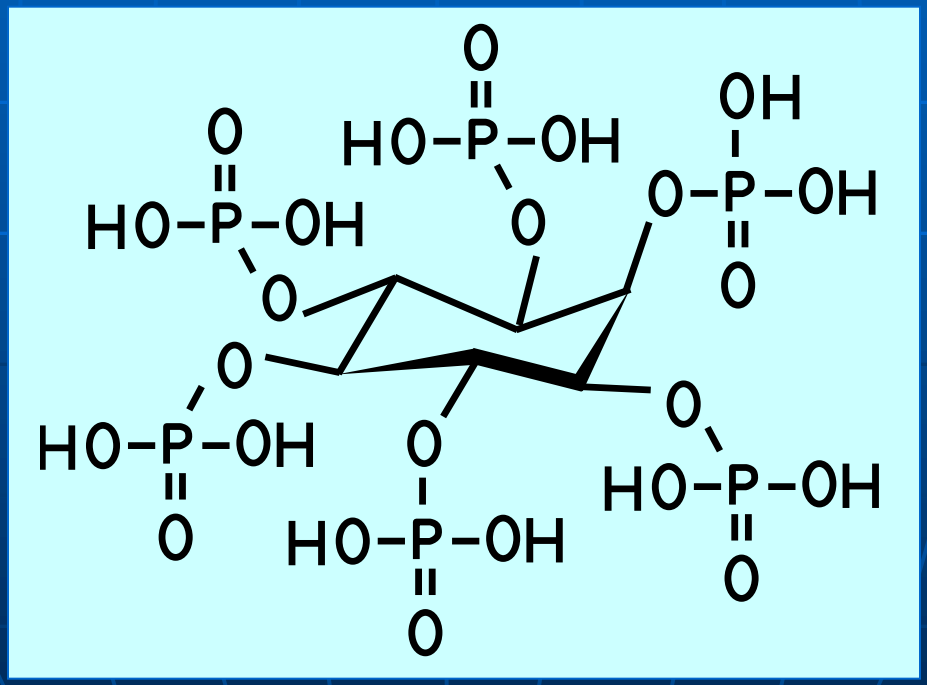
Ceruloplasmin plays a pivotal role in copper transport in blood, and as ferroxidase it is also involved in the cellular iron homeostasis.

IRON ABSORPTION

The recommended daily dose is 10 mg for men, 15 mg for woman and 30 mg for pregnant woman.

The iron availability in plant foods such as peas, beans, wheat, bread and rice is generally quite low (1 to 10%). The iron bioavailability in animal foods (heme iron) is significantly higher.

In addition to the minor presence of heme iron, in vegetal food the phytic acid has been identified as responsible for the inhibition of iron absorption. The phytate or inositol esaphosphoric acid is present and ranges between 1 and 5% by weight in the tissues of legumes, grains and vegetal food.



Scheda applicativa

MARKER DIAGNOSTICI DI CARENZA ALIMENTARE DI FERRO

I soggetti a maggiore rischio di anemia da deficienza di ferro sono i bambini in età compresa tra i sei mesi e i quattro anni e durante la prima adolescenza a causa della loro rapida crescita. Anche le donne durante la gravidanza possono incorrere in stati di carenza di ferro a causa dell'aumento del volume del sangue, delle richieste di ferro del feto e della placenta. Inoltre, anche la perdita di sangue durante il parto può essere causa di carenza del metallo. I primi segni diagnostici di carenza di ferro implicano diminuzioni delle forme di deposito del ferro (la ferritina). Poiché una piccola porzione della ferritina intracellulare lascia le cellule, i livelli di ferritina sierica riflettono i livelli di ferritina nei depositi di ferro dei tessuti. Un livello di ferritina sierica inferiore a 12 ng/ml è un chiaro segno di carenza di ferro. Una volta valutata in questo modo la presenza di una carenza di ferro è necessario procedere con altri tre tipi di test diagnostici in grado di valutare meglio la sua severità. Il primo è la misura della saturazione della transferrina. Questo test misura la proporzione di transferrina presente come forma apo (priva di ferro). Normalmente dal 20 al 25% della transferrina è presente in forma olo, saturata con il ferro, quando la saturazione scende sotto il 16% potrebbe esserci una disfunzione a livello del rifornimento di ferro durante la sintesi dei globuli rossi (eritropoiesi) nel midollo. Il secondo tipo di indagine diagnostica è la misura della quantità di apo-eme nei globuli rossi. Normalmente i globuli rossi contengono circa 350 ng di apo-eme per millilitro di cellule sedimentate. Livelli più alti di 1000 ng/ml cellule sono chiari segni di carenza di ferro protratta per un lungo periodo. Il terzo test diagnostico è la stima del volume medio corpuscolare (MCV). Quest'ultima analisi valuta il volume medio dei globuli rossi e consente di discriminare tra diagnosi di carenza di ferro, con globuli rossi ipocromici e piccoli (anemia microcitica) dalla anemia megaloblastica dovuta a carenza di folato e vitamina B_{12}.

COPPER AND ZINC

Copper is involved in redox reactions and it is in Cu^{+} or in Cu^{2+} form.

Zinc is not an acceptor or electron donor
And it is only in Zn^{2+} form.
Zinc has Lewis acid properties and so a catalytic activity.

Copper and Zinc are complexed with proteins and not in free ionic form.

Principal Zinc Proteins

OXIDOREDUCTASE	
Alcohol dehydrogenase	catabolism alcohol
Cu, Zn-superoxide dismutase (SOD) cytoplasmic	Dismutation of superoxide radicals
TRANSFERASE	
Poly(ADP-ribose) polymerase nuclear	DNA Repair
a-D-mannosidase	Degradation of oligosaccharides and glycoproteins
Aminopeptidase	Protease
Angiotensin	Regulation of blood pressure and salt balance
Carbossipeptidase A, B	Proteins digestion

HYDROLASE	
Alkaline phosphatase	Hydrolysis of phosphate groups
5'-nucleotidase	Hydrolysis of the phosphate from the 5'-monophosphate nucleotides
Fructose-1,6-bisphosphatase	Gluconeogenesis
AMP deaminase	AMP Transformation in IMP
LYASE	
Carbonic anhydrase	Interconversion CO2 and bicarbonate

OTHER	
Aminolevulinic acid dehydratase	heme biosynthesis
Sphingomyelinase	Hydrolysis of sphingomyelin
Glyoxalase	Detoxification of aldehydes
Transcription factor SP1	Regulation of transcription
Transcription factor TFIIIA	Regulation of transcription
Metallothionein	Storage and detoxification of zinc and other metals
Insulin in secretory vesicles	Zinc is used to compact the insulin molecules

Principal Copper Proteins

Ceruloplasmin	iron oxidation, storage and transport of copper
Cu, Zn-superoxide dismutase	Dismutation of superoxide radicals
Tyrosinase	Hydroxylation of tyrosine
Dopamine-β-hydroxylase	Hydroxylation dopamine
Lysyl oxidase	Collagen Synthesis
Cytochrome c oxidase	Respiratory chain
Amino oxidase	Catabolism of histamine and other related molecules
Metallothionein	Storage and detoxification of zinc and other metals

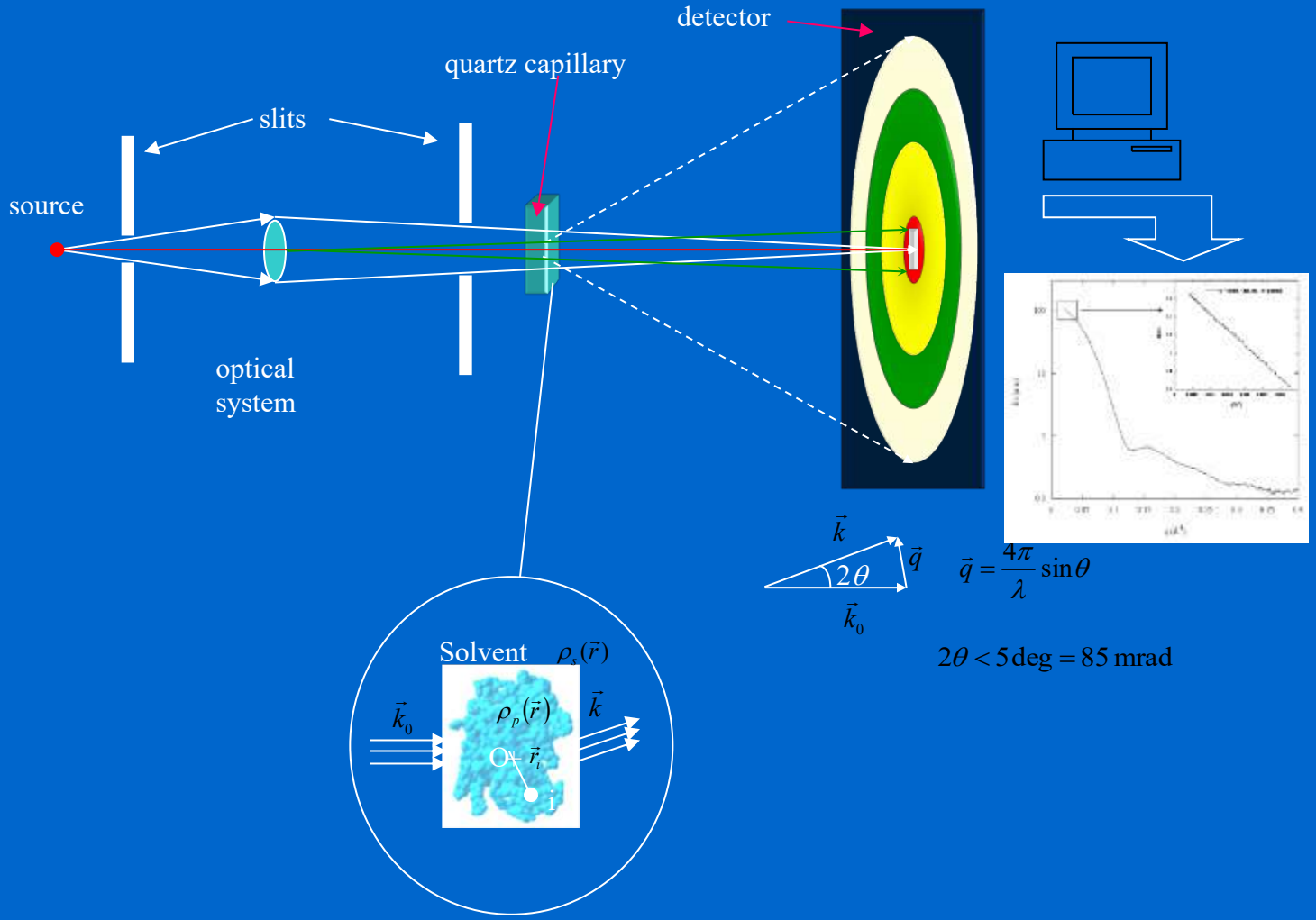
COPPER AND ZINC ABSORPTION

- ✓ The recommended daily dose is 15 mg for zinc
- ✓ The recommended daily dose for copper is between 1.5 a 3.0 mg/day.

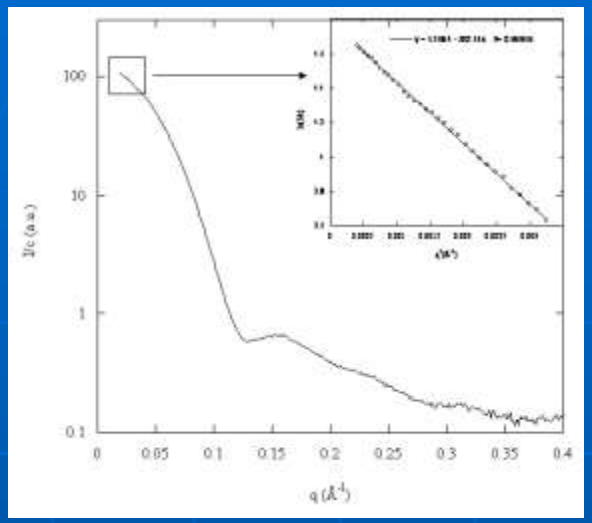
Some modulators for zinc absorption

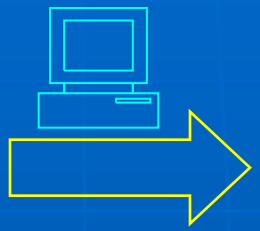
Effector	Absorption
EDTA, ortho-ossichinolina	+ +
Phytates	- -
abundant proteins in the diet	+
Lysine, cysteine, glycine, glutamic acid	+
Low level of iron in the diet	+
High level of iron in the diet	-
Lactose	+
Histidine	+
Ascorbic acid	+
High level of calcium in the diet	-
Copper	-
Cellulosio	-

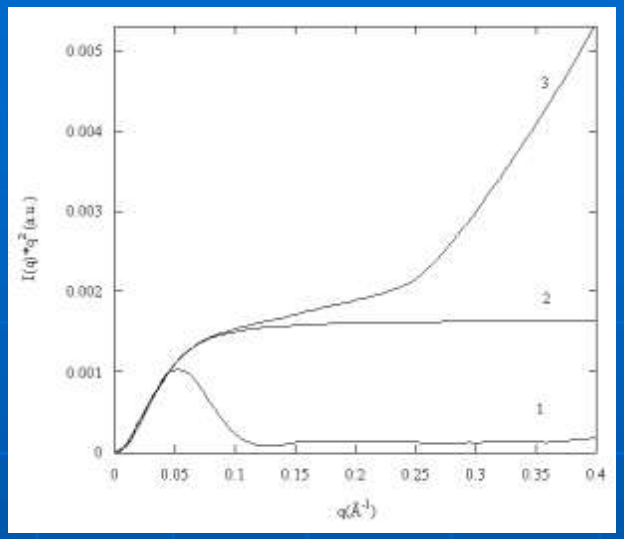
SMALL ANGLE X-RAY SCATTERING

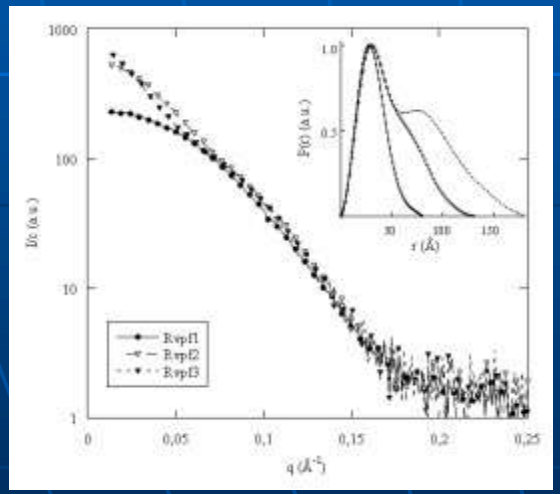


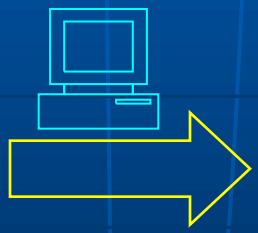
Direct structural parameters and 3D modeling

