Optimizing Nutrition for Better Living

Controlling Oxidation & Inflammation while Promoting Repairs

Disclosures: none

Objectives:

1. Review oxidant defense mechanisms: Vitamin C & E, Selenium, & alpha Lipoic Acid
2. Review the role of omega-3 FAs in immunity, tissue repair, & prostaglandin metabolism
3. Review the role of gamma-linolenic acid in inflammation & prostaglandin metabolism

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Ingest Adequate Protein:

Calculations: 1) 1.2 grams of protein per Kg lean body mass

2) Body weight (lbs) x 0.6

1 ounce of meat, poultry, fish, or an egg is about 5 grams of protein

Other Sources:

"Fairlife Nutrition Plan Shake" 11.5 ounce bottles [Amazon ($2.95), Sam's, Costco]

Protein 30 gm, Fat 2.5 gm, Carbs 1 gm, Fiber 5 gm

Calcium 730 mg, Phosphorus 500 mg, Potassium 460 mg

"Premier Protein" 11 ounce bottles ($2.24 each) Walmart: Equate $1.75

Protein 30 gm, Fat 3 gm, Carbs 4 gm, Fiber 1 gm

Calcium 650 mg, Phosphorus 550 mg, Potassium 320 mg

"Body Fortress Whey Protein" 1 scoop (45 gm) (2 to 3 daily) ***Patients with Kidney Disease***

Protein 30 gm, Fat 4 gm, Carbs 6 gm, Fiber 1 gm

Calcium 140 mg, Phosphorus 380 mg, Potassium 310 mg ($1.22 / 30 grams)

Calcium ~1,000 mg daily

Vitamin D supplement to bring 25-OH Vitamin D to 40-100 ng/ml

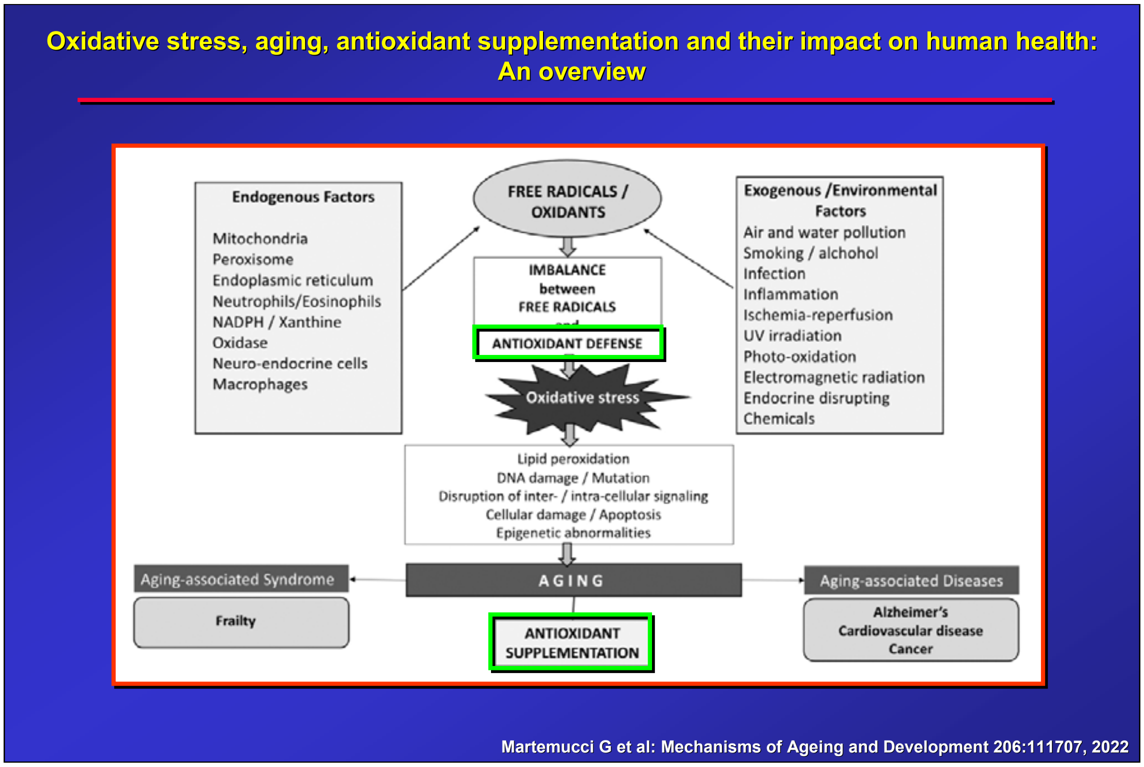


Fig. 1. The diagram represents the role of free radicals on oxidative stress and their detrimental effects on biomolecules. A number of endogenous and exogenous factors are able to generate free radicals/oxidants promoting oxidative stress, damaging biomolecules, affecting inter- and intracellular signaling and gene expression, and causing cell damage and apoptosis. The interplay between these events ultimately lead to aging, increasing both frailty and aging associated diseases.

A B S T R A C T Aging is characterized by a progressive loss of tissue and organ function due to genetic and environmental factors, nutrition, and lifestyle. Oxidative stress is one the most important mechanisms of cellular senescence and increased frailty, resulting in several age-linked, non-communicable diseases. Contributing events include genomic instability, telomere shortening, epigenetic mechanisms, reduced proteome homeostasis, altered stem-cell function, defective intercellular communication, progressive deregulation of nutrient sensing, mitochondrial dysfunction, and metabolic unbalance. These complex events and their interplay can be modulated by dietary habits and the ageing process, acting as potential measures of primary and secondary prevention. Promising nutritional approaches include the Mediterranean diet, the intake of dietary antioxidants, and the restriction of caloric intake. A comprehensive understanding of the ageing processes should promote new biomarkers of risk or diagnosis, but also beneficial treatments oriented to increase lifespan.

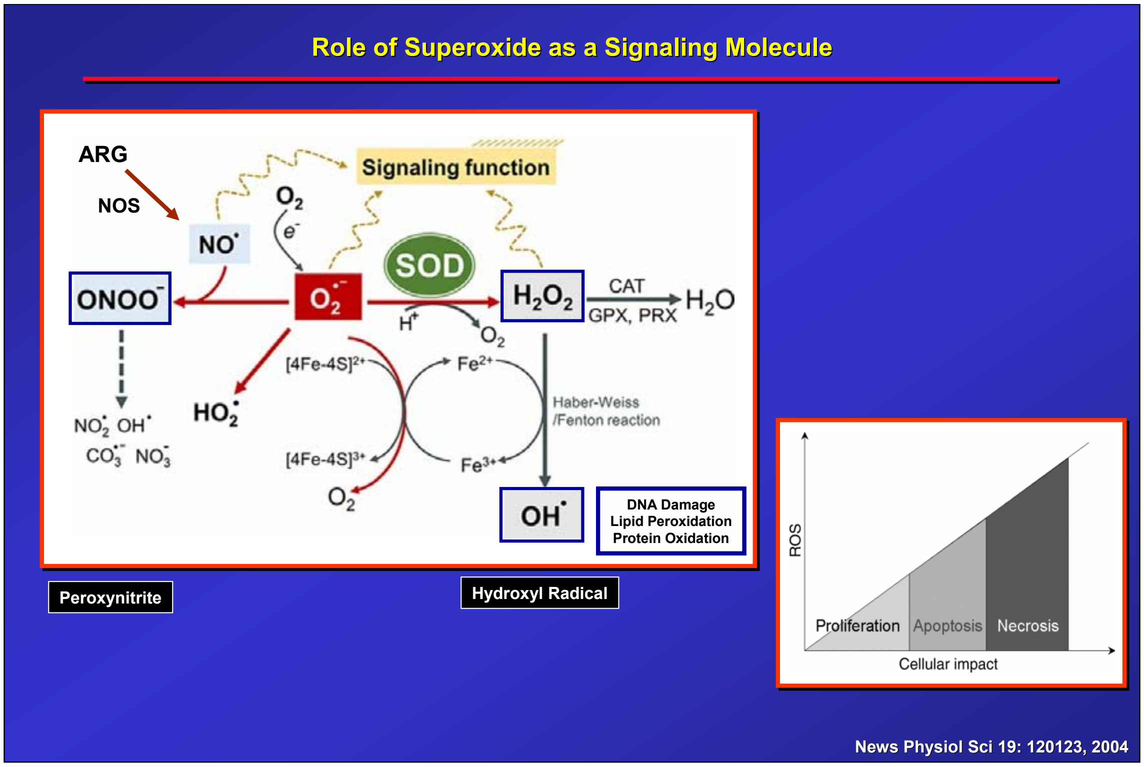
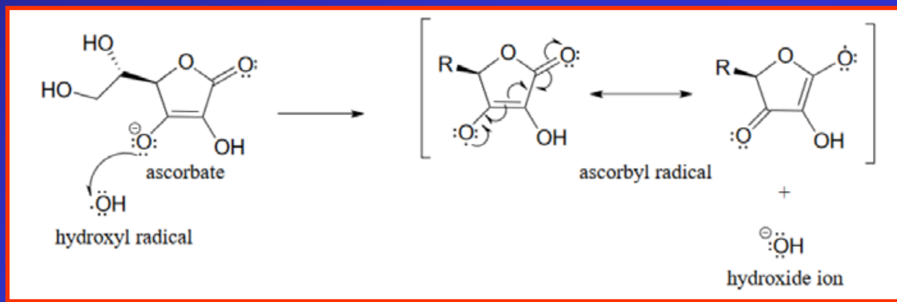
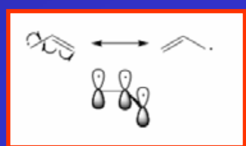


FIGURE 1. Radical flux. Oxygen is reduced to superoxide (O2·) by different cellular mechanisms. O2· is reduced to hydrogen peroxide (H2O2) by superoxide dismutase (SOD). H2O2 is reduced to water either by glutathione peroxidase (GPx) using glutathione (GSH) as substrate or by catalase (CAT). In the presence of free iron or copper, H2O2 undergoes the Fenton reaction to form the hydroxyl radical (OH·). When nitrogen monoxide (NO) is formed by NO synthase (NOS), O2· will react with NO to form peroxynitrite (ONOO). Both OH· and ONOO are very reactive and can modify cellular macromolecules and cause toxicity. The less-reactive molecules O2· and H2O2 can serve as cellular signaling molecules.

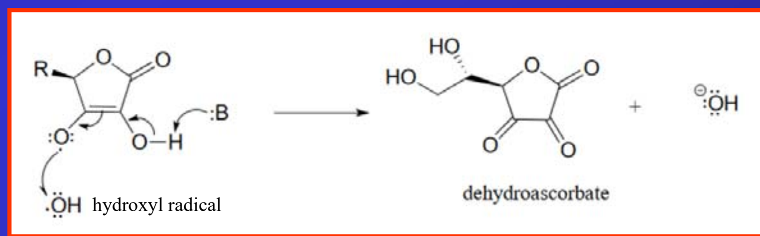
Vitamin C (Ascorbate) Eliminates 2 Radicals

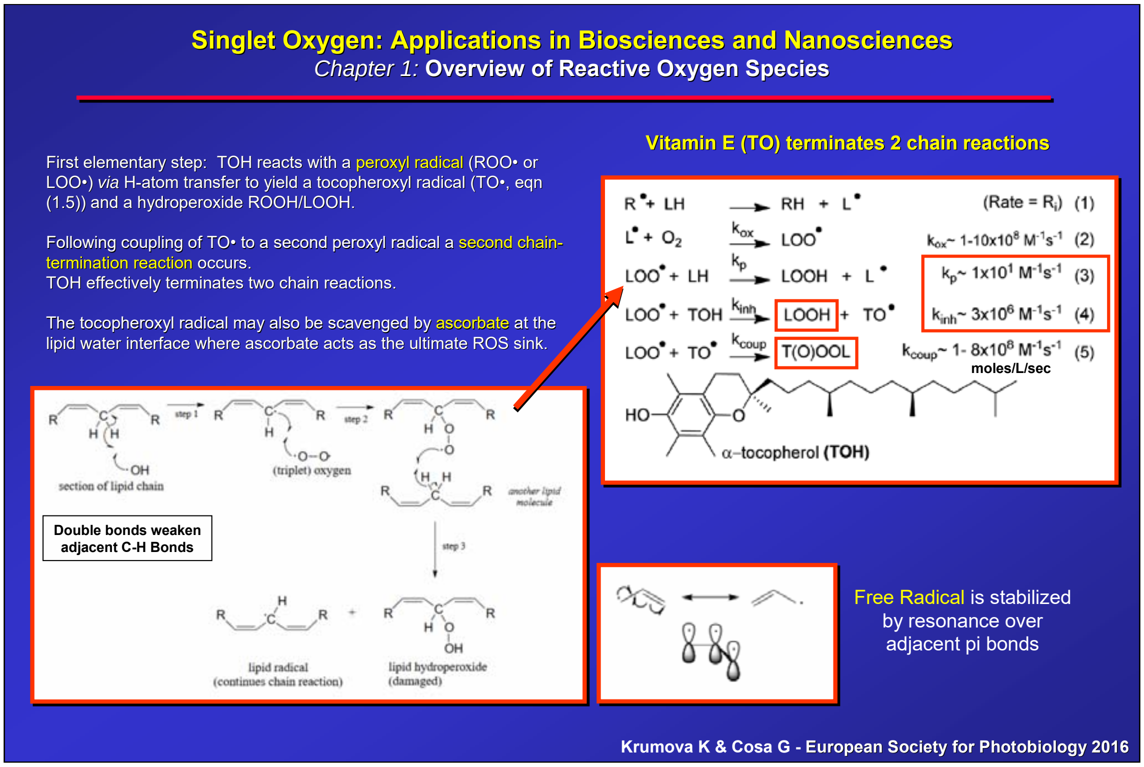
Preventing Excess Oxidation





Free Radical is stabilized by resonance over adjacent pi bonds



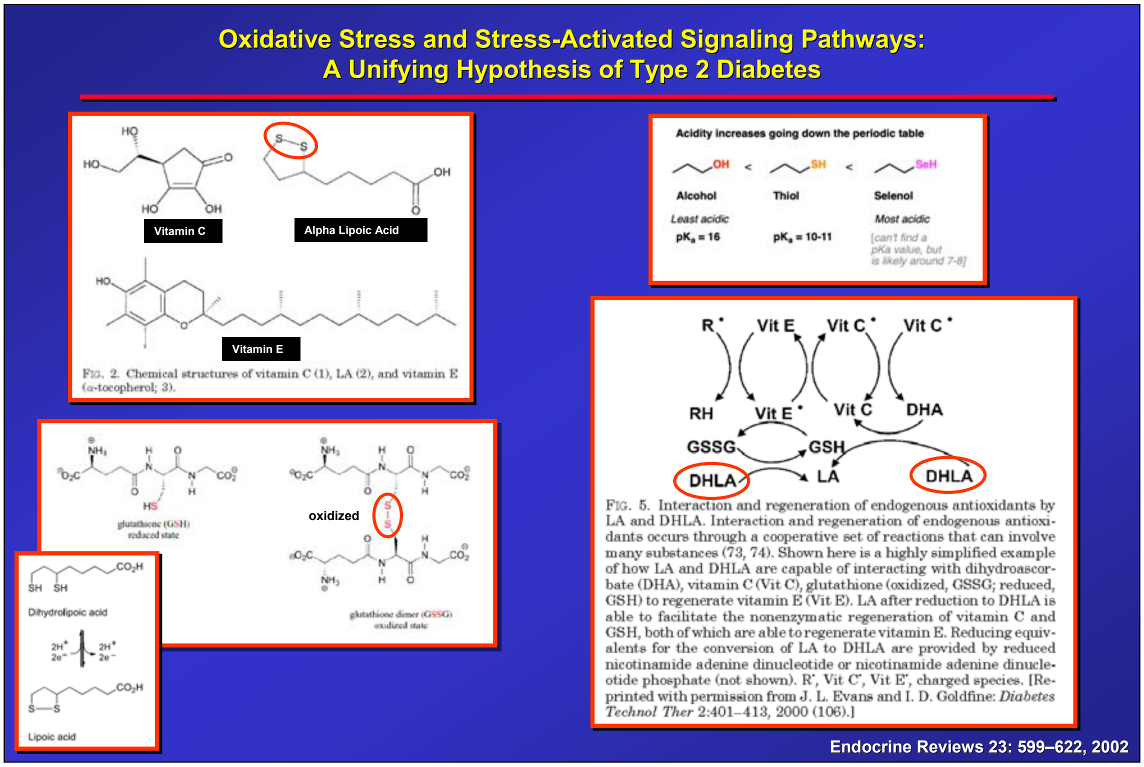


Molecular targets, lipids: a significant body of work, both in model membrane systems and in live cells, has examined the role lipid peroxyl radicals play in damaging the cell lipid milieu. **Autoxidation of polyunsaturated fatty acid residues** is initiated by a free radical such as the hydroxyl radical, which upon reaction with fatty acids generate lipid carbon centered radicals (eqn (1.1), Scheme 1.3). Lipid carbon centered radicals in turn readily trap molecular oxygen under physiological conditions to form lipid **peroxyl radicals**, effective chain carriers in the lipid chain auto-oxidation (eqn (1.2) and (1.3), Scheme 1.3). In the oxidation process, fatty acyl chains mostly in their *cis* configuration are either **converted to the *trans* configuration**, or form corresponding **hydroperoxides** and **alcohols** or may **fragment** into electrophilic αβ-unsaturated aldehydes, among others. Peroxidation and destruction of the *cis* double bonds may in turn lead to a reduction in the **membrane fluidity** and appearance of **liquid-order domains**. Auto-oxidation of polyunsaturated fatty acid residues ultimately generates a variety of secondary cytotoxic products that account for pathological effects, *e.g.*, neurodegenerative diseases, atherosclerosis, and cell apoptosis. Importantly, polyunsaturated fatty acids within the inner mitochondrial membrane are particularly vulnerable to ROS elicited oxidative damage.

Benzylic and allylic radicals are more stable than alkyl radicals due to resonance effects - an unpaired electron (just like a positive or negative charge) can be delocalized over a system of conjugated π bonds. An allylic radical, for example, can be pictured as a system of three parallel p orbitals sharing three electrons.

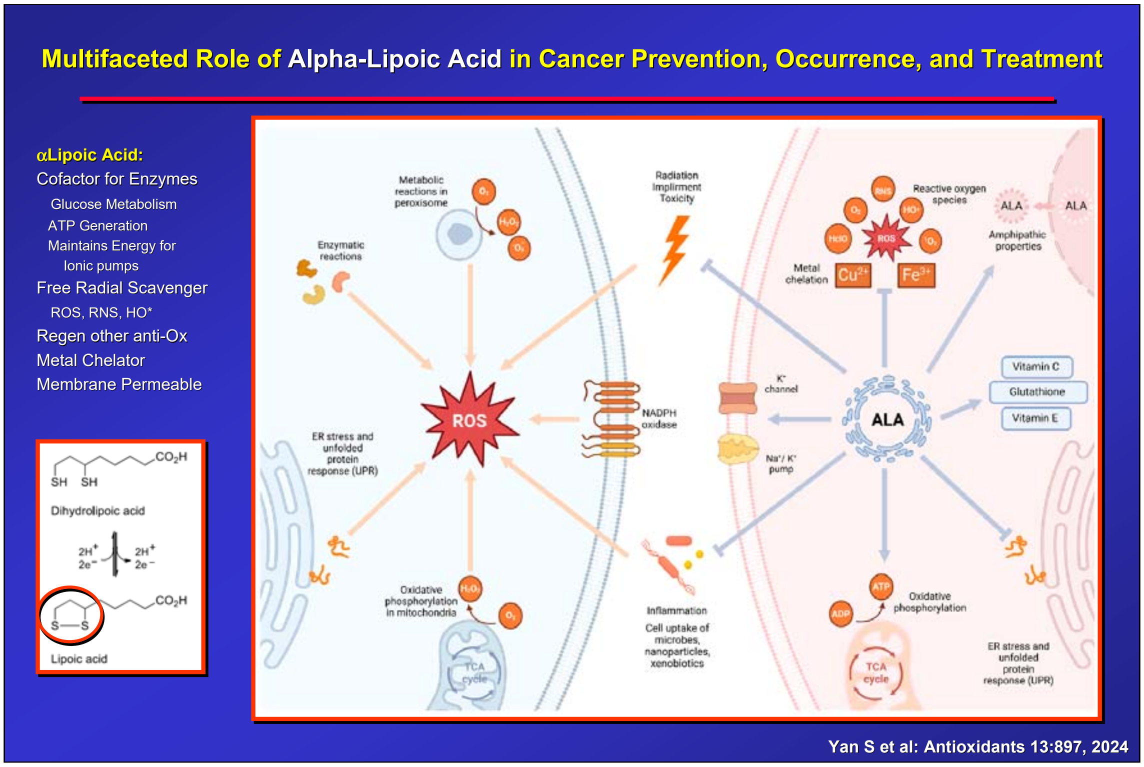
1.4.2. ROS Scavengers and Antioxidants Enzymatic and nonenzymatic antioxidant systems in cells regulate the concentration of ROS. Notably, McCord and Fridovich in the late 1960s discovered a variety of enzymes that were found to be responsible for detoxification of oxygen in aerobes but were absent in anaerobes (leading to oxygen-induced damage in these organisms). The finding of superoxide dismutase (SOD) was a landmark discovery in the field of free-radical biology. The presence of such enzymes suggested that if the ROS were not scavenged, they would critically injure cells. SOD ensures that the level of O2•− remains below 0.1 nM in *E. coli*. Over the years, new examples of enzymatic antioxidant systems have emerged. They include **catalase**, and **glutathione** and **NADH peroxidase**. The latter ensure that the steady-state concentration of H2O2 within *E. coli* does not exceed 20 nM.

Examples of nonenzymatic antioxidants include glutathione, vitamin C (both water soluble) and α-tocopherol (lipid soluble, see also Scheme 1.3). All three antioxidants scavenge free radicals. **Ascorbic acid yields ascorbyl radicals that readily disproportionate so no secondary free-radical byproducts are formed**. In the case of glutathione, the **thyil radical formed** may be a concern as it may react with lipids and proteins yet it **readily forms a disulfide bond with the thiolate of a second glutathione molecule**. The disulfide radical anion is next scavenged by oxygen yielding an inert disulfide. A member of the vitamin E family of compounds, **α-tocopherol (TOH)** has long been recognized as the **most active naturally occurring lipid soluble antioxidant** (Scheme 1.1 and Figure 1.1). The paradigm of TOH antioxidant activity in auto-oxidation reactions has been laid out in a number of studies conducted over the past 30 years in homogeneous solution and in the presence of initiators (see Scheme 1.3, reactions 1.4 and 1.5).