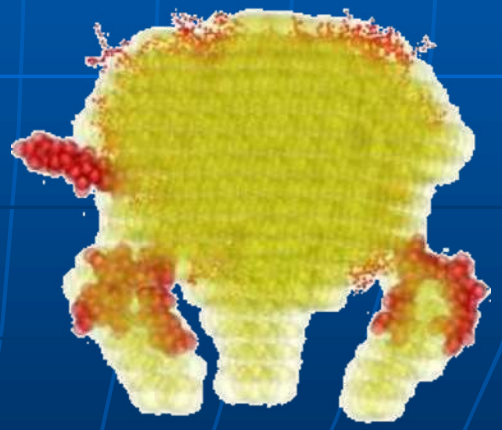








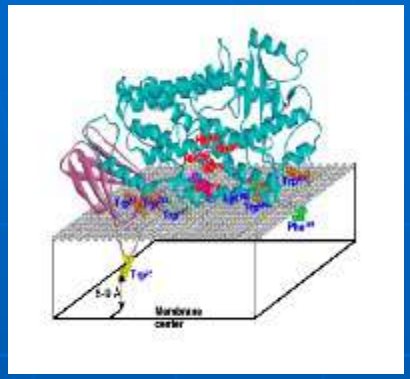




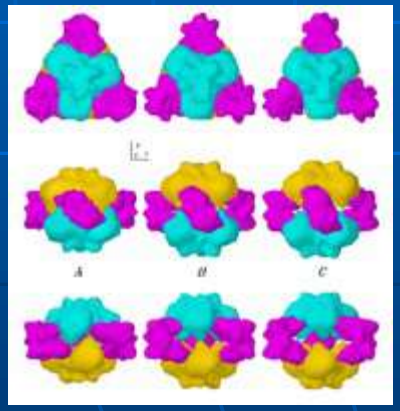
Studies by SAXS: conformational changes of biological macromolecules

Applications to proteins

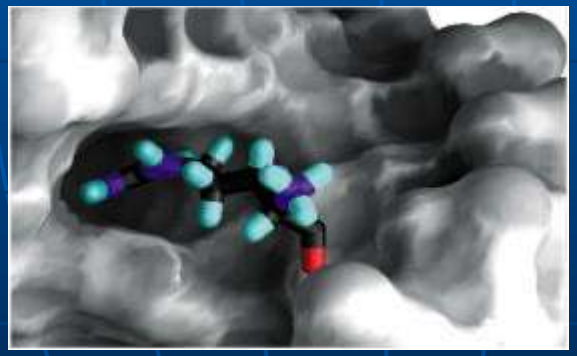
folding-unfolding



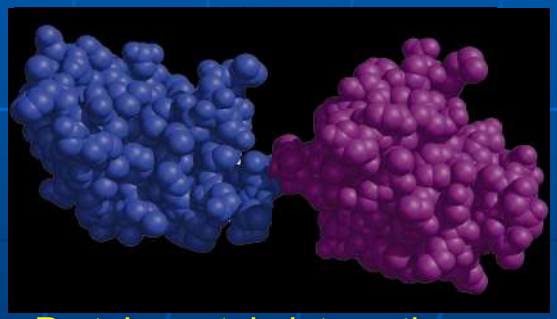
Protein-lipids interactions



Oligomeric organization

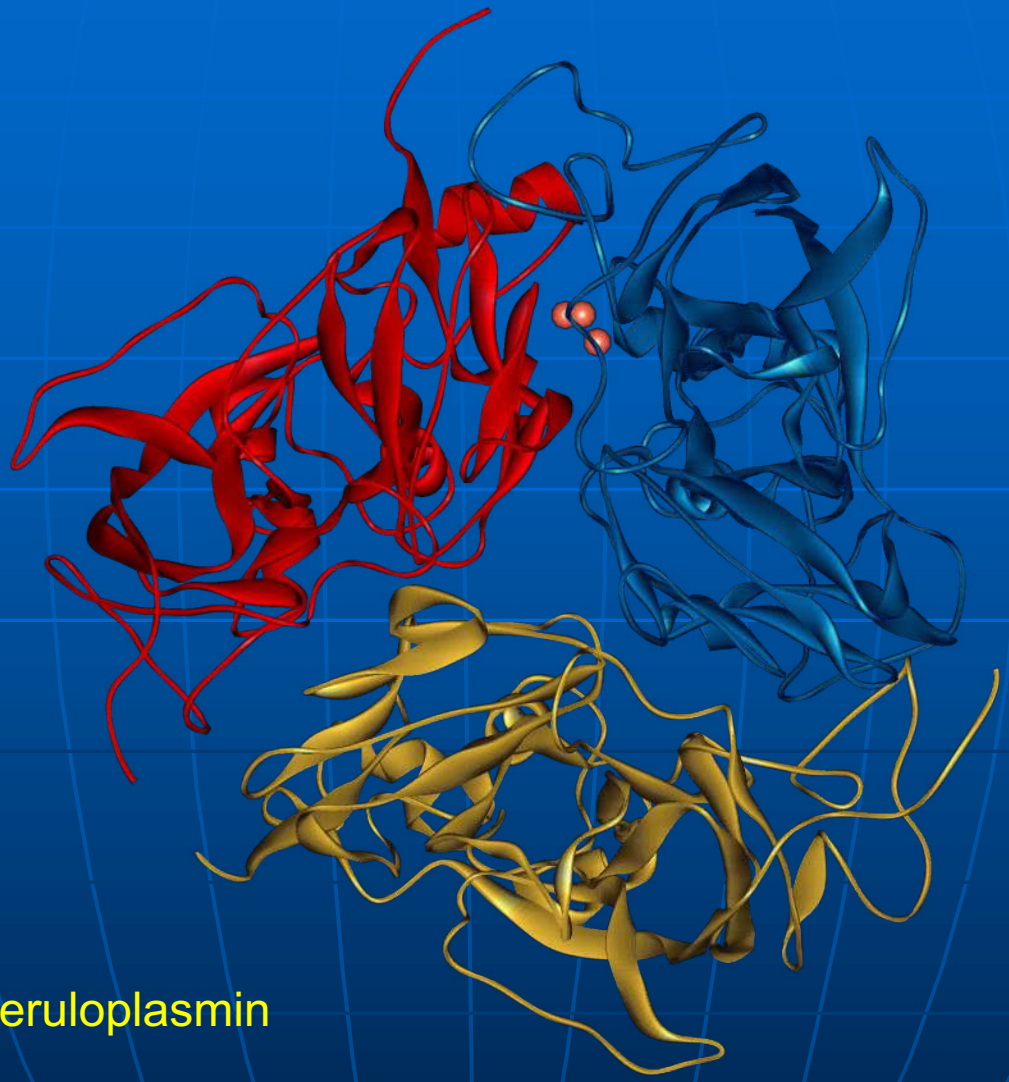


Protein-ligand interactions



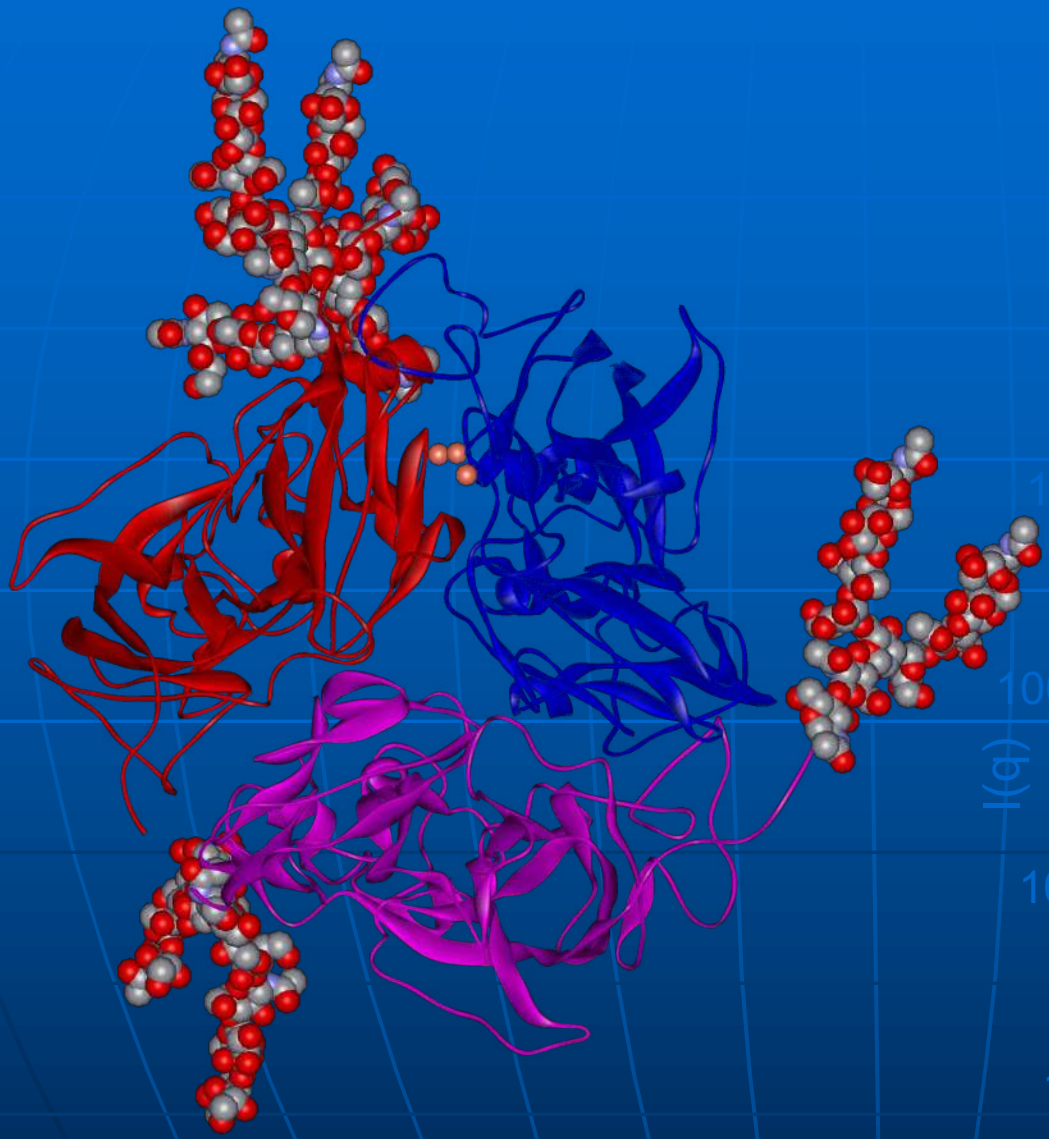
Protein-protein interactions

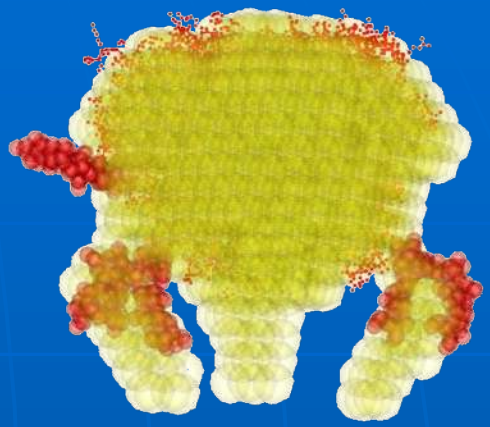
Structural and functional role of the copper ligand in human ceruloplasmin