In both type 1 and type 2 diabetes, the late diabetic complications in nerve, vascular endothelium, and kidney arise from chronic elevations of glucose and possibly other metabolites including free fatty acids (FFA). Recent evidence suggests that common stress-activated signaling pathways such as nuclear factor-κB, p38 MAPK, and NH2-terminal Jun kinases/stress-activated protein kinases underlie the development of these late diabetic complications. In addition, in type 2 diabetes, there is evidence that the activation of these same stress pathways by glucose and possibly FFA leads to both insulin resistance and impaired insulin secretion. Thus, we propose a unifying hypothesis whereby hyperglycemia and FFA induced activation of the nuclear factor-κB, p38 MAPK, and NH2-terminal Jun kinases/stress-activated protein kinases stress pathways, along with the activation of the advanced glycosylation end-products/receptor for advanced glycosylation end-products, protein kinase C, and sorbitol stress pathways, plays a key role in causing late complications in type 1 and type 2 diabetes, along with insulin resistance and impaired insulin secretion in type 2 diabetes. Studies with antioxidants such as vitamin E, α-lipoic acid, and *N*-acetylcysteine suggest that new strategies may become available to treat these conditions. (*Endocrine Reviews* 23: 599–622, 2002)

Electronegativity, symbolized as χ, is the tendency for an atom of a given chemical element to attract shared electrons when forming a chemical bond. An atom's electronegativity is affected by both its atomic number and the distance at which its valence electrons reside from the charged nucleus.



Abstract: Alpha-lipoic acid (ALA) is a naturally occurring compound synthesized by mitochondria and widely distributed in both animal and plant tissues. It primarily influences cellular metabolism and oxidative stress networks through its antioxidant properties and is an important drug for treating metabolic diseases associated with oxidative damage. Nevertheless, research indicates that the mechanism by which ALA affects cancer cells is distinct from that observed in normal cells, exhibiting pro-oxidative properties. We specifically focus on the interactions between ALA and various carcinogenic and anti-carcinogenic pathways and discuss ALA's pro-oxidative capabilities in the unique redox environment of cancer cells.

Introduction: Alpha-lipoic acid (ALA) and its reduced form, dihydrolipoic acid (DHLA), are natural antioxidants that are widely distributed in both plants and animals. Since its discovery in 1937, ALA has garnered increasing attention due to its simple structure and complex functions. In humans, ALA primarily originates from two sources: endogenous de novo synthesis and exogenous intake. As a key compound, ALA is an essential **cofactor** for the activity of four **mitochondrial enzyme complexes** involved in energy metabolism: **pyruvate dehydrogenase** (PDHC), the **glycine cleavage system** (GCS), **branched-chain 2-ketoacid dehydrogenase** (BCKDH), and **2-ketoglutarate dehydrogenase** (2-KGDH). As a mitochondrial nutrient, ALA not only is a crucial component of glucose metabolism and ATP generation but also has **antioxidant** functions, protecting normal cells from the damaging effects of free radicals, also known as reactive oxygen species (ROS). Additionally, ALA acts as a **metal chelator** in certain metal ion accumulation phenomena, and its amphipathic characteristics make it a suitable chelating agent in nanotherapy, aiding drugs in achieving cellular selectivity and safety. Given these features, ALA has been found to be applicable in the treatment of chronic diseases related to inflammation and oxidative stress. In recent years, the **anticancer effects** of ALA have also been demonstrated, including the promotion of oxidation, inhibition of pro-cancer pathways, and activation of tumor suppressor genes, and is posited to play roles in various stages of cancer development.

Figure 2. Sources of ROS and the antioxidative effects of ALA. Sources of ROS: The blue section on the left side of the figure outlines the extracellular and intracellular sources of ROS production. Extracellular sources of ROS include environmental pollutants, radiation, toxic stimuli, microbial infections, and the inflammation they induce. Intracellularly, both normal and abnormal cellular activities can induce ROS production, including mitochondrial oxidative phosphorylation, ER stress and the UPR, enzymatic reactions, peroxisome metabolism, and NADPH oxidase activity.

Antioxidative effects of ALA: The red section on the right side of the figure summarizes the role of ALA as a multifunctional antioxidant in protecting cells from excessive ROS damage. ALA can directly quench various reactive oxygen species, including **ROS, RNS, HO•,** HClO, and 1O2. It also provides indirect antioxidant protection through metal chelation (e.g., Cu2+ and Fe3+) and the regeneration of certain endogenous antioxidants (e.g., vitamin E, vitamin C, and glutathione). Additionally, ALA leverages its amphiphilic properties to distribute in both hydrophilic environments (e.g., plasma) and lipophilic environments (e.g., cell membranes). Finally, ALA's antioxidative action effectively **protects mitochondria**, ensuring efficient ATP production and thereby maintaining the proper function of the **sodium–potassium pump**

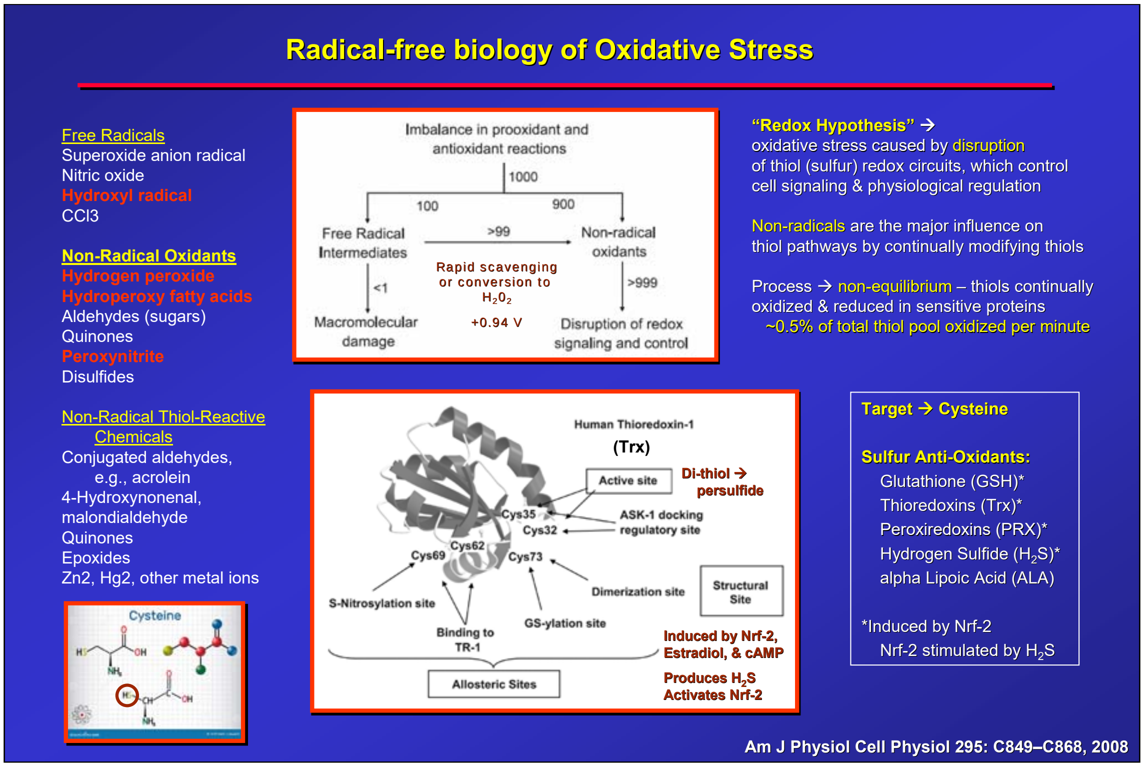


Fig. 1. Two mechanisms of oxidative stress. Oxidative stress is defined as an imbalance in pro-oxidants and antioxidants, which results in macromolecular damage and disruption of redox signaling and control. Considerable research has focused on free radical (1-electron) mechanisms in macromolecular damage (*left*). The present review focuses on an alternative mechanism that involves **2-electron oxidants** (*right*). Based on the xanthine oxidase kinetics, which show that univalent reduction of O_2 to superoxide anion radical is always a minor fraction of the bivalent reduction to H2O2, one can anticipate that **2-electron oxidants predominate during oxidative stress**. Thus for an arbitrary rate of oxidant generation equal to 1,000, perhaps 900 would go directly to 2-electron oxidants and only 100 would be 1-electron oxidants. **Free radical scavenging mechanisms are efficient and convert free radicals into nonradical oxidants**. The rate of oxidant production would be partitioned; if the radical scavengers are 99% effective, then there would be a rate of macromolecular damage of 1 and a rate of 2-electron oxidant production equal to 999. Thus if non-radical oxidants contribute to disease pathology by disruption of redox signaling and control mechanisms, **the pathology can correlate with macromolecular damage and yet be relatively insensitive to free radical scavengers**. Non-radical oxidants (e.g., conjugated aldehydes) also cause macromolecular damage, which can disrupt redox signaling and control pathways.

Jones DP. Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* 295: C849–C868, 2008. First published August 6, 2008; doi:10.1152/ajpcell.00283.2008.— Free radical-induced macromolecular damage has been studied extensively as a mechanism of oxidative stress, but large-scale intervention trials with free radical scavenging antioxidant supplements show little benefit in humans. The present review summarizes data supporting a complementary hypothesis for oxidative stress in disease that can occur without free radicals. This hypothesis, which is termed the "redox hypothesis," is that **oxidative stress occurs as a consequence of disruption of thiol redox circuits**, which normally function in cell signaling and physiological regulation. The redox states of thiol systems are sensitive to two electron oxidants and controlled by the **thioredoxins (Trx), glutathione (GSH), and cysteine (Cys).** Trx and GSH systems are maintained under stable, but non-equilibrium conditions, due to a **continuous oxidation of cell thiols** at a rate of about **0.5% of the total thiol pool per minute**. Redox-sensitive thiols are critical for signal transduction (e.g., H-Ras, PTP-1B), transcription factor binding to DNA (e.g., Nrf-2, nuclear factor-κB), receptor activation (e.g., IIb3 integrin in platelet activation), and other processes. Non-radical oxidants, including peroxides, aldehydes, quinones, and epoxides, are generated **enzymatically** from both endogenous and exogenous precursors and **do not require free radicals as intermediates** to **oxidize or modify these thiols**. Because of the non-equilibrium conditions in the thiol pathways, aberrant generation of non-radical oxidants at rates comparable to normal oxidation may be sufficient to disrupt function. Considerable opportunity exists to elucidate specific thiol control pathways and develop interventional strategies to restore normal redox control and protect against oxidative stress in aging and age-related disease.

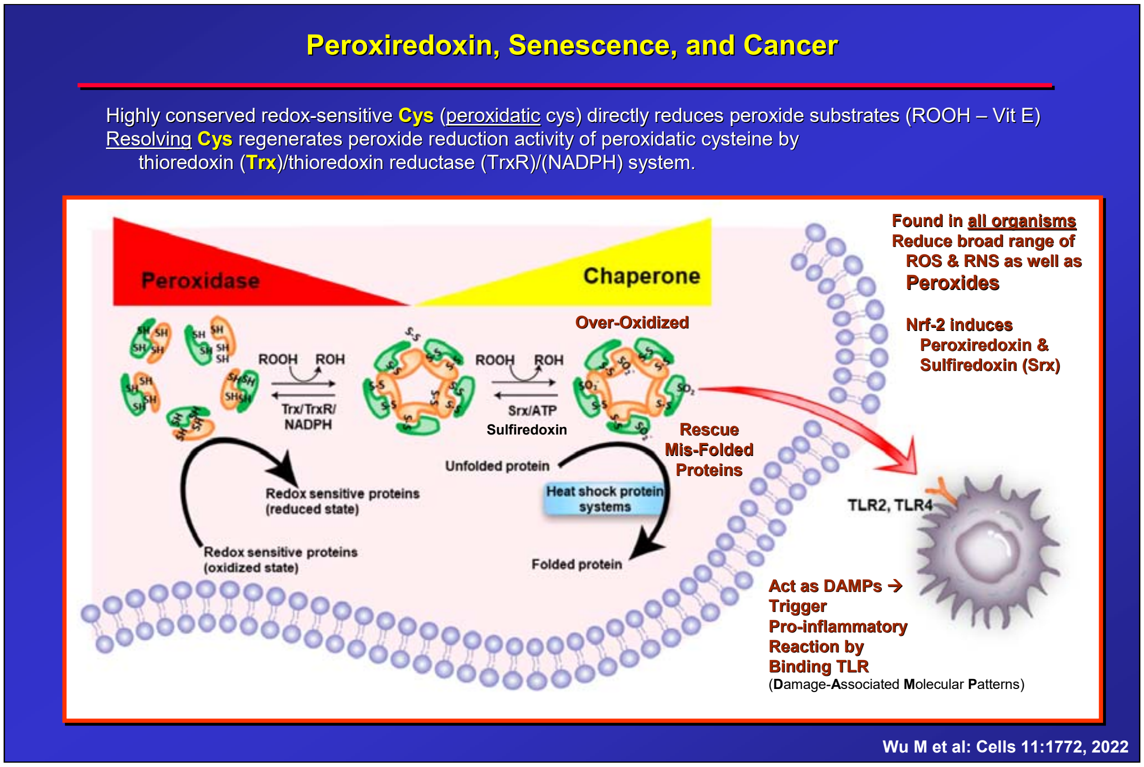


Figure 1. Schematic illustration of the general functions of peroxiredoxins. The highly conserved redox-sensitive cysteine residue (**peroxidatic cysteine**) in peroxiredoxin **directly reduces various peroxide substrates (ROOH).** A resolving cysteine can regenerate the peroxide reduction activity of the peroxidatic cysteine by thioredoxin (Trx)/thioredoxin reductase (TrxR)/nicotinamide adenine dinucleotide phosphate (NADPH) system. The **peroxidase activity of peroxiredoxin can be easily inactivated by hyperoxidation of the peroxidatic cysteine to cysteine sulfinic acid** (Cys-SO2-). A special oxidoreductase sulfiredoxin (SRX) is required to restore the peroxidase activity of hyperoxidized peroxiredoxins by reducing sulfinic acid of peroxiredoxin back to thiol in an ATP-dependent manner. Interestingly, hyperoxidated peroxiredoxin is required for the recruitment of cytosolic molecular chaperone HSP70 and disaggregase HSP104 to rescue misfolded proteins from aggregates. Extracellular peroxiredoxins function as DAMPs by triggering a proinflammatory response via binding to TLR2/TLR4 receptor.

Abstract: Peroxiredoxins are multifunctional enzymes that play a key role in protecting cells from stresses and maintaining the homeostasis of many cellular processes. **Peroxiredoxins were firstly identified as antioxidant enzymes that can be found in all living organisms.** Later studies demonstrated that peroxiredoxins also act as redox signaling regulators, chaperones, and proinflammatory factors and play important roles in oxidative defense, redox signaling, protein folding, cycle cell progression, DNA integrity, inflammation, and carcinogenesis. The **versatility of peroxiredoxins** is mainly based on their unique active center cysteine with a wide range of redox states and the ability to switch between low- and high-molecular-weight species for regulating their peroxidase and chaperone activities. Understanding the molecular mechanisms of peroxiredoxin in these processes will allow the development of new approaches to enhance longevity and to treat various cancers. In this article, we briefly review the history of peroxiredoxins, summarize recent advances in our understanding of peroxiredoxins in aging- and cancer-related biological processes, and discuss the future perspectives of using peroxiredoxins in disease diagnostics and treatments.

2. Identification of Peroxiredoxins as Antioxidant Enzymes

The first peroxiredoxin was identified in **budding yeast** Saccharomyces cerevisiae as a sulfur radical scavenger in 1987. It was named thiol-specific antioxidant (TSA) at that time as this antioxidant enzyme required thiol for its activity. Peroxiredoxins display several characteristics different from classical antioxidant enzymes. Firstly, they **do not require any redox cofactors** for function (such as copper/zinc for superoxide dismutase and heme for cytochrome c peroxidase). Secondly, a **unique peroxidatic cysteine** in the catalytic center of peroxiredoxins is responsible for their basic functions in oxidant defense. Thirdly, these antioxidant enzymes **can reduce a broad spectrum of oxidants** including reactive oxygen species (ROS) and reactive nitrogen species (RNS) via the **evolutionarily conserved redox-active cysteine**. These thiol-dependent peroxidases can be **found in all organisms**, and **multiple isoenzymes** are commonly found in one species.

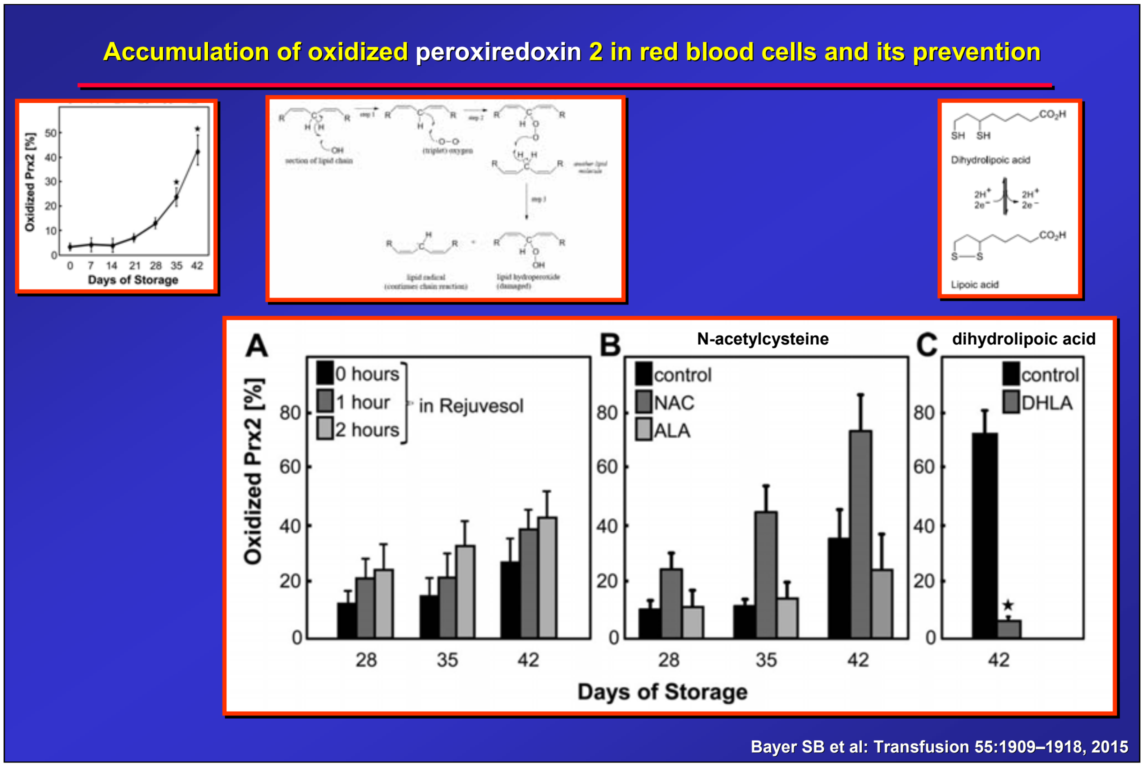


Fig. 3. Prx2 oxidation in RBCs treated with Rejuvesol, NAC, α-lipoic acid, or DHLA. RBCs were stored as in Fig. 1.

(A) Samples were incubated with 24 mL of Rejuvesol (1:8 Rejuvesol:RBCs) for up to 2 hours. Results are the mean and SE of three independent experiments. $p<0.05$ between 0- and 2-hour treatment groups (repeated-measures two-way ANOVA, with Holm-Sidak method).

(B) Samples were incubated with 5 mmol/L NAC or 250 mmol/L ALA (0.33% ethanol) for 1 hour. Results are the mean and SE of three independent experiments. $p<0.05$ between control and NAC and between ALA and NAC (repeated-measures two-way ANOVA, with Holm-Sidak method).

(C) Samples were treated with 250 mmol/L DHLA (0.005% DMSO) as in B. *$p<0.013$ in comparison with control (paired t test). Results are the mean and SE of three independent experiments.

Abstract Background: The thiol protein peroxiredoxin 2 (Prx2) is a major red blood cell (RBC) antioxidant that breaks down hydroperoxides and in the process is converted to an oxidized disulfide. Our objective was to determine whether Prx2 becomes oxidized during storage of RBCs, to understand the underlying mechanism, and to find ways of preventing the accumulation of the oxidized form.

Study design and Methods: RBCs were stored for up to 6 weeks under simulated blood banking conditions and Prx2 oxidation was monitored by nonreducing gel electrophoresis. The ability of the cells to reverse Prx2 oxidation after storage and to respond to added hydrogen peroxide was also evaluated.

Results: Prx2 remained predominantly reduced during the first 3 weeks of storage, and then the oxidized form accumulated progressively. In contrast to fresh cells, oxidation was not reversed by incubation with glucose. Storage of RBCs in a high-pH, low-chloride, and high phosphate/bicarbonate buffer (EAS-76v6) largely prevented accumulation of oxidized Prx for at least 6 weeks, and dihydrolipoic acid (DHLA), but not Rejuvesol, N-acetylcysteine, or α-lipoic acid, was able to reverse or protect against Prx2 oxidation. Additional, Prx2 oxidation occurred when hydrogen peroxide was added. However, this was reversible, suggesting that the reductive capacity was compromised in some but not in all cells.

Conclusion: Prx2 remains mostly reduced in a high-pH storage solution with buffering capacity. Addition of DHLA to stored RBCs might be advantageous. Prx2 redox status could be used as a biomarker for the quality of stored RBCs.