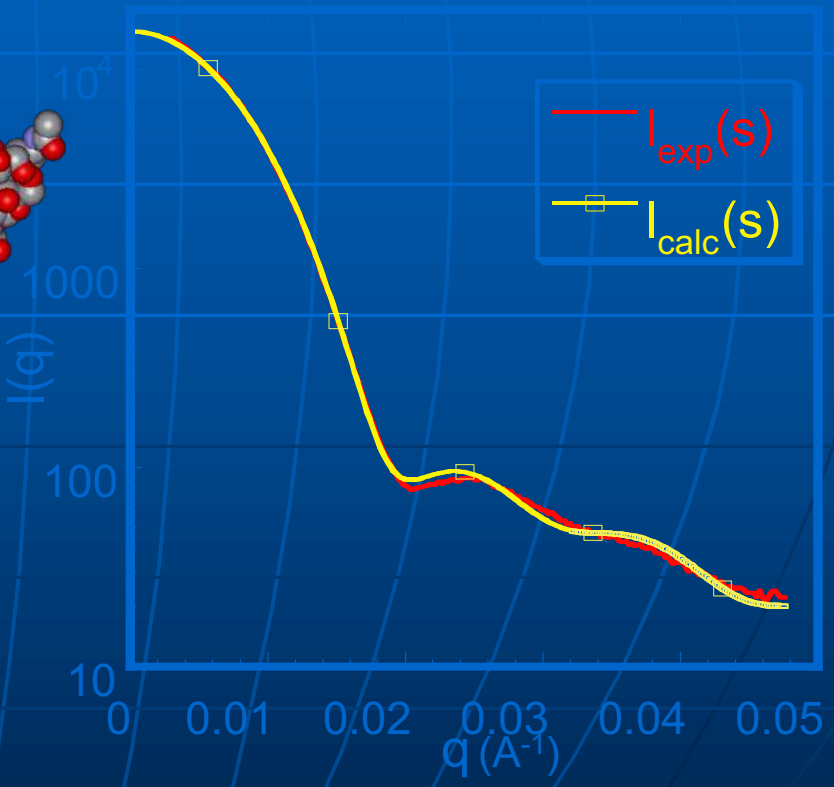


Human Ceruloplasmin

Crystallographic data versus SAXS data

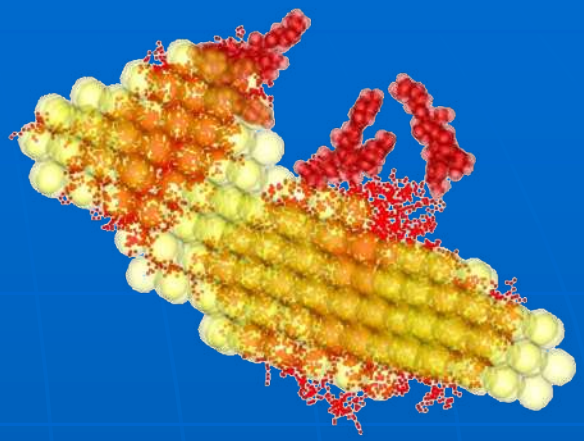


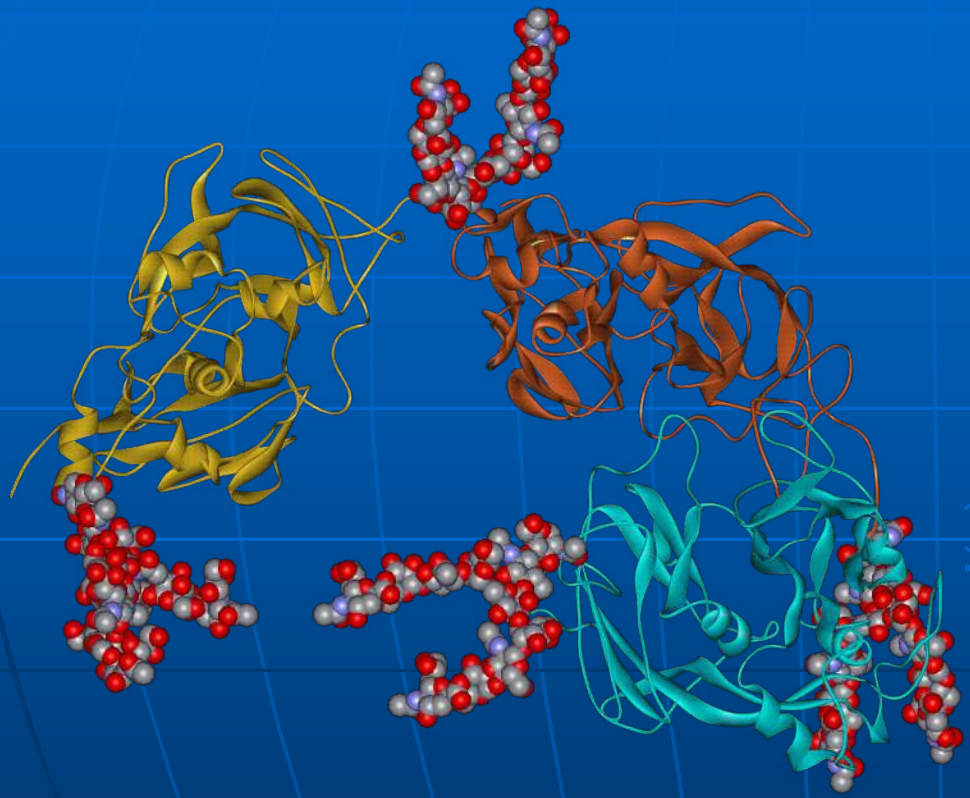


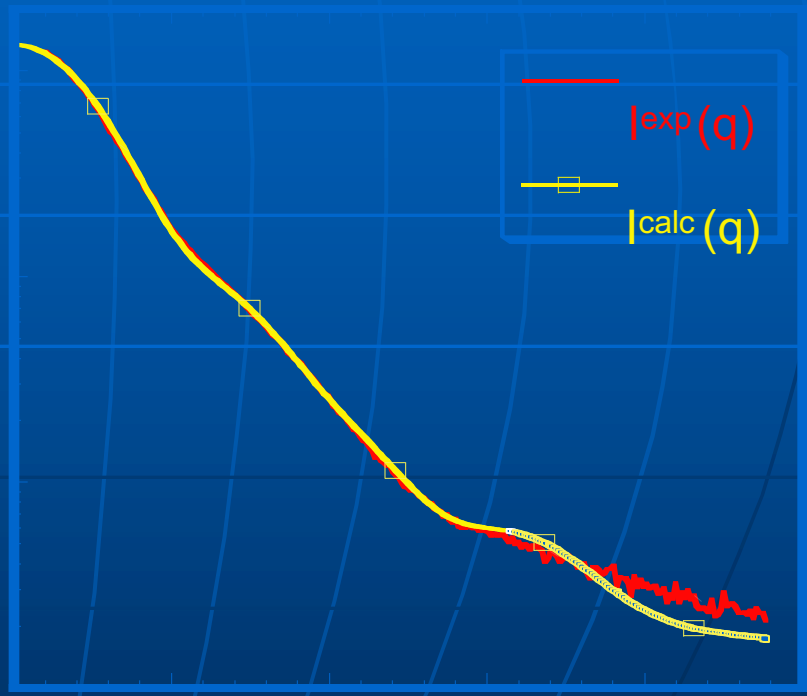


Vachette & Dainese et al., (2002) JBC. 277, 40823.

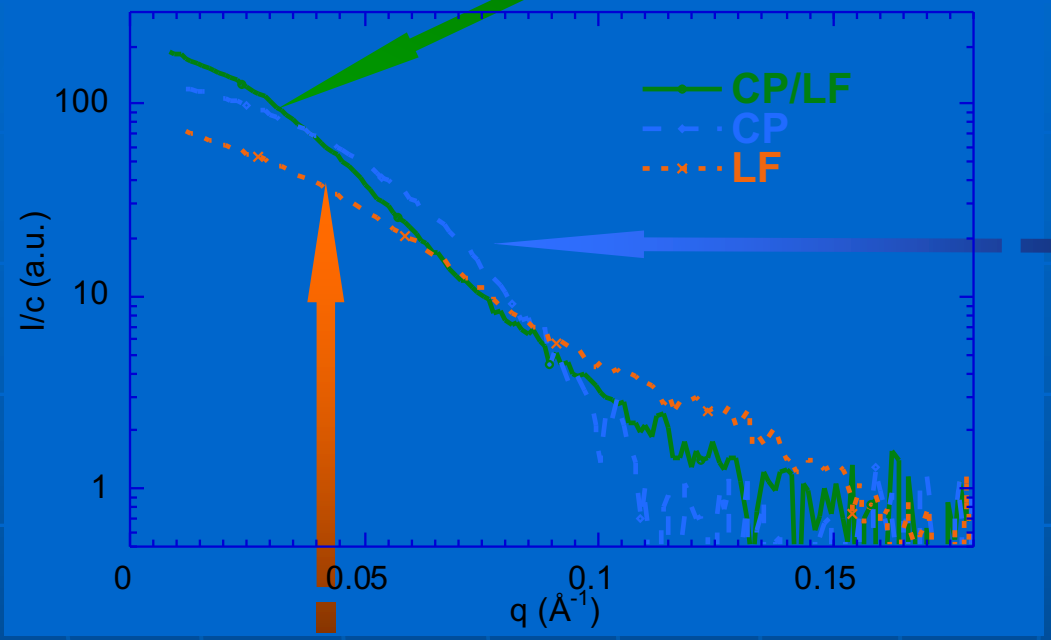
Structure of the apo-ceruloplasmin by SAXS

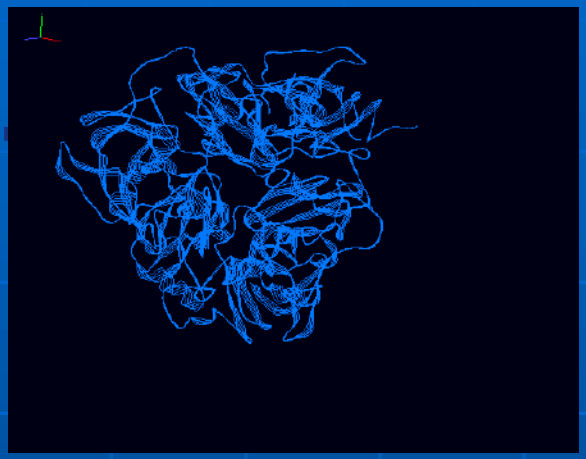


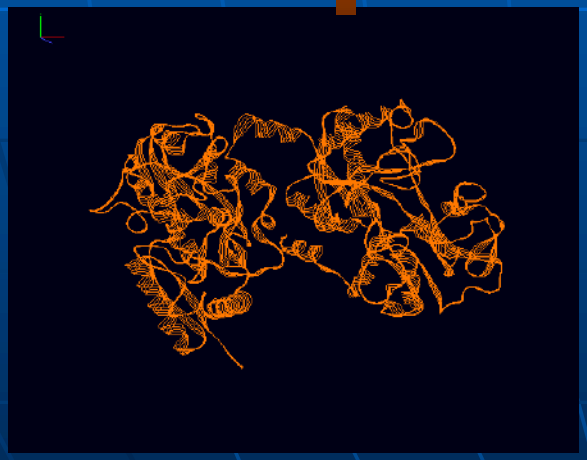




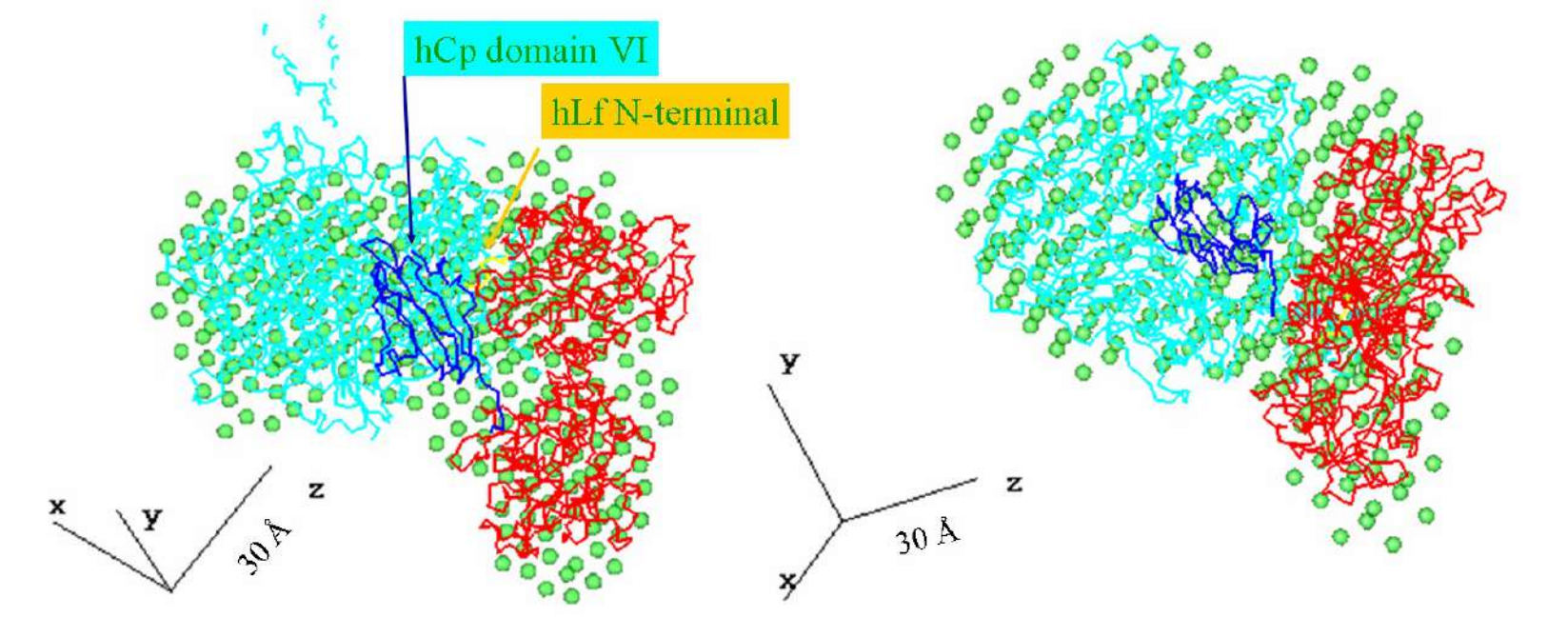
Interaction between human ceruloplasmin and lactoferrin







Ceruloplasmin has the correct orientation to facilitate the iron incorporation into lactoferrin



doi:10.1016/j.jmb.2007.05.089 J. Mol. Biol. (2007) 371, 1038–1046

JMB

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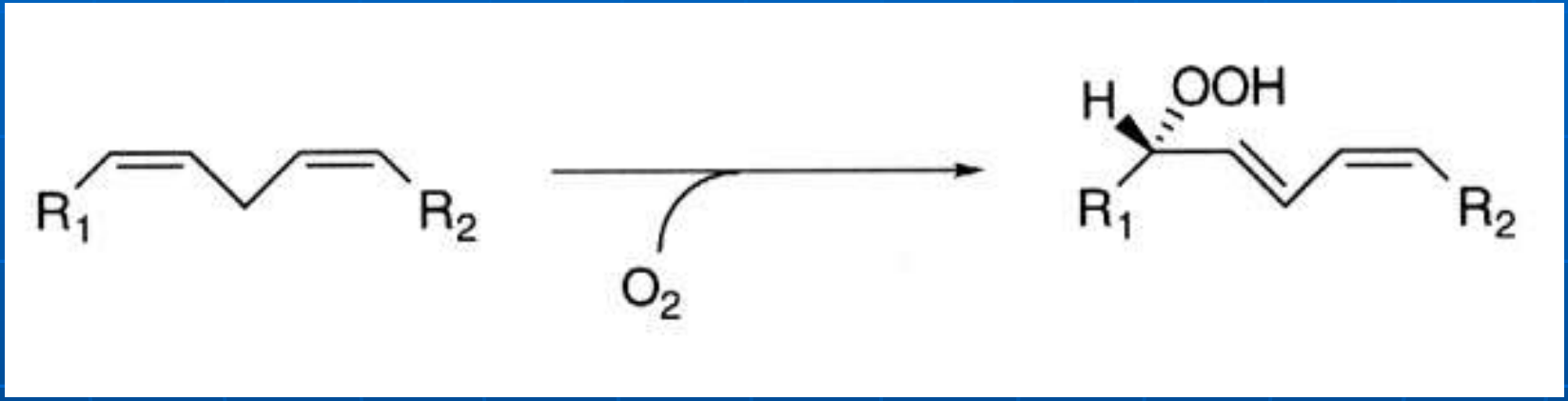
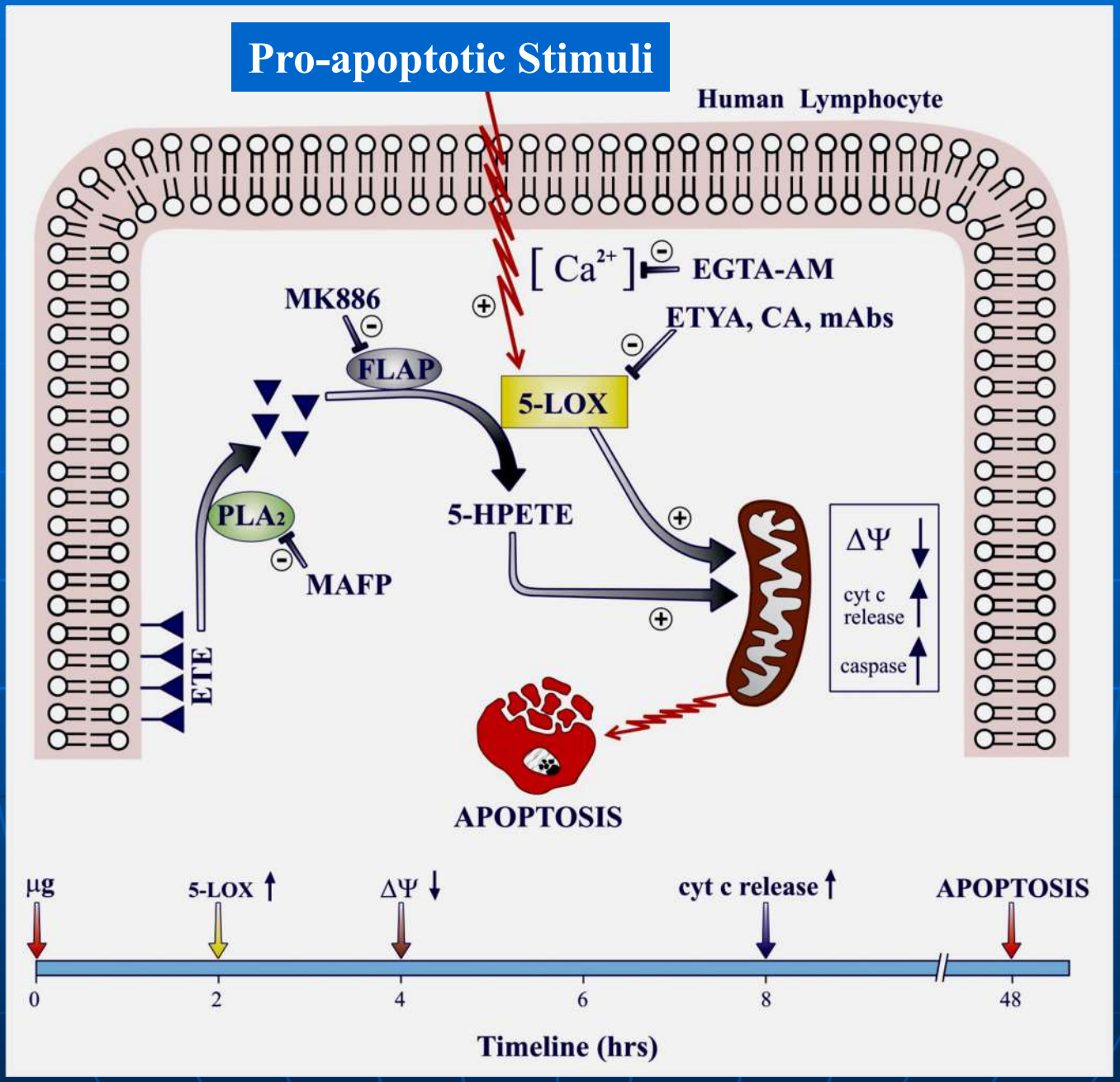
Structural Characterization of the Ceruloplasmin: Lactoferrin Complex in Solution