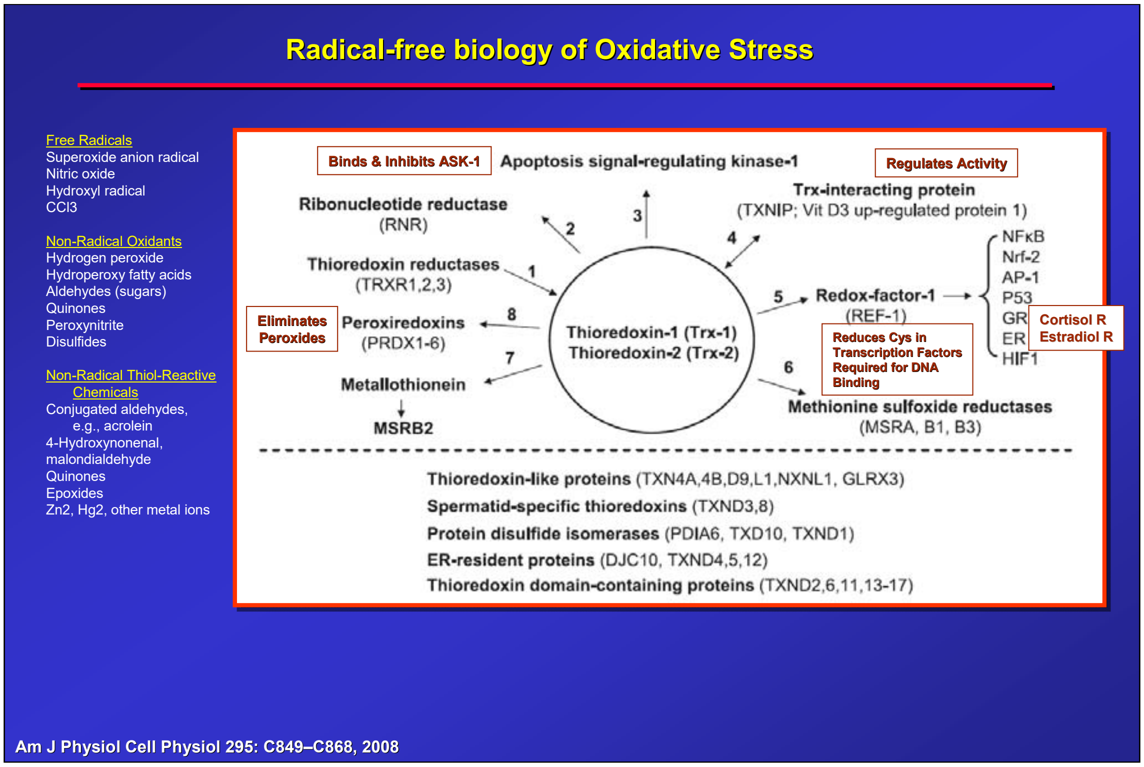


Fig. 5. A partial list of proteins in the Trx redox network.

1) Thioredoxins are reduced by thioredoxin reductases (TRXR1,2,3).

2) Trx functions as a reductant for ribonucleotide reductase (RNR).

3) Reduced Trx-1 or Trx-2 binds to **apoptosis** signal-regulating kinase-1 (ASK-1), inhibiting its function.

4) Trx-1 also binds to other proteins, including the vitamin D3-binding protein (VDUP1; TXNIP). Interaction with such proteins regulates activity and may determine distribution between cytoplasm and nuclei.

5) In cell nuclei, Trx-1 reduces redox factor-1 (REF-1), which is the **DNA repair/redox enzyme**, AP endonuclease. **REF-1 maintains conserved Cys residues of transcription factors in the reduced form required for DNA binding**. These transcription factors include nuclear factor (NF)-kB, Nrf-2, AP-1, P53, glucocorticoid receptor (GR), estrogen receptor (ER), and HIF-1.

6) Trx-1 is a reductant for methionine sulfoxide reductases (MSR) and also for

7) a pathway for MSRB2 reduction mediated by metallothionein in the metal-free thionein form.

8) Trx supports **six peroxiredoxins** (PDRX) that have heterogeneous subcellular distributions but a common activity to eliminate peroxides. Redox functions can be provided by Trx-related proteins (TRP14, TRP32, nucleeoredoxin and Trx-related transmembrane protein), which contain a Trx motif and may have evolved to recognize distinctive groups of proteins from those recognized by Trx-1.

A large number of Trx-like proteins are known (below dotted line), and many of these are likely to be redox active, either in pathways dependent on Trx or in parallel pathways, which evolved to provide additional specificity beyond that provided by the Trx proteins.

Protein designations and common names are from the UniProtKB/Swiss-Prot database. TXN4A, Trx-like protein 4A (spliceosomal U5 snRNP 15 kDa; Dim-1); TXN4B, Trx-like protein 4B (Dim1-like protein); TXND9, ATP-binding protein associated with cell differentiation (APACD); TXNL1, Trx-like protein 1 (32 kDa); NXNL1, nucleoredoxin-like protein 1 (Trx-like protein 6); GLRX3, glutaredoxin-3, PKC-theta-interacting protein; TXND3, spermatid-specific Trx-2; TXND8, spermatid-specific thioredoxin-3 (Trx-6); PDIA6 Protein disulfide-isomerase A6; TXD10, Trx-related transmembrane protein 3 (PDI); TXND1, transmembrane Trx-related protein; DJC10, ER-resident protein; ERdj5, microthioredoxin; TXND4, endoplasmic reticulum protein ERp44; TXND5, endoplasmic reticulum protein ERp46; TXD12, Trx domain-containing protein 12 (ERp18); TXND, Trx domain-containing proteins.

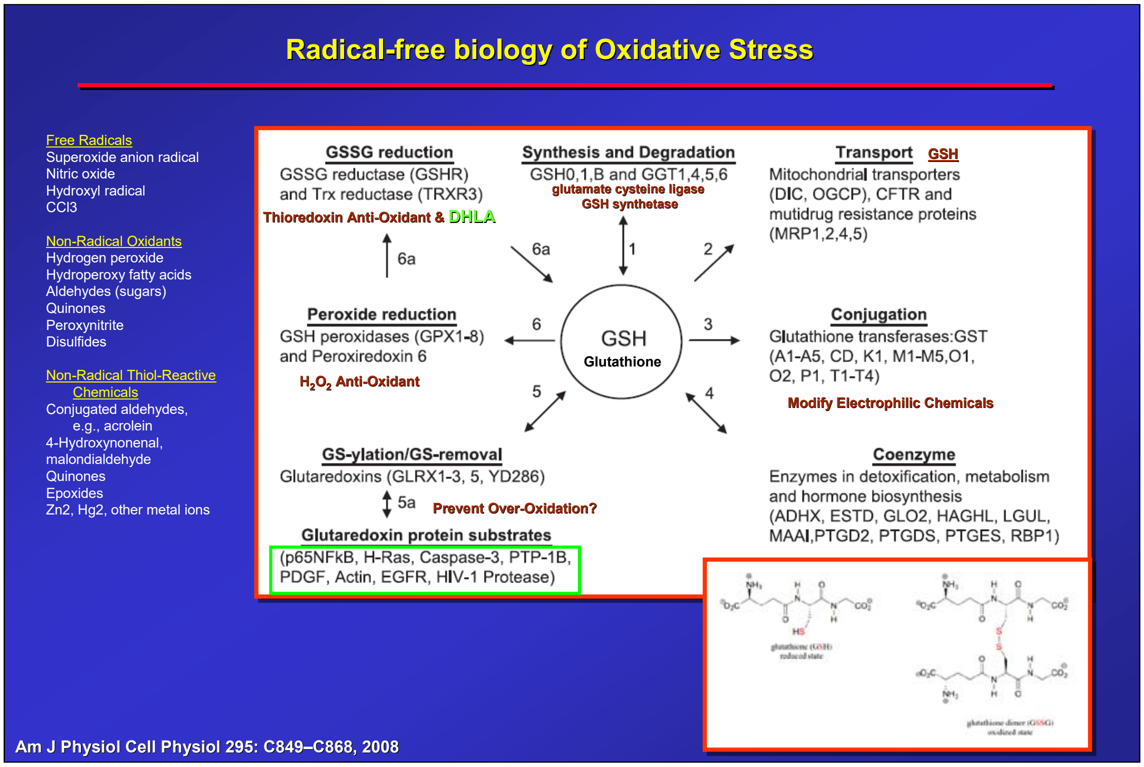


Fig. 4. Glutathionine (GSH) redox network. A partial list of GSH-dependent proteins illustrates the need for research to understand the integrated function of these redox systems.

1) GSH is synthesized by a two-step pathway in which abundance of two enzymes, glutamate cysteine ligase (GSH0, GSH1) and GSH synthetase (GSHB), determine synthesis rate. GSH is degraded by gamma-glutamyltransferase (GGT) at the surface of the brush border of the kidney, small intestine, and a number of other tissues, and probably also in the cisternae of the secretory pathway.

2) GSH is transported out of cells by several multidrug resistance proteins (MRP). The chloride channel, which is mutated in cystic fibrosis (CFTR), also transports GSH, and GSH is transported into mitochondria by the dicarboxylate carrier (DIC) and a monocarboxylate carrier (OGCP). GSH is transported into the cisternae of the endoplasmic reticulum, but the molecular nature of the transporter is not known.

3) GSH is used by a number of GSH transferases (GST), which include microsomal and non-microsomal locations, to modify electrophilic chemicals. These are thought to largely function in **detoxification**, but some also act on biosynthetic intermediates for **prostaglandins** and **leukotrienes**. A fraction of GSH is present as *S*-nitroso-GSH, a transnitrosylating agent generated from nitric oxide or its metabolites.

4) GSH functions in metabolism as a **coenzyme** for formaldehyde dehydrogenase, glyoxylase, and other metabolic reactions. In these reactions, GSH is cyclically removed by one reaction and regenerated in a second reaction.

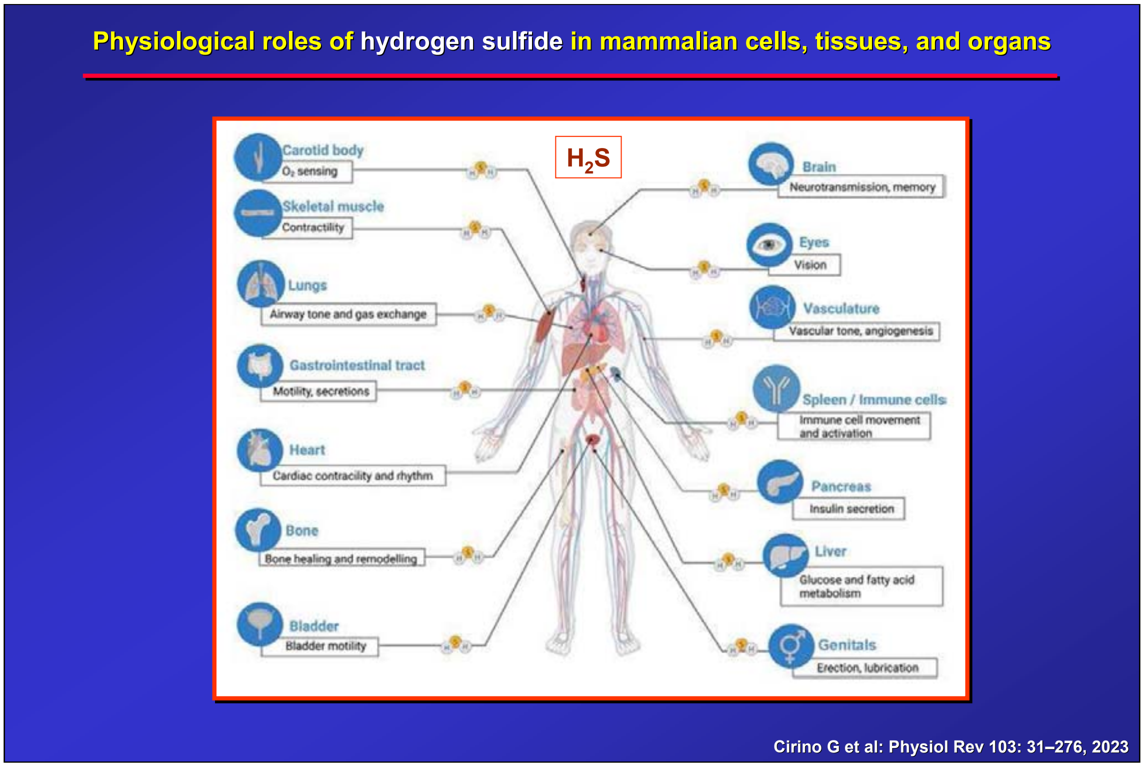
5) Several thiol transferases, also known as glutaredoxins, catalyze introduction and removal of GSH.

5a) Several proteins are regulated by GS-ylation, and many others undergo GS-ylation under oxidative stress conditions.

6) GSH is used as a reductant for selenium-dependent GSH peroxidases (GPX) and selenium-independent peroxiredoxin-6 (PRX6) and some GSH transferases (GST).

6a) The product of these oxidative reactions, GSSG, is reduced back to GSH by GSSG reductase (GSHR) in most tissues. In sperm, thioredoxin reductase-3 (TRXR3) has activity toward both Trx and GSH. The proteins included in this figure are present in multiple cellular compartments and are differentially expressed in cells so that development of functional maps will require tissue-specific measurements of individual reaction rates. Protein designations and common names are from the UniProtKB/Swiss-Prot database.

Abbreviations are as follows: GSH0, Glu-Cys ligase, regulatory; GSH1, Glu-Cys ligase, catalytic; GSHB, GSH synthetase; GGT1,4, 5, 6, -glutamyltransferase; DIC, mitochondrial dicarboxylate carrier (SLC25A10); OGCP, mitochondrial 2-oxoglutarate/malate carrier; CFTR, cystic fibrosis transmembrane conductance protein; MRP, multidrug resistance-associated protein; MRP2, canalicular multispecific organic anion transporter 1; GST, GSH transferase; ADHX, alcohol dehydrogenase class-3; ESTD, *S*-formyl-GSH hydrolase; GLO2, Glyoxalase II; HAGHL, hydroxyacylGSH hydrolase-like; LGUL, lactoylGSH lyase; MAAI , maleylacetoacetate isomerase; PTGD2, GSH-requiring prostaglandin D synthase; PTGDS, prostaglandin-H2 D-isomerase; PTGES, prostaglandin E synthase; RBP1, RalA-binding protein 1 (RalBP1); GLRX, glutaredoxin and glutaredoxin-related proteins; YD286, glutaredoxin-like protein; GPX, GSH peroxidase; GSHR, GSSG reductase; TRXR3, thioredoxin reductase 3.



Abstract Over the last two decades, hydrogen sulfide (H2S) has emerged as an **endogenous regulator** of a broad range of physiological functions. H2S belongs to the class of molecules known as **gasotransmitters**, which typically include nitric oxide (**NO**) and carbon monoxide (**CO**). Three enzymes are recognized as endogenous sources of H2S in various cells and tissues: cystathionine γ-lyase (**CSE**), cystathionine β-synthase (**CBS**), and 3-mercaptopyruvate sulfurtransferase (**3-MST**). The present article reviews the regulation of these enzymes as well as the pathways of their enzymatic and nonenzymatic degradation and elimination. The multiple interactions of H2S with other labile endogenous molecules (e.g., **NO**) and reactive oxygen species are also outlined. Next, the various biological targets and signaling pathways are outlined, with special reference to H2S or oxidative posttranscriptional modification (**persulfidation** or **sulfhydration**) of proteins and the effect of H2S on various channels and intracellular second messenger pathways, the regulation of gene transcription and translation, and the regulation of cellular bioenergetics and metabolism. The pharmacological and molecular tools currently available to study H2S physiology are also reviewed, including their utility and limitations. In subsequent sections, the role of H2S in the regulation of various physiological and cellular functions is reviewed, including the regulation of membrane potential, **endo- and exocytosis**, regulation of various cell organelles (**endoplasmic reticulum, Golgi, mitochondria**), regulation of cell **movement**, cell **cycle**, cell **differentiation**, and physiological aspects of regulated cell **death**. Next, the physiological roles of H2S in various cell types and organ systems are overviewed, including the role of H2S in red blood cells, immune cells, the central and peripheral nervous systems (with focus on neuronal transmission, learning, and memory formation), and regulation of vascular function (including angiogenesis as well as its specialized roles in the **cerebrovascular**, **renal**, and **pulmonary vascular beds**) and the role of H2S in the regulation of special senses, vision, hearing, taste and smell, and **pain-sensing**. Finally, the roles of H2S in the regulation of various organ functions (lung, heart, liver, kidney, urogenital organs, reproductive system, bone and cartilage, skeletal muscle, and endocrine organs) are presented, with a focus on physiology (including physiological aging) but also extending to some common pathophysiological conditions. From these data, a wide array of significant roles of H2S in the physiological regulation of all organ functions emerges and the characteristic bell-shaped biphasic effects of H2S are highlighted. In addition, key pathophysiological aspects, debated areas, and future research and translational areas are identified.

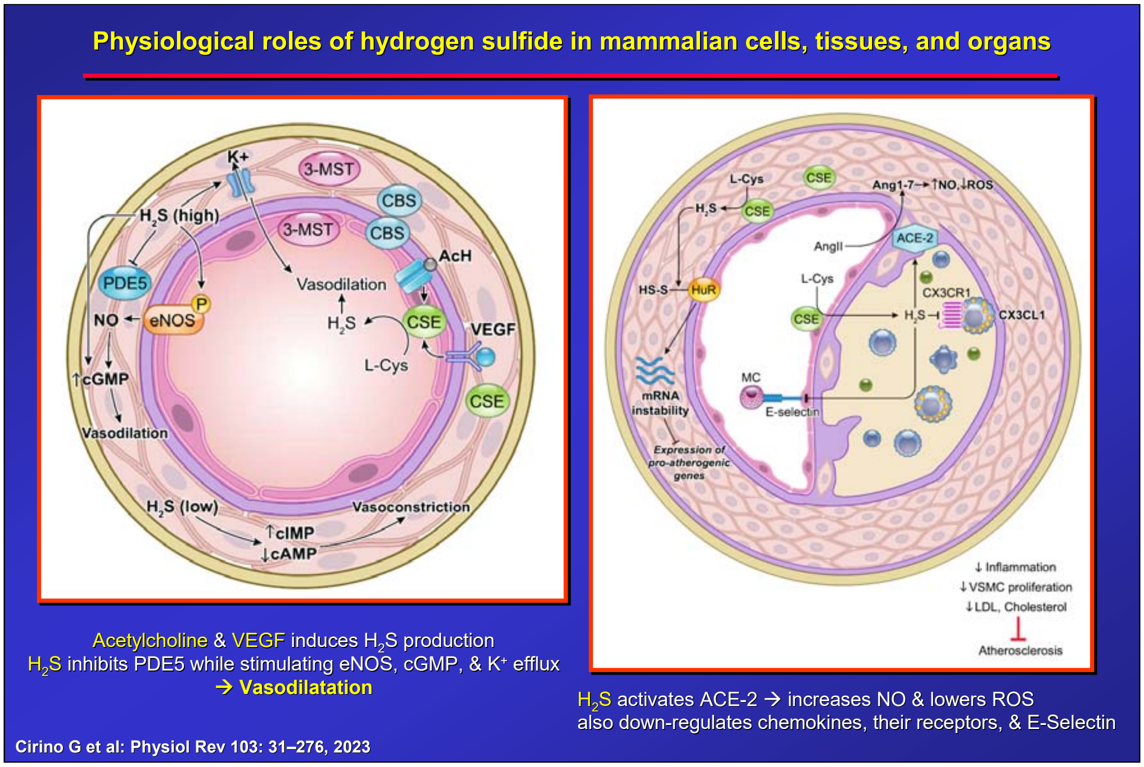


FIGURE 15. Common pathways involved in the physiological regulatory effect of hydrogen sulfide (H2S) on the vascular tone.

Low H2S levels promote vasoconstriction by increasing cIMP and decreasing cAMP levels. **High levels of H2S** lead to **vasodilation** via multiple mechanisms including 1) activation of endothelial nitric oxide synthase (**eNOS**) (by phosphorylation on S1177), which increases nitric oxide (NO) production and elevates intracellular cGMP levels, 2) **inhibition of phosphodiesterase** (PDE)5, which **prolongs the half-life of cGMP**, and 3) activation of ATP-sensitive K1 (**KATP) channels**, followed by hyperpolarization of the vascular smooth muscle membrane. Classic endothelium-dependent vaso-relaxants like acetylcholine (**ACh**) or **VEGF** bind to their cognate receptors and **enhance** endogenous **H2S production via cystathionine-c-lyase (CSE) activation**. Additional ion channels, phospholipase A2, the Cl/HCO3 exchanger, and metabolic inhibition have also been implicated in the vasoactive action of H2S. The other 2 main H2S-producing enzymes, cystathionine-b-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST), are also expressed in both endothelial cells and smooth muscle cells, but their role in regulating vascular tone remains to be defined. Reduced CBS function or expression leads to increased homocysteine and impaired endothelium-dependent relaxation. 3-MST has been described to have a role in the regulation of tone in selected vascular beads (e.g., in the coronary artery).

FIGURE 16. Role of hydrogen sulfide (H2S) in atherosclerosis.

Cystathionine-c-lyase (CSE)-derived H2S attenuates pro-atherogenic pathways and pro-atherogenic gene expression in multiple ways. Some of these mechanisms include:

1) **Activation** of angiotensin-converting enzyme (**ACE**)-2 and **increased conversion rates of the proatherogenic ANG II to the antiatherogenic ANG(1–7).** The elevation of ANG(1–7) leads to enhanced nitric oxide (NO) and reduced reactive oxygen species (ROS) levels.

2) **Attenuation** of proinflammatory chemokines and cognate receptors in monocytes/macrophages (**CX3CR1, CX3CL1**)

3) **Down regulation** of **E-selectin** and attenuated adhesion of circulating monocytes (MCs). At the molecular level, the CSE-derived H2S sulfhydrates and inactivates human antigen R (HuR). Dimeric active HuR binds to and stabilizes mRNA transcripts of proatherogenic genes.

VSMC, vascular smooth muscle cell.