Annalaura Sabatucci[1], Patrice Vachette[3,4], Vadim B. Vasilyev[5]
Mariano Beltramini[2], Alexey Sokolov[5], Maria Pulina[5]
Benedetto Salvato[2], Clotilde B. Angelucci[1], Mauro Maccarrone[1]
Ivo Cozzani[1] and Enrico Dainese[1]*

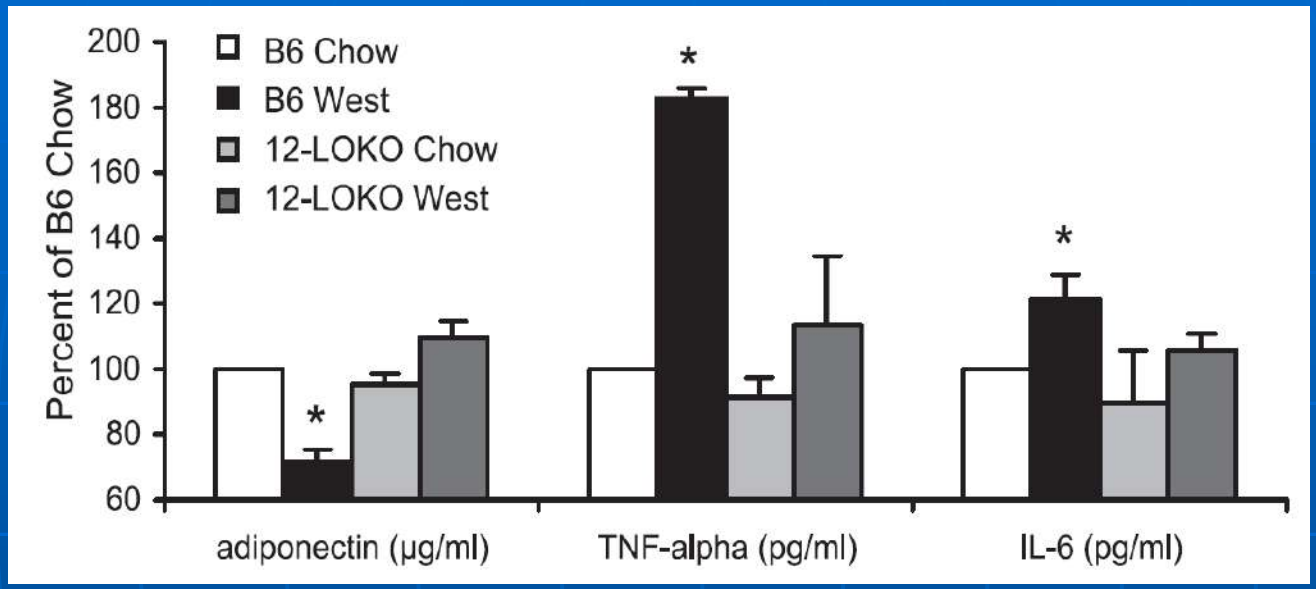
Lipid peroxidation catalyzed by lipoxygenase




Pro-apoptotic Stimuli
Human Lymphocyte
[Ca2+]
EGTA-AM
MK886
ETYA, CA, mAbs
FLAP
5-LOX
PLA2
5-HPETE
MAFP
ETE
ΔΨ
cyt c release
caspase
APOPTOSIS
µg
5-LOX
ΔΨ
cyt c release
APOPTOSIS
0
2
4
6
8
48
Timeline (hrs)

The role of lipoxygenase in CVD

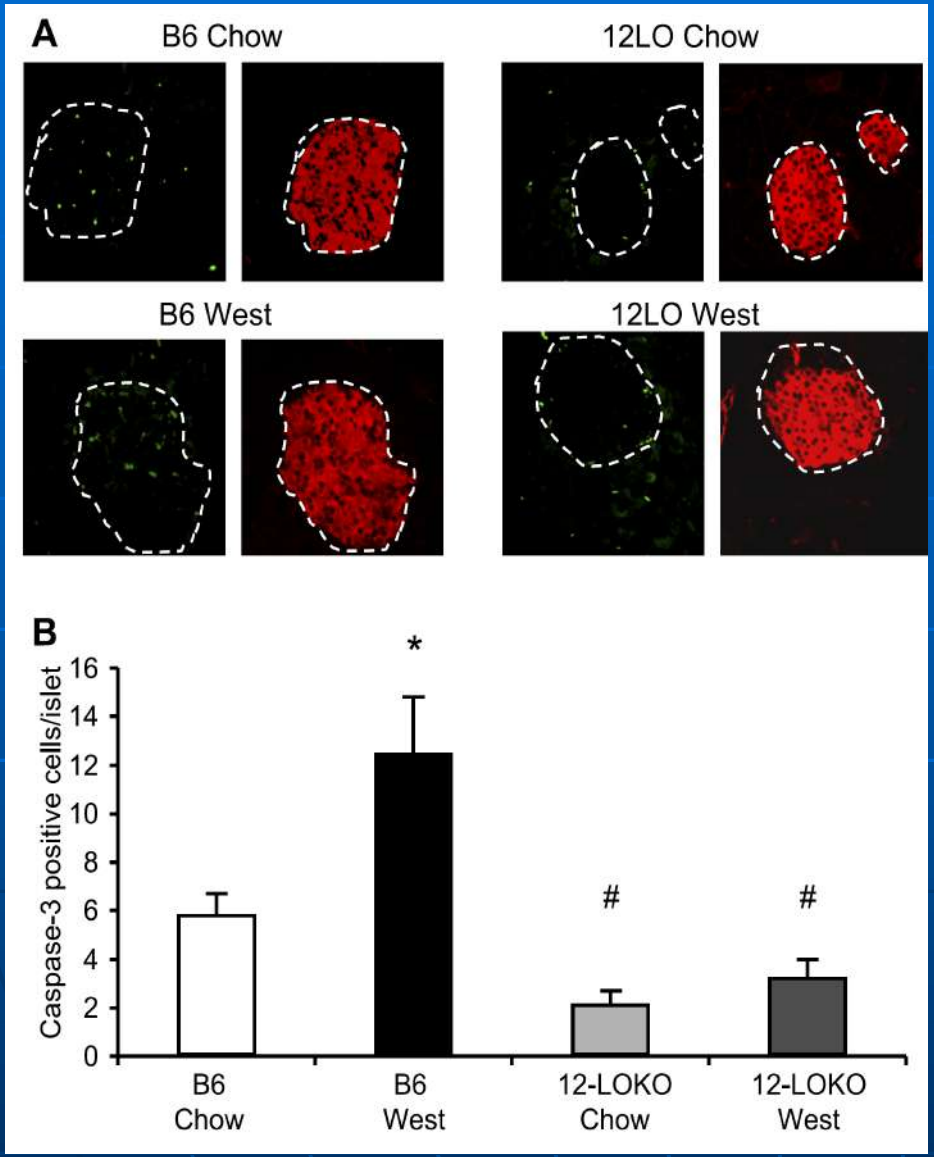
Studies on 12/15-lipoxigenase knock-out



Craig et al,. (2008) Am J Physiol Endocrinol Metab 295: E1065.

Circulating proinflammatory cytokines, tumor necrosis factor-α and interleukin-6, are increased in western B6 mice but not 12-LOKO mice, whereas the protective adipokine, adiponectin, is decreased only in western B6 mice.

The role of lipoxygenase in CVD



12/15-Lipoxygenase activity is significantly elevated by western diet in islets from B6 mice. Pancreatic islets from 12-LOKO mice do not show western-diet-induced islet hyperplasia or increases in caspase-3 apoptotic staining observed in western-fed B6 mice.

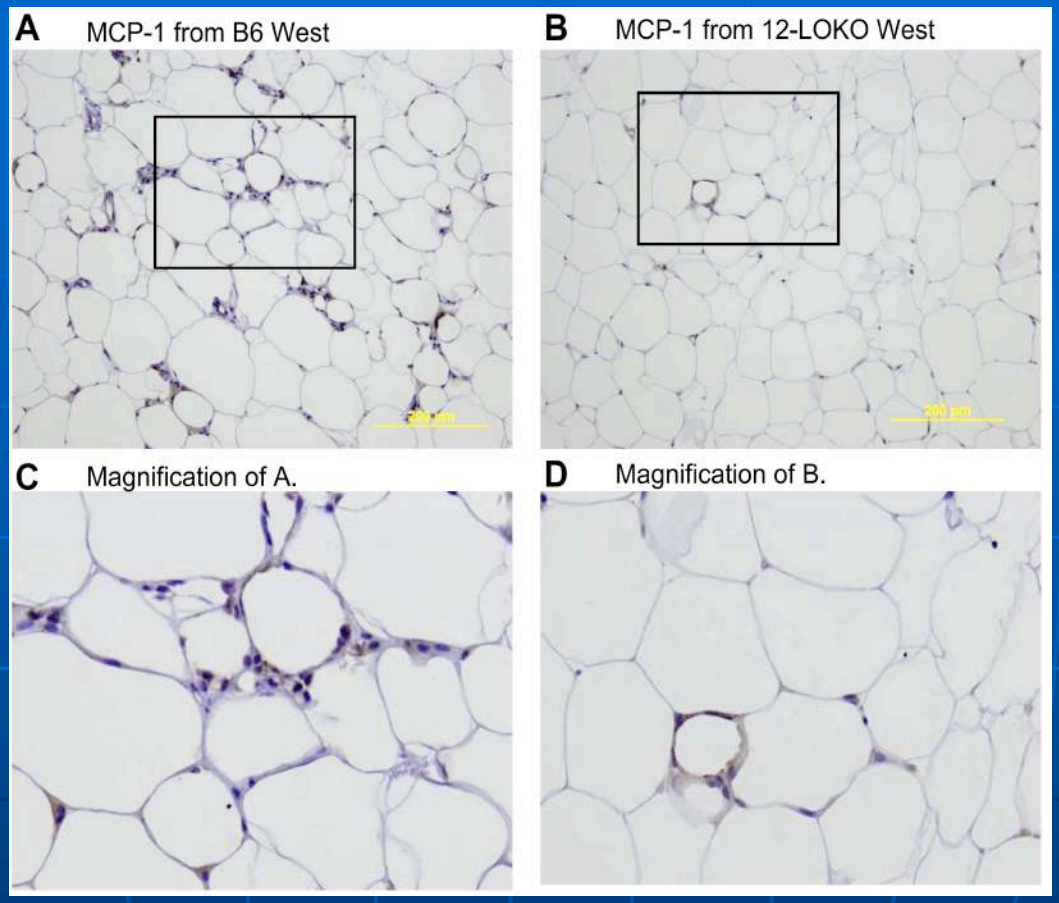
Thus, apoptosis is increased among western-fed B6 mice but not 12-LOKO mice.

Islets from 12-LOKO mice are also protected from reduced glucose-stimulated insulin secretion observed in islets from western-fed B6 mice.

Craig et al,. (2008) Am J Physiol Endocrinol Metab 295: E1065.

The role of lipoxygenase in CVD

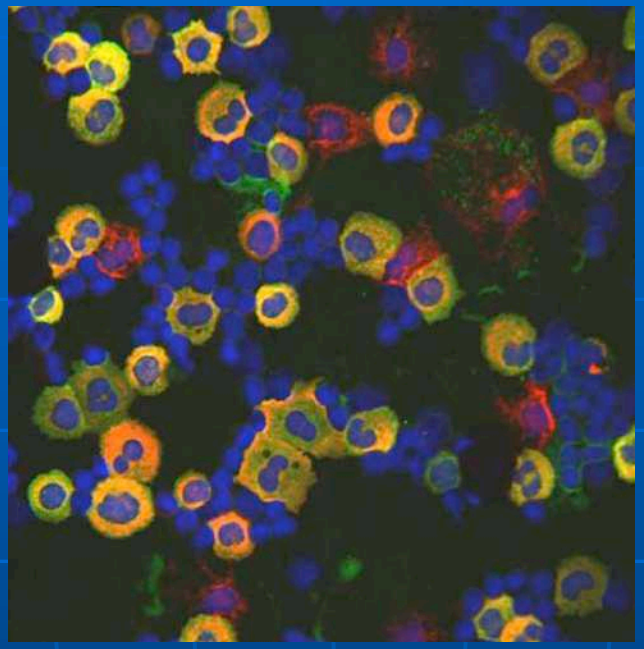
12/15-Lipoxygenase activation plays a crucial role in western-diet-induced damage in visceral fat and islets.