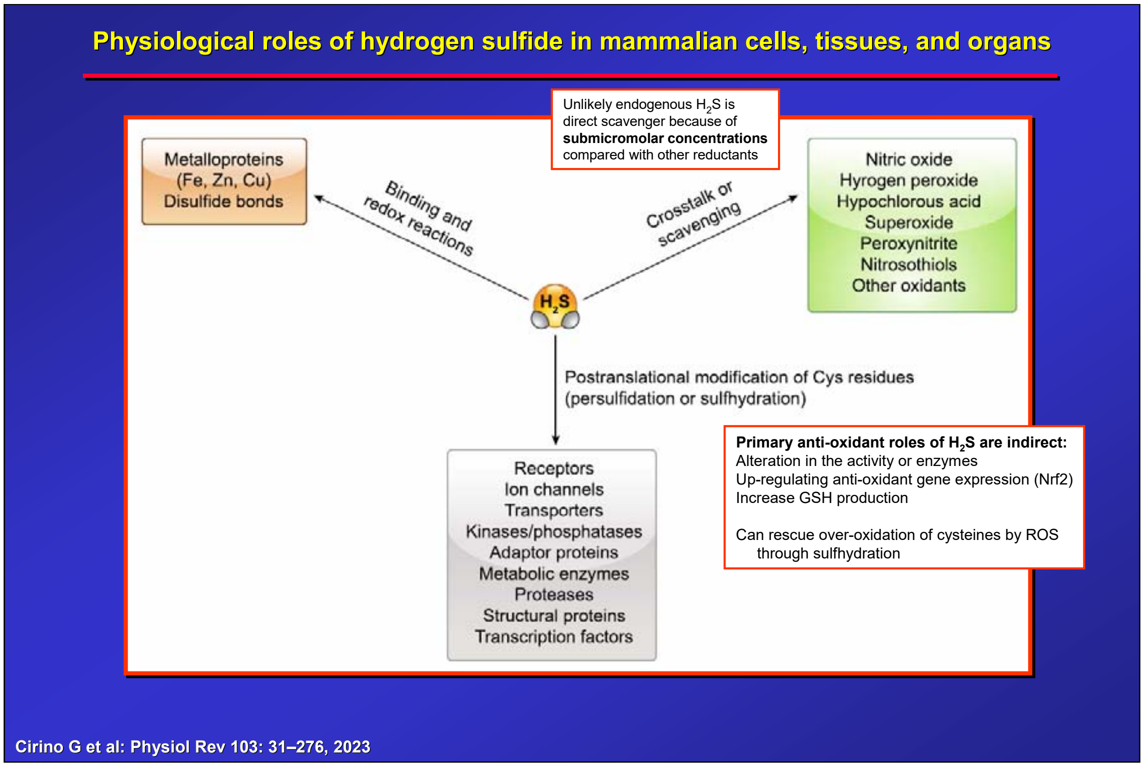


FIGURE 10. Role of hydrogen sulfide (H2S) in the regulation of cell signaling.

H2S signals in mammalian cells via 3 distinct mechanisms. H2S can react directly with oxidants or free radicals to scavenge them or to form other signaling species. After binding to protein metal centers, H2S leads to their reduction or is subsequently delivered to specific targets. H2S can also modify protein **cysteine residues to form persulfides**.

H2S has been demonstrated to react with reactive oxygen species, including **superoxide anions, hydroxyl radicals, peroxynitrite, hydrogen peroxide**, and hypochlorite. The rate constants for some of these reactions are comparable to those of low-molecular-weight thiols. However, it is unlikely that endogenous H2S acts as a direct scavenger of oxidants in biological systems because of its **submicromolar concentrations**, compared with other reductants that are three or four orders of magnitude more abundant. Thus, the antioxidant roles of H2S are probably indirect, mediated by **alteration in the activity or enzymes**, upregulation of antioxidant gene expression [such as that occurring through **Nrf2**], and **increase GSH production**. In addition, H2S can **rescue over-oxidation of cysteines by ROS**, through sulfhydration, preventing cytoprotective signaling and extending life span. H2S also reacts directly with NO to form nitroxyl and generates nitrosothiols after reacting with nitrosylated proteins. **Reduction of disulfide bonds by H2S** has also been shown to be important in the regulation of receptor tyrosine kinase signaling, at least in the case of epidermal growth factor receptor and vascular endothelial growth factor.

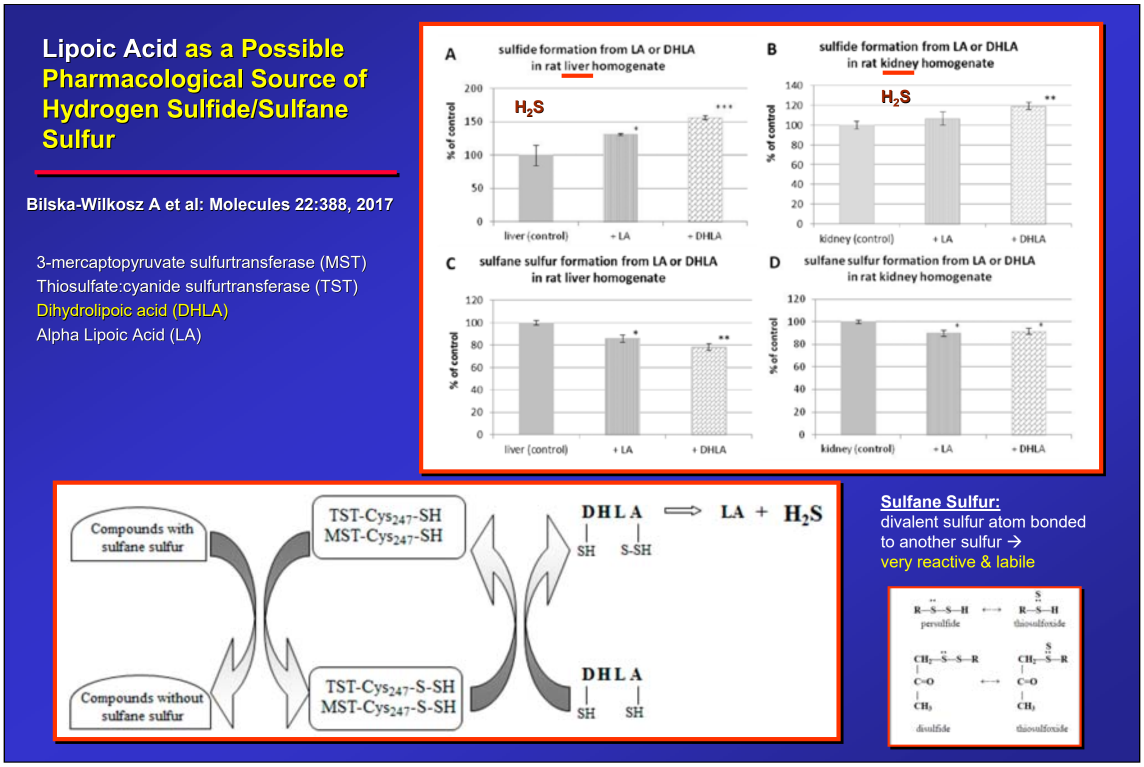
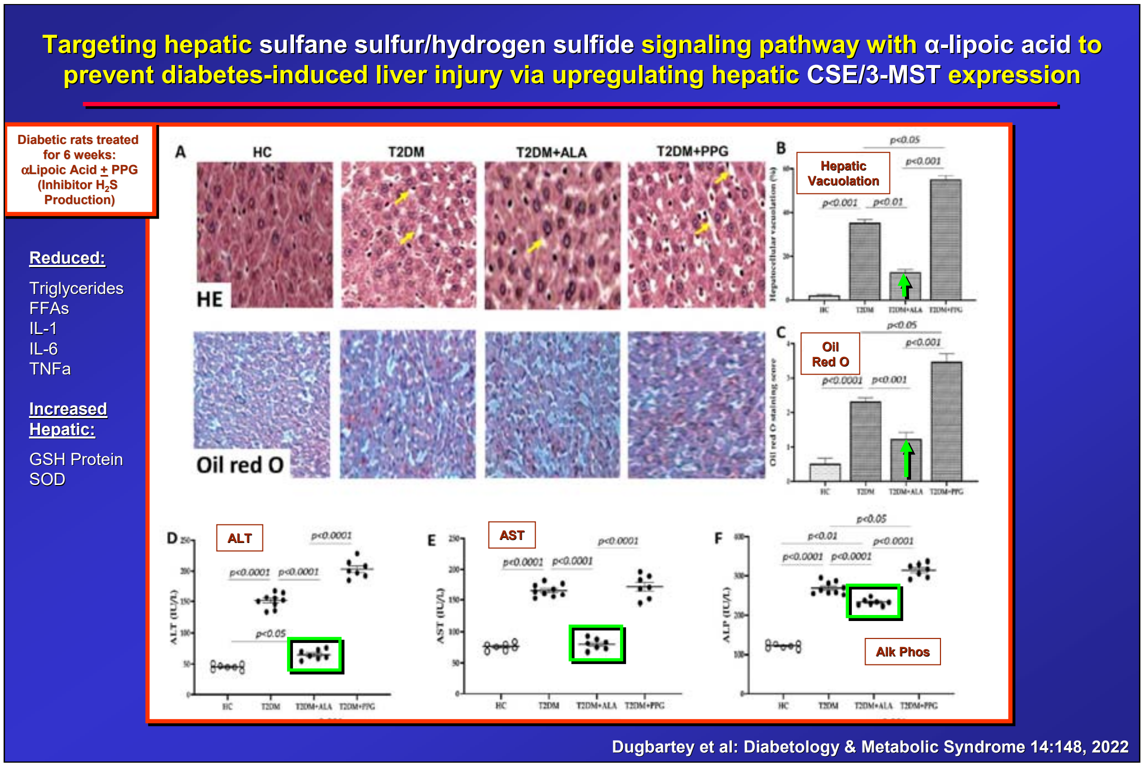


Figure 5: Level of hydrogen sulfide (**A**,**B**) and sulfane sulfur (**C**,**D**) formed in vitro enzymatically in the presence of rat liver or kidney homogenate from fresh LA or dihydrolipoic acid (DHLA) protected from light compared to endogenous hydrogen sulfide and sulfane sulfur level in homogenates (control). The results are presented as the means ± SD. Statistical significance compared to the control (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 6: Role of LA/DHLA system in the interconversion of H2S and sulfane sulfur in the rat liver and kidneys. DHLA accepts sulfur atoms from sulfane sulfur-containing persulfide group of Cys-247, located in the active center of 3-mercaptopyruvate sulfurtransferase (MST) and/or thiosulfate:cyanide sulfurtransferase, rhodanese (TST). In this process, DHLA hydropersulfide is formed from which sulfur is released in the form of H2S, and LA is produced concomitantly.

Abstract: The aim of the present study was to verify whether lipoic acid (LA) itself is a source of H2S and sulfane sulfur. It was investigated in vitro non-enzymatically and enzymatically (in the presence of rat tissue homogenate). The results indicate that both H2S and sulfane sulfur are formed from LA non-enzymatically in the presence of environmental light. These results suggest that H2S is the first product of non-enzymatic light-dependent decomposition of LA that is, probably, next oxidized to sulfane sulfur-containing compound(s). The study performed in the presence of rat liver and kidney homogenate revealed an increase of H2S level in samples containing LA and its reduced form dihydrolipoic acid (DHLA). It was accompanied by a decrease in sulfane sulfur level. It seems that, in these conditions, DHLA acts as a reducing agent that releases H2S from an endogenous pool of sulfane sulfur compounds present in tissues. Simultaneously, it means that exogenous LA cannot be a direct donor of H2S/sulfane sulfur in animal tissues. The present study is an initial approach to the question whether LA itself is a donor of H2S/sulfane sulfur.