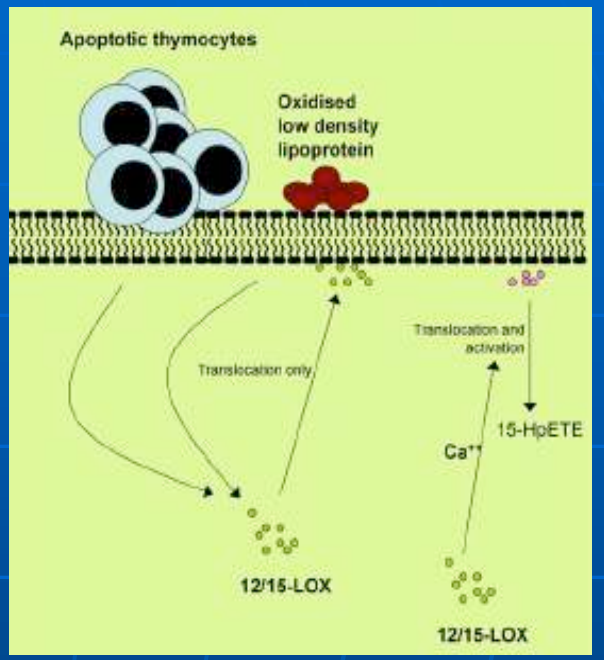


Craig et al,. (2008) Am J Physiol Endocrinol Metab 295: E1065.

In visceral fat, macrophage numbers and monocyte chemoattractant protein-1 expression are elevated in western B6 mice but not 12-LOKO mice.

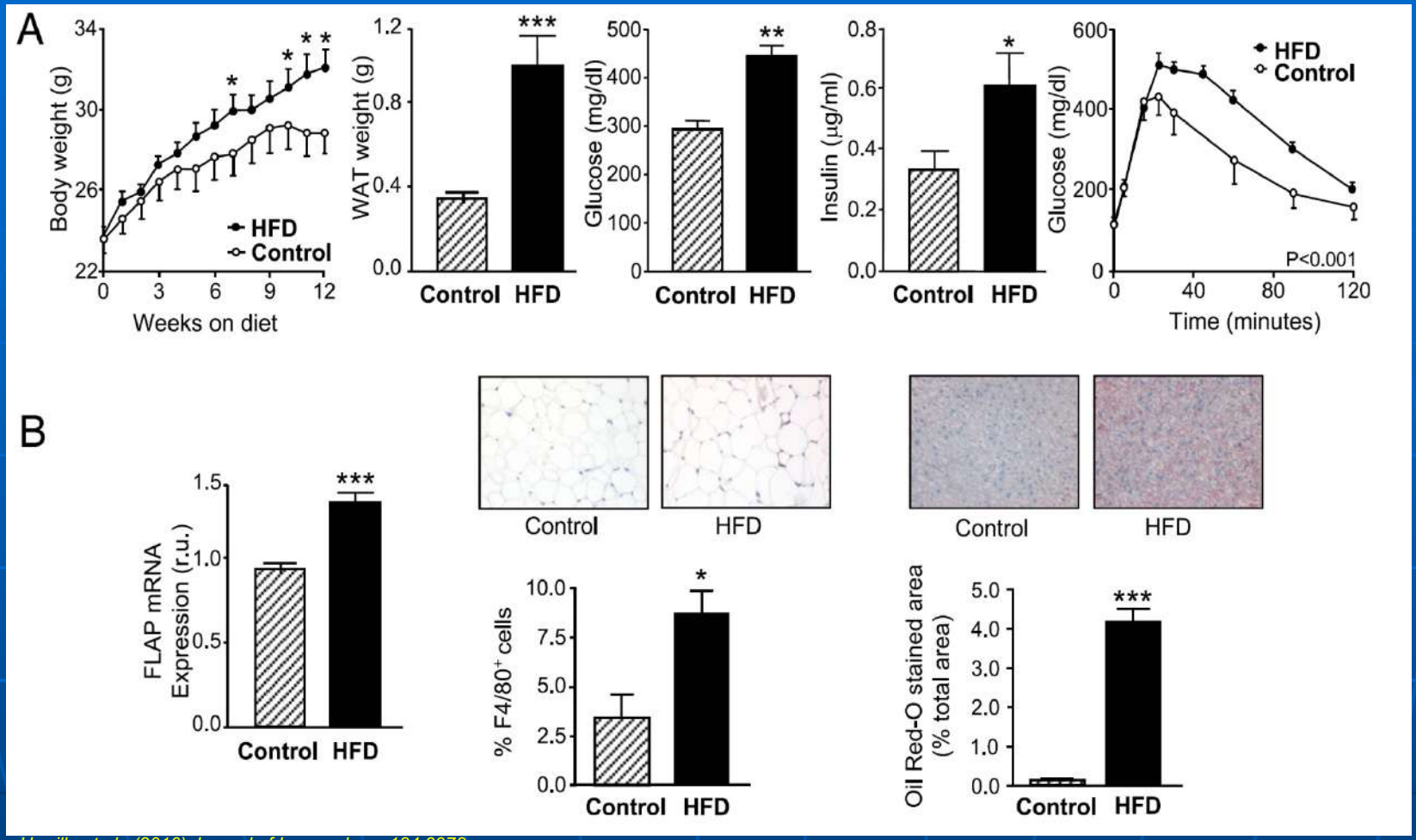
The role of 12/15 lipoxygenase





Activation and translocation of lipoxygenase in macrophages is mediated by the binding of oxidized LDL to macrophages.

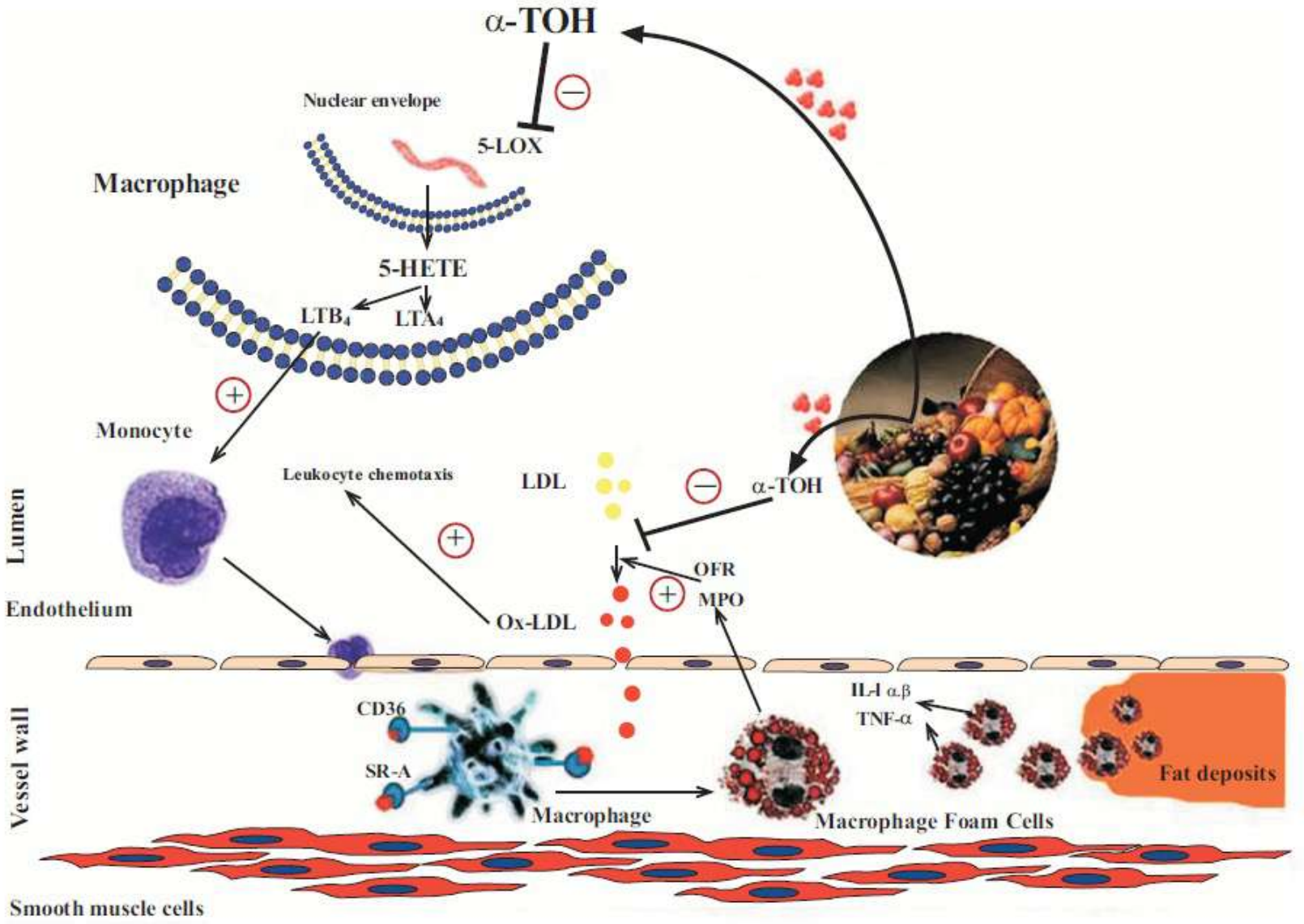
The role of lipoxygenase in CVD



Horrillo et al., (2010) Journal of Immunology, 184:3978.

In mice with dietary obesity, elevated 5-lipoxygenase-activating protein (FLAP) expression in adipose tissue is paralleled with macrophage infiltration, increased circulating FFA levels, and hepatic steatosis, phenomena that can be reversed by FLAP inhibition.

Vitamin E (α-TOH) and 5-lipoxygenase



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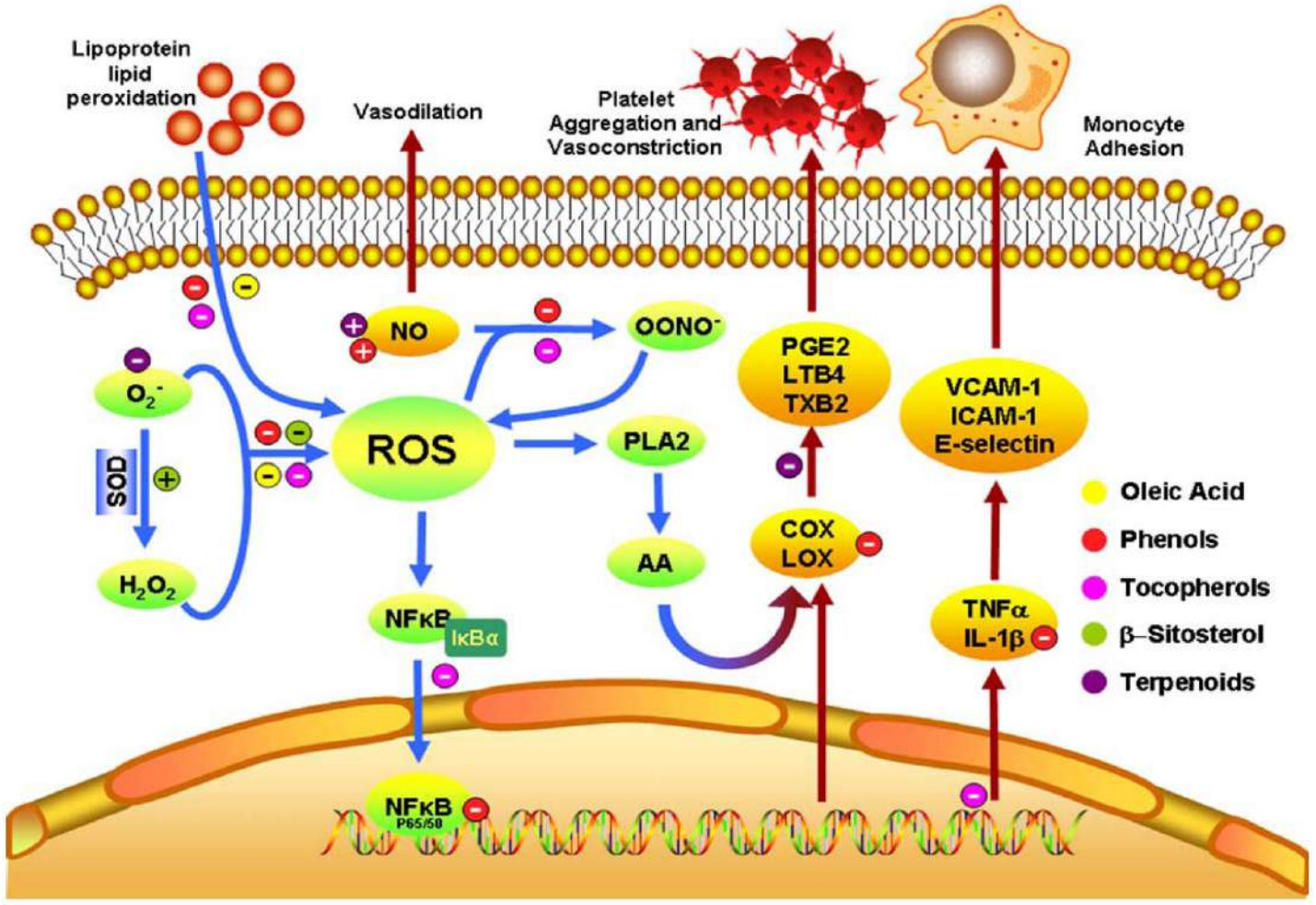
Journal of Nutritional Biochemistry

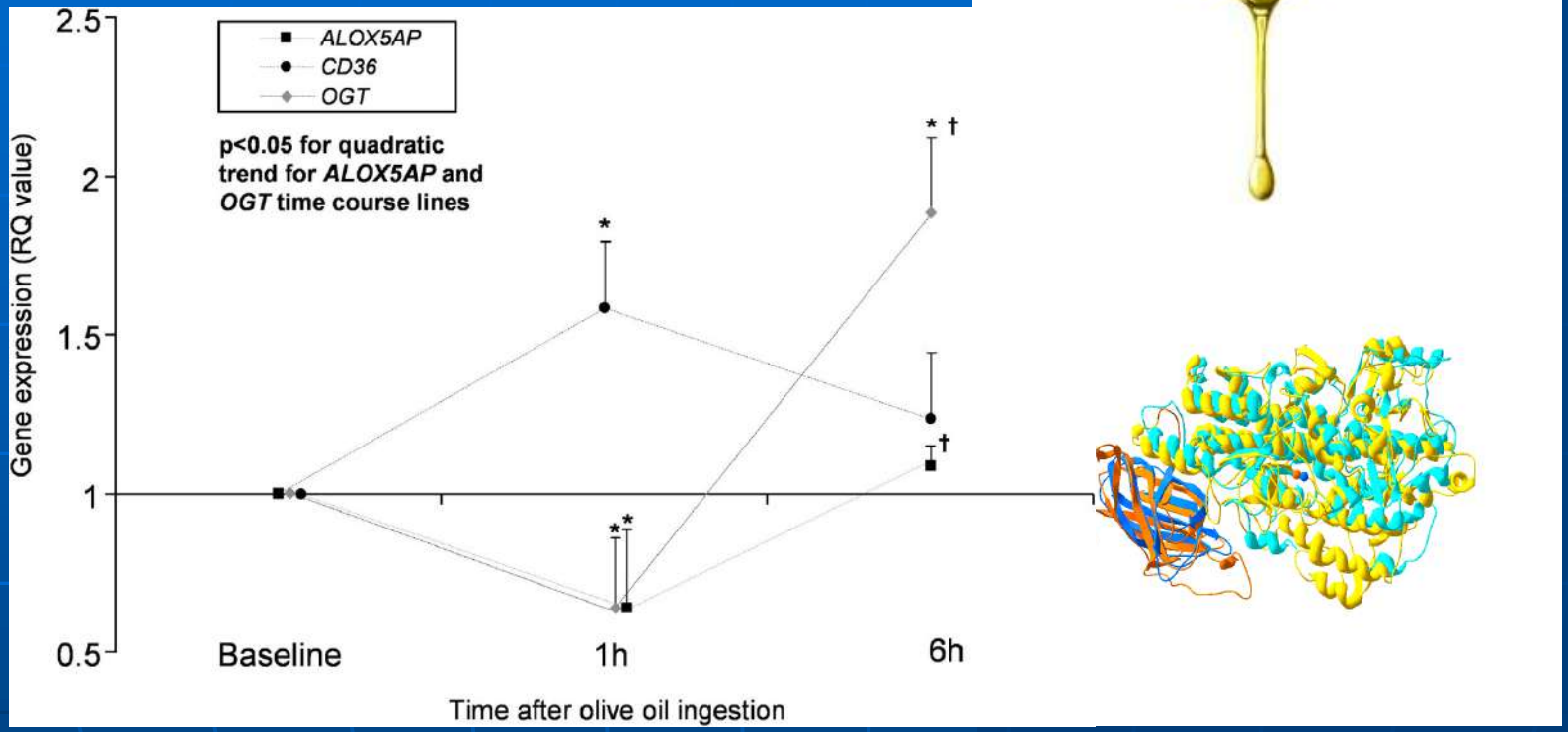
REVIEWS: CURRENT TOPICS

The role of virgin olive oil components in the modulation of endothelial function

Javier S. Perona, Rosana Cabello-Moruno, Valentina Ruiz-Gutierrez*
Nutrition and Lipid Metabolism Group, Instituto de la Grasa (CSIC), Seville 41012, Spain
Received 30 September 2005; received in revised form 27 October 2005; accepted 1 November 2005

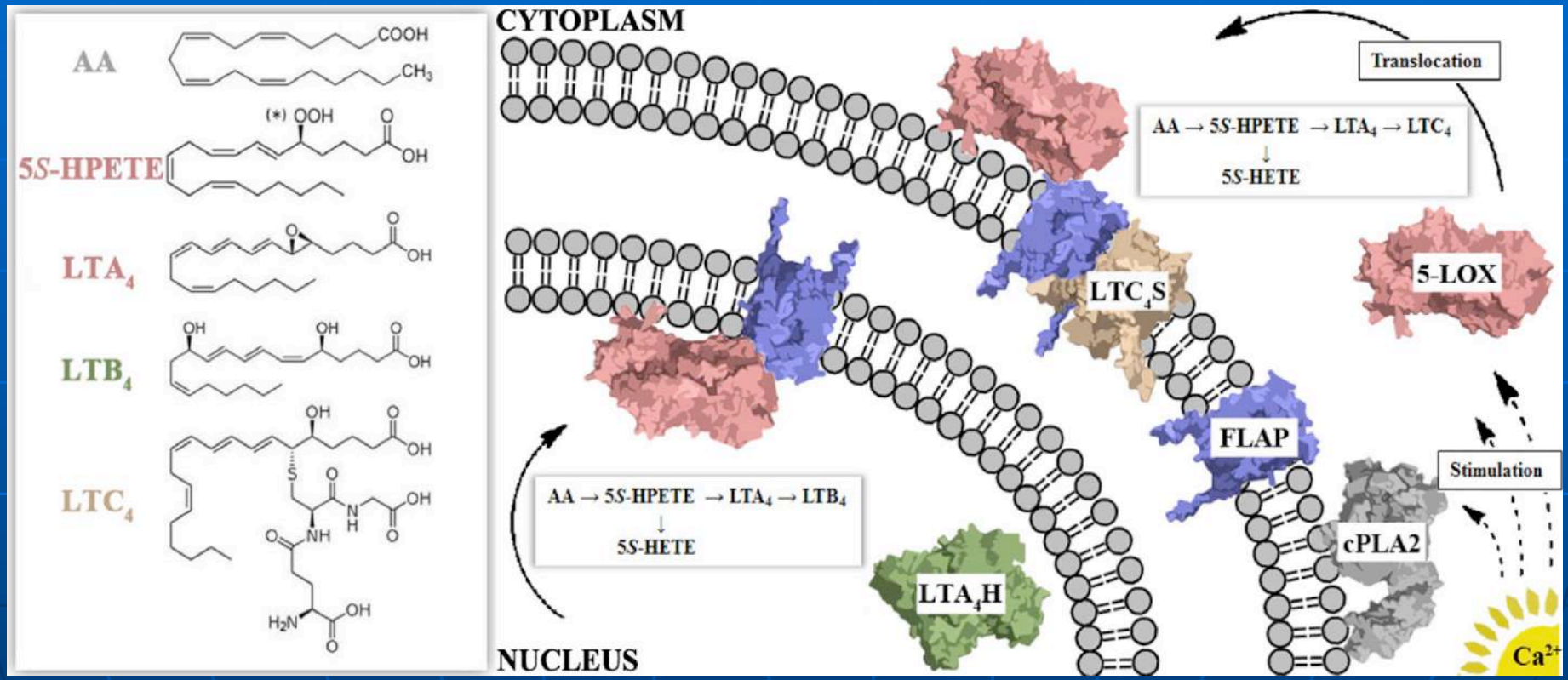


Olive oil against inflammation



Time course of changes in the expression of insulin sensitivity-related genes after an acute load of virgin olive oil. A downregulation was observed in O-linked-N-acetylglucosamine transferase and 5-lipoxygenase-activating protein (FLAP) genes.

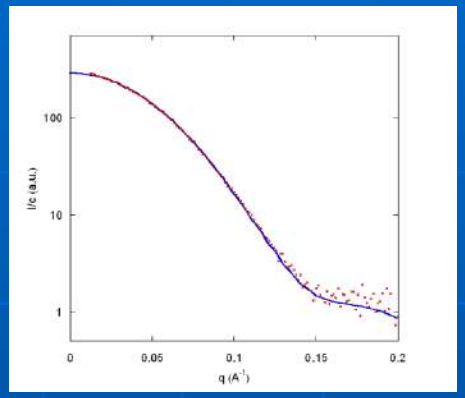
Initial events in leukotriens biosynthesis: the role of five lipoxygenase activating protein (FLAP)

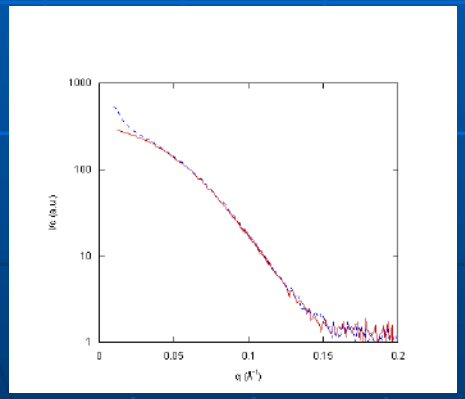


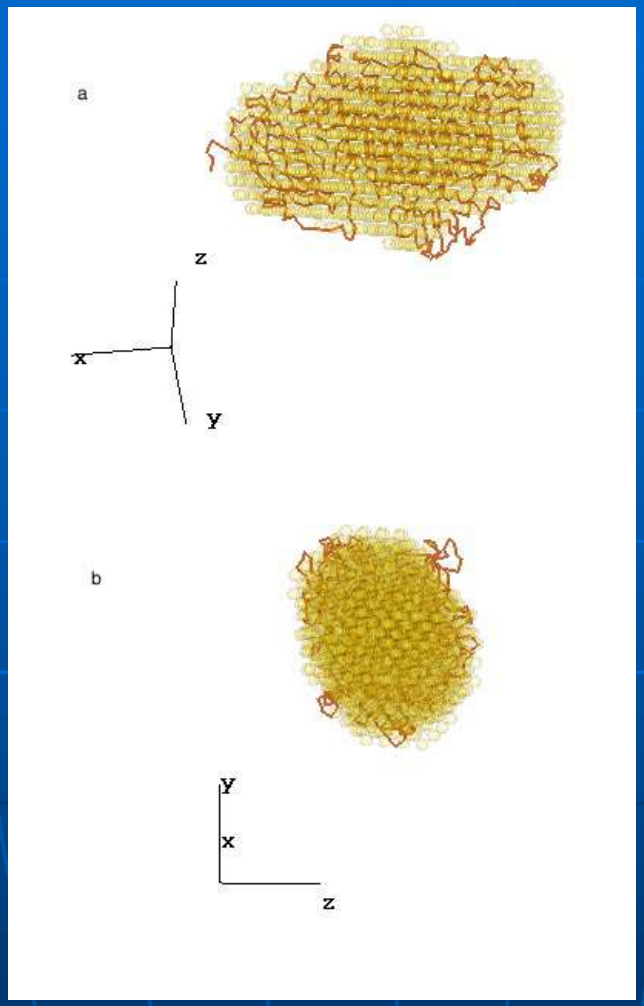
Iron and inflammatory diseases

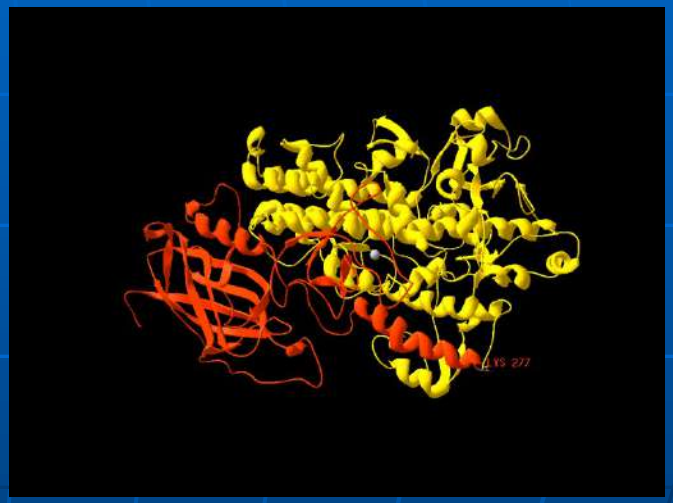
- High iron content has also been reported in in atherosclerotic lesions. However, the precise mechanism(s) underlying the relationship between iron and 5-LOX are as yet unclear.
- The effect of inflammation on the regulation of iron metabolism is widely recognized, and a dysregulated iron homeostasis is a cornerstone of acute and chronic inflammatory processes involving cell-mediated immunity.
- Iron-associated oxidant damage is intimately involved with neurological disorders, from Alzheimer's disease to multiple sclerosis, where the accumulation of iron within microglia/macrophages has been documented.
- To gain insight into the mechanism(s) by which iron activates lipoxygenases, and in particular to assess whether iron effects are principally due to direct enzyme activation and membrane association, we studied the role of iron in the a purified lipoxygenase, and we exposed cultured macrophages to exogenous iron.

The structural features of lipoxygenases



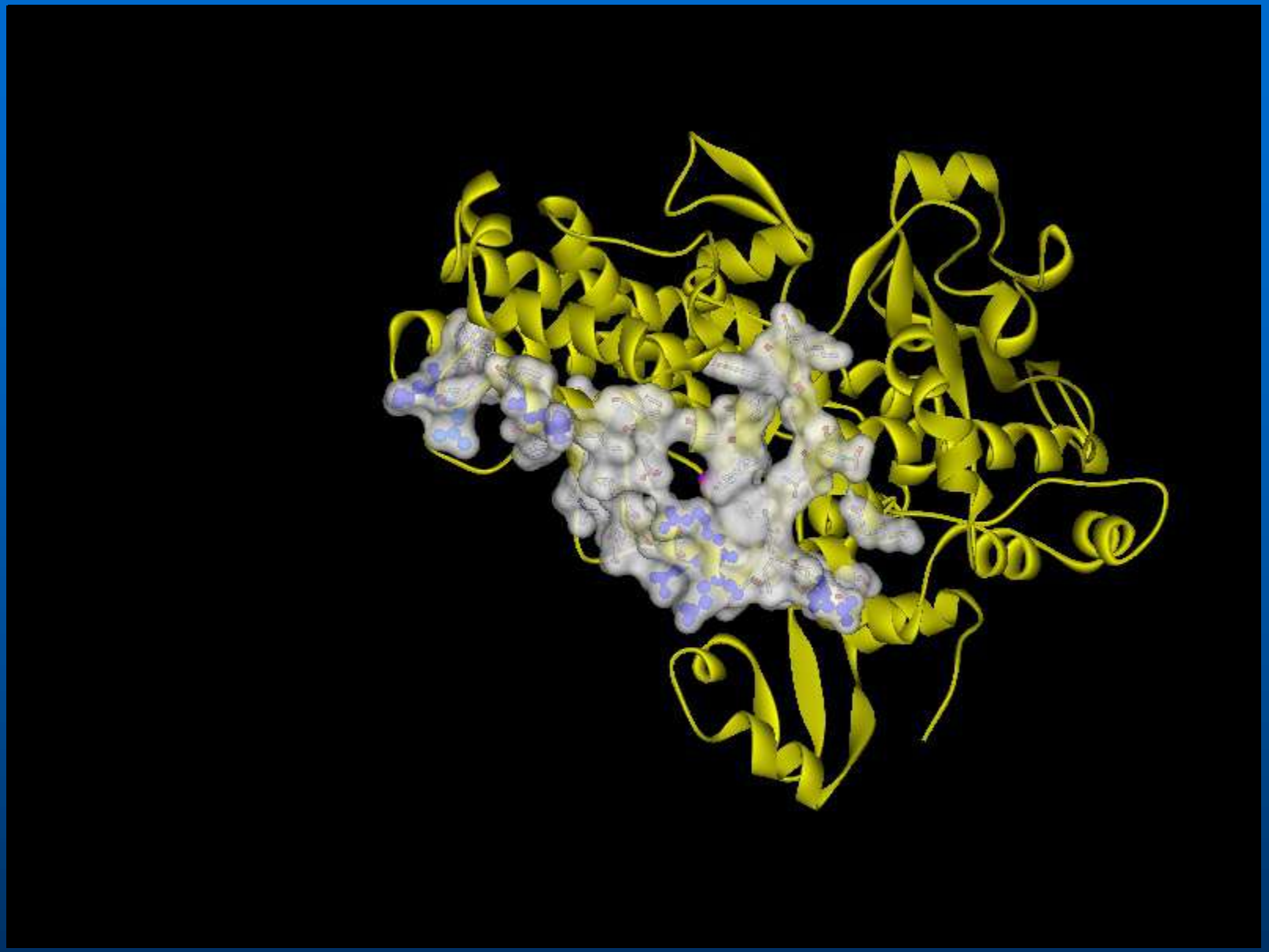






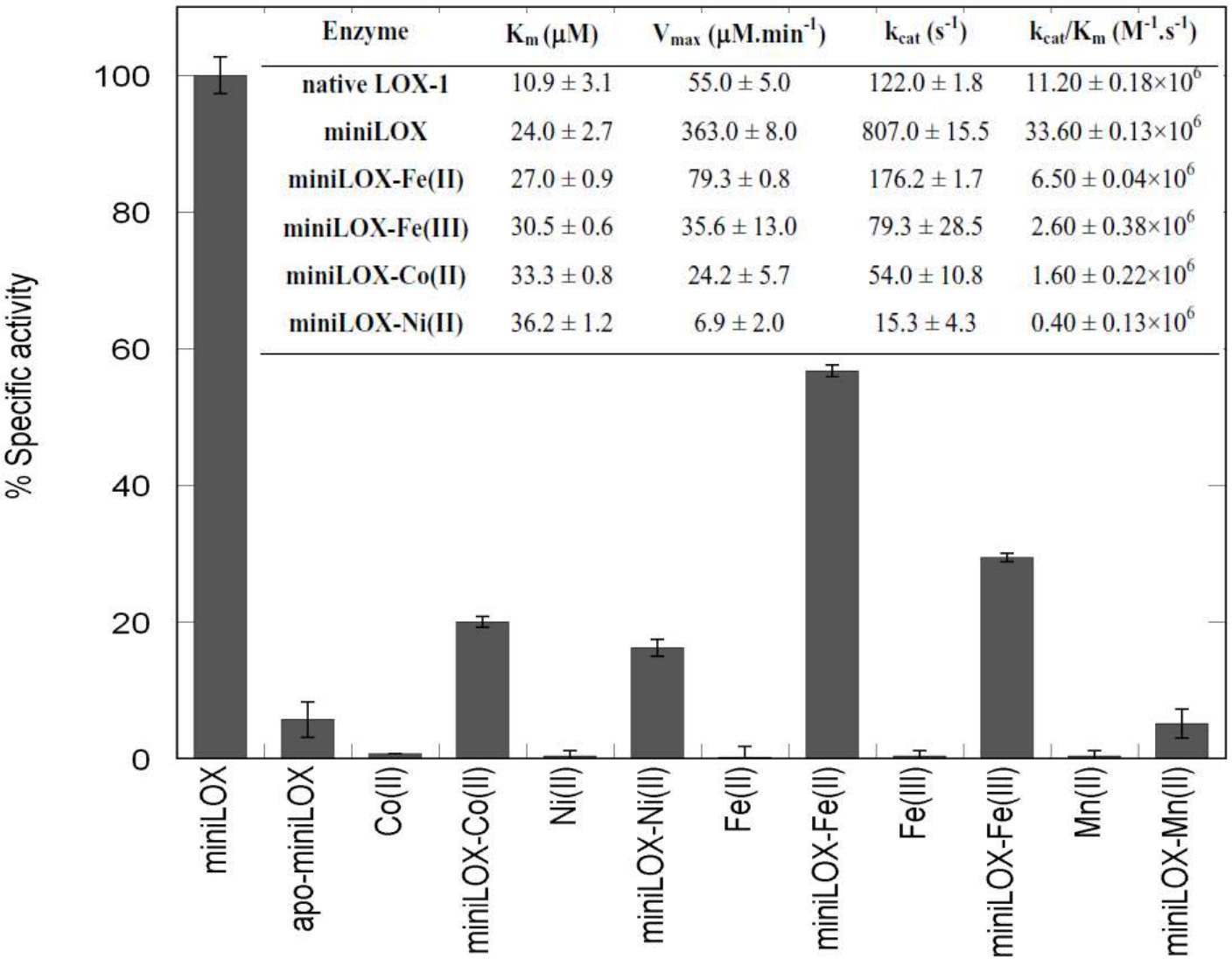
Dainese et al., (2005) *JMB* 349,143-152.

We took advantage of a “mini-lipoxygenase”



Iron extraction, reconstitution and substitution have been successfully applied to miniLOX

Enzyme	K_m (μM)	V_{max} (μM.min^{-1})	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1}.s^{-1})
native LOX-1	10.9 ± 3.1	55.0 ± 5.0	122.0 ± 1.8	11.20 ± 0.18×10^6
miniLOX	24.0 ± 2.7	363.0 ± 8.0	807.0 ± 15.5	33.60 ± 0.13×10^6
miniLOX-Fe(II)	27.0 ± 0.9	79.3 ± 0.8	176.2 ± 1.7	6.50 ± 0.04×10^6
miniLOX-Fe(III)	30.5 ± 0.6	35.6 ± 13.0	79.3 ± 28.5	2.60 ± 0.38×10^6
miniLOX-Co(II)	33.3 ± 0.8	24.2 ± 5.7	54.0 ± 10.8	1.60 ± 0.22×10^6
miniLOX-Ni(II)	36.2 ± 1.2	6.9 ± 2.0	15.3 ± 4.3	0.40 ± 0.13×10^6

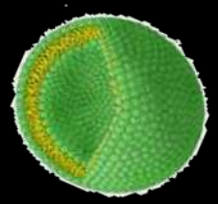


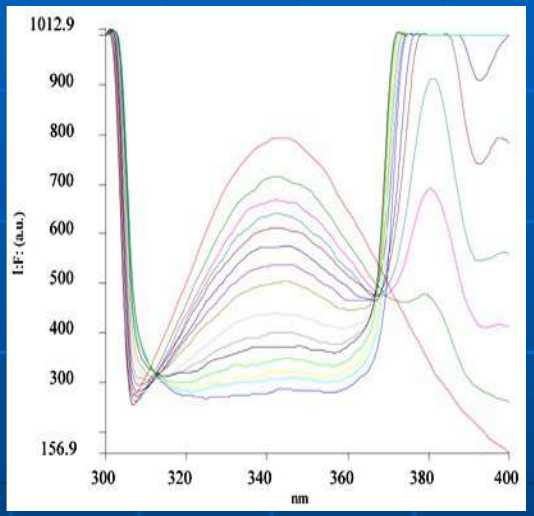
Iron affects the membrane binding of lipoxygenase

F1 = fluorescence donors (Trp of the protein)
F2 = fluorescence acceptors (Dansyl-DHPE)

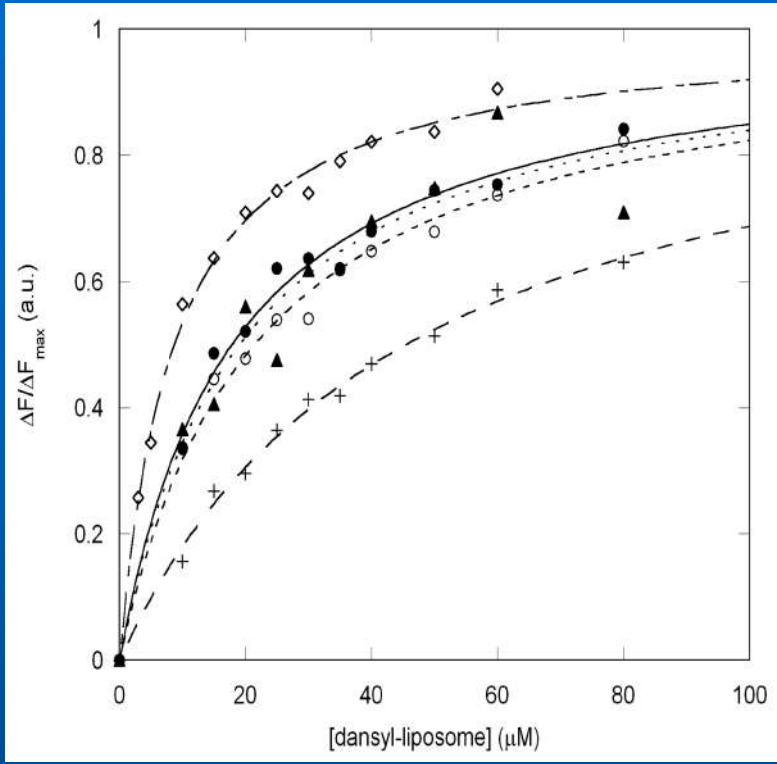
FRET

excitation → *F1* → *F2* → *F2 emission*
F1 quenching





Membrane binding affinity is modulated by the iron content



Protein form	K_d (μM)
(●) LOX-1	17.9 ± 2.0
(◊) miniLOX	9.2 ± 1.0
(+) apo-miniLOX	45.4 ± 4.3
(○) miniLOX-Fe(II)	21.4 ± 2.4
(▲) miniLOX-Fe(III)	18.9 ± 5.5

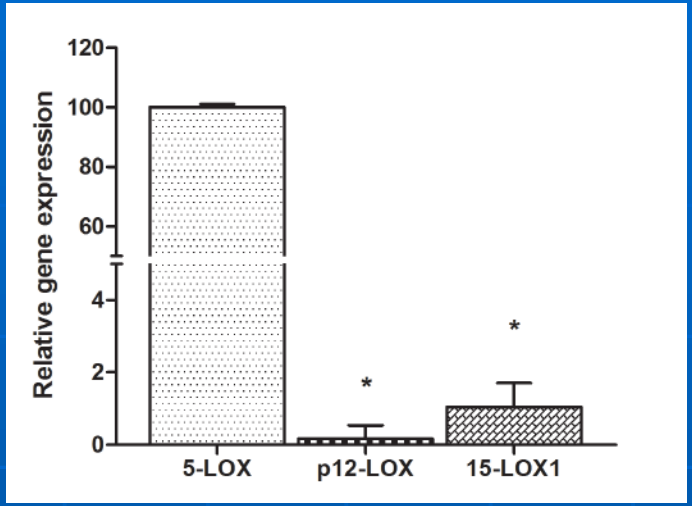
The FASEB Journal • Research Communication

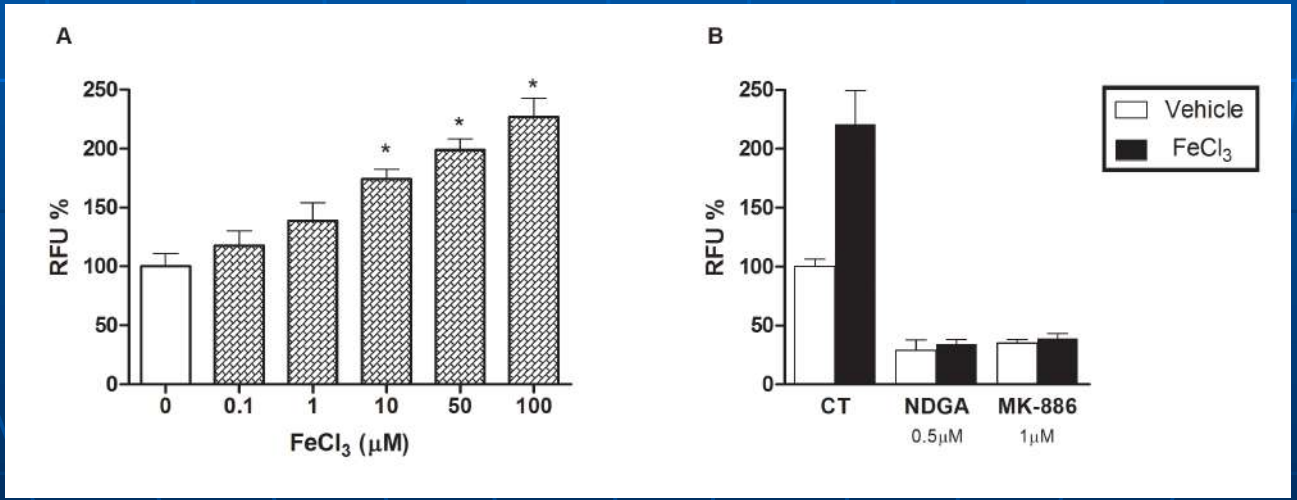
A novel role for iron in modulating the activity and membrane-binding ability of a trimmed soybean lipoxygenase-1

Enrico Dainese,*,[1,2] Clotilde B. Angelucci,*,[1] Annalaura Sabatucci,* Vincenzo De Filippis,[†] Giampiero Mei,[‡] and Mauro Maccarrone*,[§,2]

*Department of Biomedical Sciences, University of Teramo, Teramo, Italy; [†]Department of Pharmacological Sciences, University of Padua, Padua, Italy; [‡]Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Rome, Italy; and [§]European Center for Brain Research/Santa Lucia Foundation, Rome, Italy

Effect of exogenous iron on 5-LOX activity in THP-1 macrophages





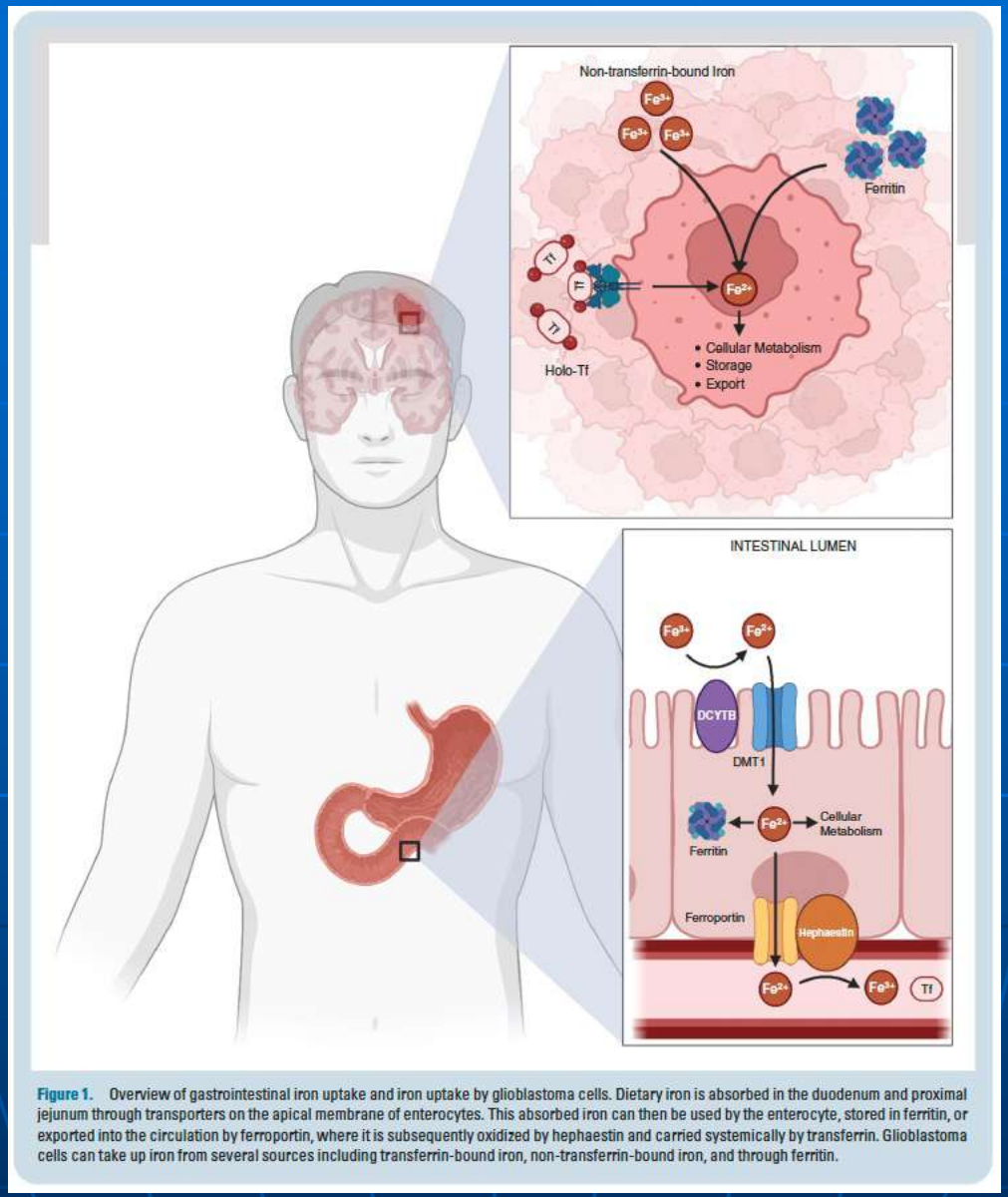
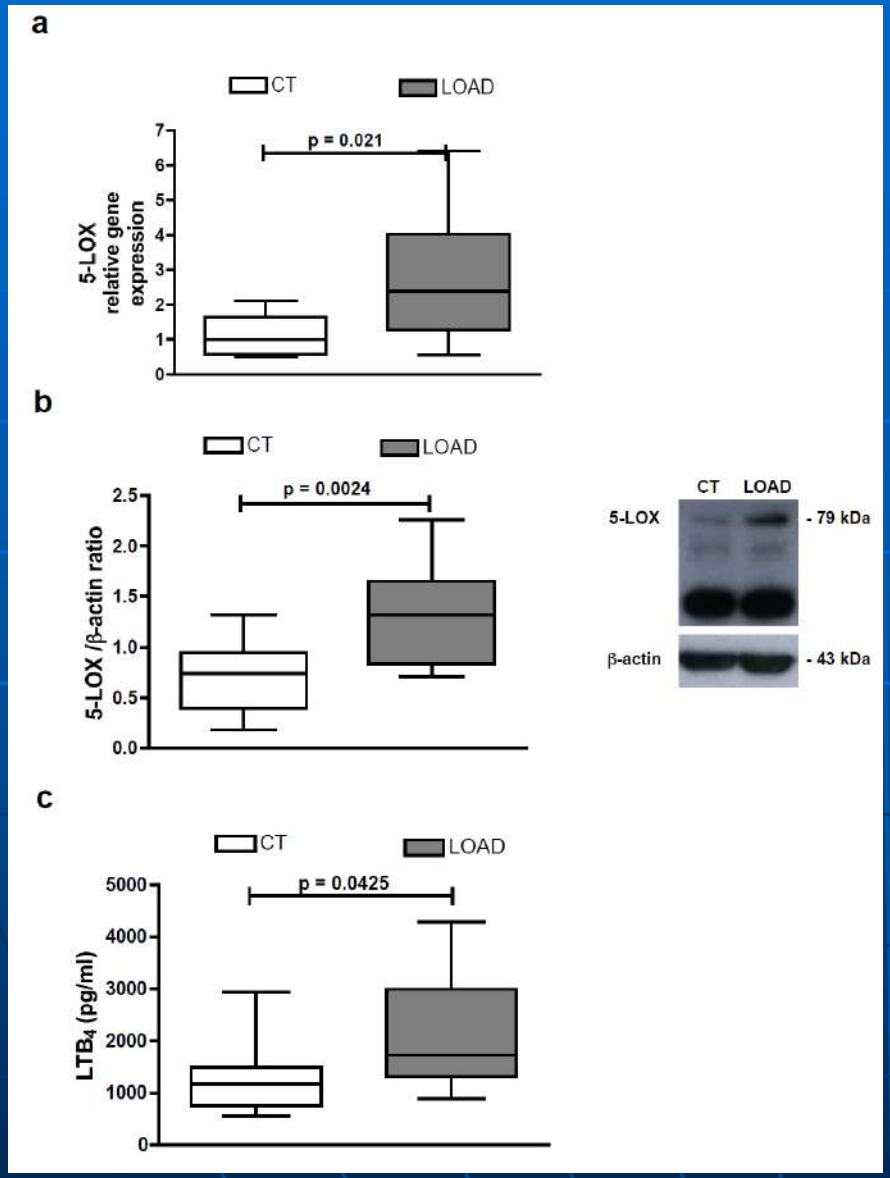
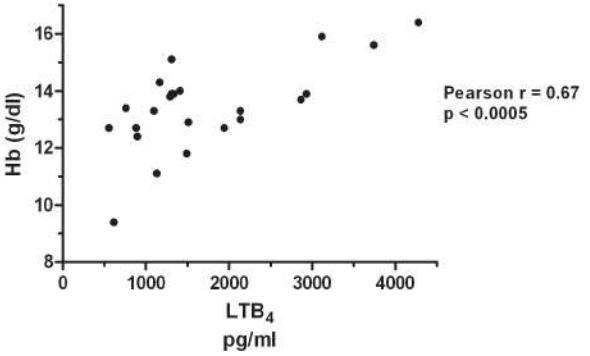


Figure 1. Overview of gastrointestinal iron uptake and iron uptake by glioblastoma cells. Dietary iron is absorbed in the duodenum and proximal jejunum through transporters on the apical membrane of enterocytes. This absorbed iron can then be used by the enterocyte, stored in ferritin, or exported into the circulation by ferroportin, where it is subsequently oxidized by hephaestin and carried systemically by transferrin. Glioblastoma cells can take up iron from several sources including transferrin-bound iron, non-transferrin-bound iron, and through ferritin.

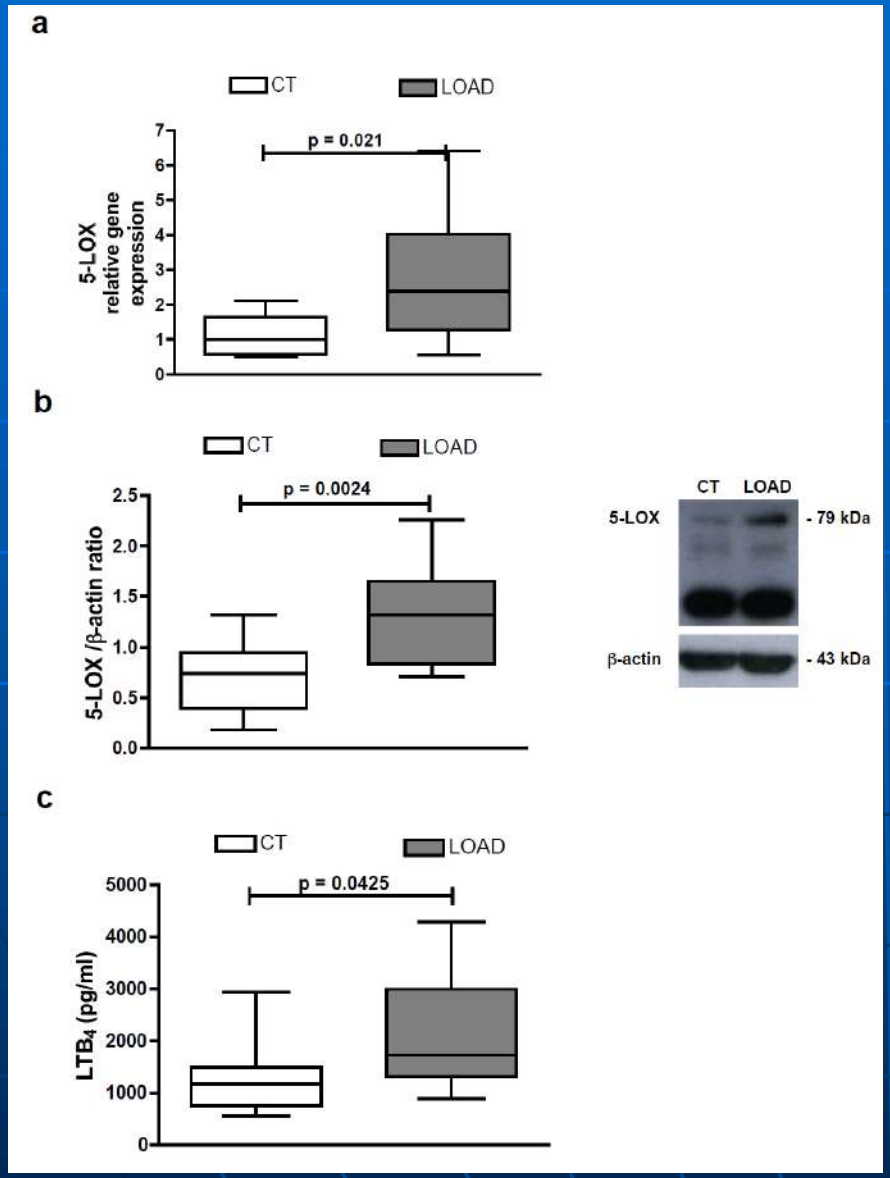
Plasma levels of hemoglobin correlate with the 5-LOX end-product LTB_4 in a population of healthy and Alzheimer's subjects

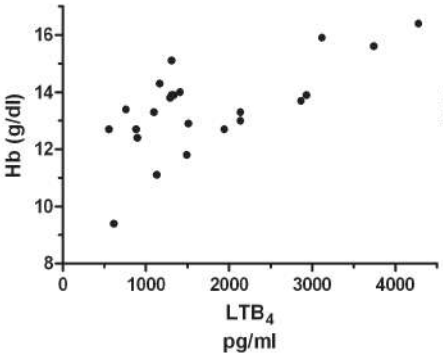




	CT	LOAD	ALL
Number of pairs	11	12	23
Pearson r	0.37	0.81	0.67
R^2	0.13	0.66	0.44
P value	0.27	0.001	0.0005

Plasma levels of hemoglobin correlate with the 5-LOX end-product LTB_4 in a population





	CT	LOAD	ALL
Number of pairs	11	12	23
Pearson r	0.37	0.81	0.67
R^2	0.13	0.66	0.44
P value	0.27	0.001	0.0005

REDOX HOMEOSTASIS

Pro-oxidant

- *Reactive oxygen species: ROS*
- *Dyslipidemia*
- *Obesity*
- *Inflammation*
- *Diabetes*
- *Excess iron*

Enzymes

- *Cicloxygenase*
- *Lipoxygenase*

Anti-oxidant

- *Vitamin E*
- *Vitamin C*
- *NADPH*
- *Glutathione*

Enzymes:

- *Glutathione peroxidase*
- *Glutathione reductase*
- *Superoxide dismutase*
- *Catalase*

CONCLUSIONS

- Epidemiological observations and metabolic investigations have consistently demonstrated that the accumulation of excess visceral fat is related to an increased risk of CVD as well as several metabolic and inflammatory perturbations;

- Inhibition of COX and LOX metabolism of arachidonic acid might help diminishing death of macrophage foam cells in atherosclerotic lesion;

- Inhibiting LOX may provide a new therapeutic approach to prevent inflammation-mediated metabolic consequences of excess fat intake;

- Olive oil is a functional food modulating FLAP and LOX, and COX activities;

- Since 12/15-LOX, 5-LOX and FLAP and eLOX3 have been implicated in the metabolism of adipose tissue, isoform-specific LOX inhibitors might constitute new anti-adipogenic drugs;

- Identification of new lead compounds (also from natural sources). Structural optimization of the lead compounds on the basis of the X-ray coordinates of different LOX-isoforms.

CONCLUSIONS

Nutritional biochemistry is a science that involves the relationship of food and nutrients to health. The specific goal of this science is to improve human health by understanding the biochemical role of each nutrient in the diet.

The bioavailability of a nutrient depends on its concentration within the food but mainly on its chemical form affecting the intestinal absorption.

This is a fundamental rule governing the absorbtion of all nutrients in food:

-Carbohydrates, Proteins, Lipids;

-Vitamins and oligoelements;

-Antioxidant molecules and **NUTRACEUTICS**

Antioxidant activities

In vitro (i.e. within the food)

-Total antioxidant activity;
- Total polyphenols content (etc.)

Food stabilization effects:
-Reduced amount of reactive oxygen species in food;
- Reduced activities of pro-oxidant enzymes in food;
-Reduced amount of lipid peroxides in food;
- High quality of food.

Intestinal barrier

In vivo (e.g. within the body)

- Specific molecules derived from digestion (not always the same observed *in vitro*);
- Effects on anti- or pro-oxidant enzymes.
- Modulation of genes involved in lipid biosynthesis

Effects on health:
- Cell antioxidant activity;
- Reduced amount of oxidized LDL;
- Contrasting CVD and cancer.