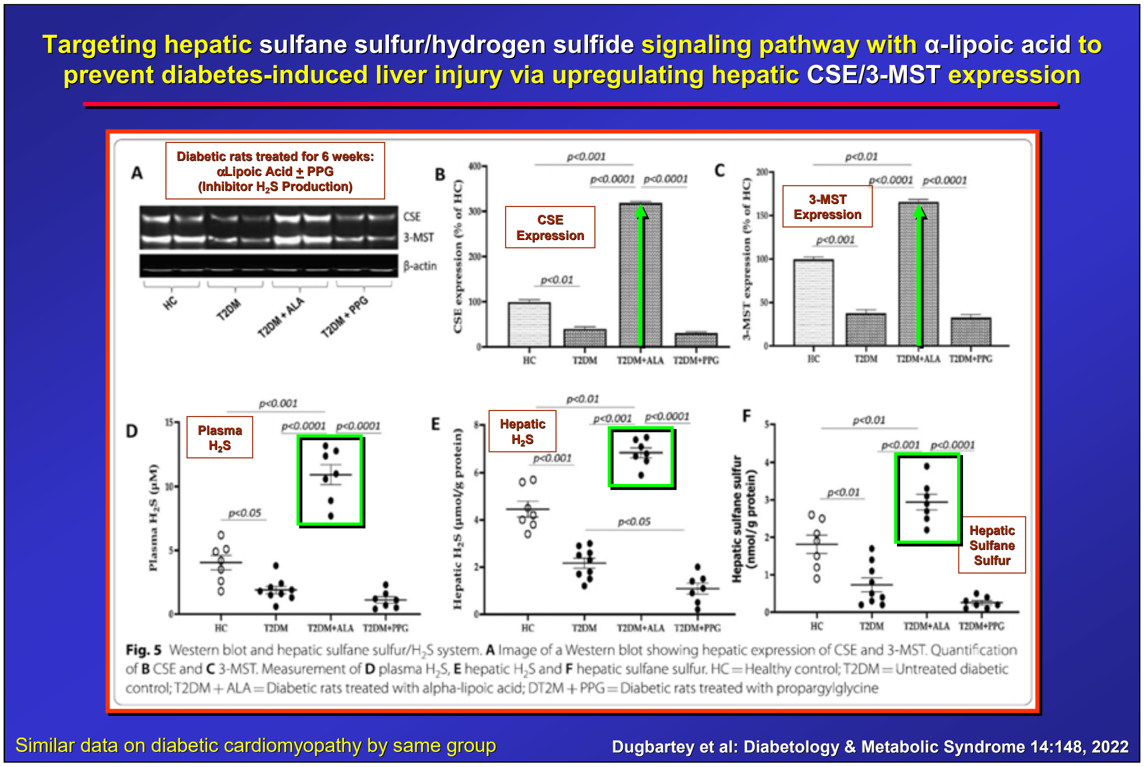


Abstract Background: Diabetes-induced liver injury is a complication of diabetes mellitus of which there are no approved drugs for effective treatment or prevention. This study investigates possible hepatoprotective effect of alpha-lipoic acid (ALA), and sulfane sulfur/hydrogen sulfide pathway as a novel protective mechanism in a rat model of type 2 diabetes-induced liver injury.

Methods: Thirty Sprague–Dawley rats underwent fasting for 12 h after which fasting blood glucose was measured and rats were randomly assigned to diabetic and non-diabetic groups. Type 2 diabetes mellitus (T2DM) was induced in diabetic group by administration of nicotinamide (110 mg/kg) and streptozotocin (55 mg/kg). Diabetic rats were treated daily with ALA (60 mg/kg/day p.o.) or 40 mg/kg/day DL-propargylglycine (PPG, an inhibitor of endogenous hydrogen sulfide production) for 6 weeks and then sacrificed. Liver, pancreas and blood samples were collected for analysis. Untreated T2DM rats received distilled water.

Results: Hypoinsulinemia, hyperglycemia, hepatomegaly and reduced hepatic glycogen content were observed in untreated T2DM rats compared to healthy control group ($p < 0.001$). Also, the pancreas of untreated T2DM rats showed severely damaged pancreatic islets while liver damage was characterized by markedly increased hepatocellular vacuolation, sinusoidal enlargement, abnormal intrahepatic lipid accumulation, severe transaminitis, hyperbilirubinemia, and impaired hepatic antioxidant status and inflammation compared to healthy control rats ($p < 0.01$). While pharmacological inhibition of hepatic sulfane sulfur/hydrogen sulfide with PPG administration aggravated these pathological changes ($p < 0.05$), ALA strongly prevented these changes. ALA also significantly increased hepatic expression of hydrogen sulfide-producing enzymes (cystathionine γ-lyase and 3-mecaptopyruvate sulfurtransferase) as well as hepatic sulfane sulfur and hydrogen sulfide levels compared to all groups ($p < 0.01$).

Conclusions: To the best of our knowledge, this is the first experimental evidence showing that ALA prevents diabetes-induced liver injury by activating hepatic sulfane sulfur/hydrogen sulfide pathway via upregulation of hepatic cystathionine γ-lyase and 3-mecaptopyruvate sulfurtransferase expressions. Therefore, ALA could serve as a novel pharmacological agent for the treatment and prevention of diabetes-induced liver injury, with hepatic sulfane sulfur / hydrogen sulfide as a novel therapeutic target.



Alpha-lipoic acid treatment improves adverse cardiac remodelling in the diabetic heart – The role of cardiac hydrogen sulfide-synthesizing enzymes Biochemical Pharmacology 203:115179, 2022

A B S T R A C T **Introduction:** Alpha-lipoic acid (ALA) is a licensed drug for the treatment of diabetic neuropathy. We recently reported that it also improves diabetic cardiomyopathy (DCM) in type 2 diabetes mellitus (T2DM). In this study, we present evidence supporting our hypothesis that the cardioprotective effect of ALA is via upregulation of cardiac hydrogen sulfide (H2S)-synthesizing enzymes.

Methods: Following 12 h of overnight fasting, T2DM was induced in 23 out of 30 male Sprague-Dawley rats by intraperitoneal administration of nicotinamide (110 mg/kg) followed by streptozotocin (55 mg/kg) while the rest served as healthy control (HC). T2DM rats then received either oral administration of ALA (60 mg/kg/day; n = 7) or 40 mg/kg/day DL-propargylglycine (PAG, an endogenous H2S inhibitor; n = 7) intraperitoneally for 6 weeks after which all rats were sacrificed and samples collected for analysis. Untreated T2DM rats served as diabetic control (DCM; n = 9).

Results: T2DM resulted in weight loss, islet destruction, reduced pancreatic β-cell function and hyperglycemia. Histologically, DCM rats showed significant myocardial damage evidenced by myocardial degeneration, cardiomyocyte vacuolation and apoptosis, cardiac fibrosis and inflammation, which positively correlated with elevated levels of cardiac damage markers compared to HC rats ($p < 0.001$). These pathological alterations worsened significantly in PAG-treated rats ($p < 0.05$). However, ALA treatment restored normoinsulemia, normoglycemia, prevented DCM, and improved lipid and antioxidant status. Mechanistically, ALA significantly upregulated the expression of cardiac H2S-synthesizing enzymes and increased plasma H2S concentration compared to DCM rats ($p < 0.001$).

Conclusion: ALA preserves myocardial integrity in T2DM likely by maintaining the expression of cardiac H2S-synthezing enzymes and increasing plasma H2S level.

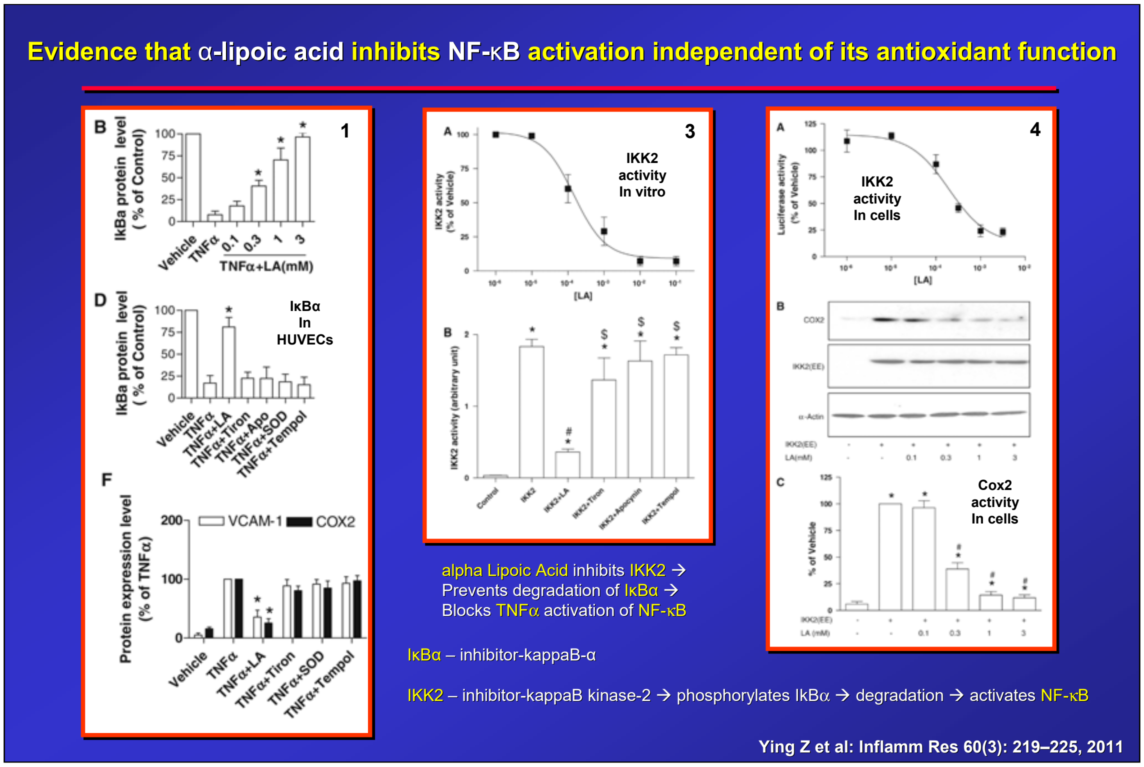


Fig 1. Antioxidant function of LA is not involved in inhibition of TNFα-induced NF-κB activation.

b. HUVECs were starved overnight and pre-treated with various concentration of LA for 20 min, followed by treatment with TNFα (10 ng/ml) for 10 min.

d. HUVECs were starved overnight and pre-treated with LA (1 mM), tiron (20 mM), apocynin (0.3 mM), PEG-SOD (100 U/ml) and tempol (0.1 mM) for 20 min, followed by treatment with TNFα (10 ng/ml) for 10 min.

f. HUVECs were starved overnight and pre-treated with LA (1 mM), tiron (20 mM), apocynin (0.3 mM), PEG-SOD (100 U/ml) and tempol (0.1 mM) for 20 min, followed by treatment with TNFα (10 ng/ml) for 30 min (NF-κB DNA binding activity assay) or 8 h (all other measurements). NF-κB DNA binding activity was assessed by EMSA. The expression of COX2 and VCAM-1 were examined by Western blot.

Fig 3. LA inhibits IKK2 in vitro.

a. The effect of LA on IKK2 activity

b The effect of antioxidants, n = 5. *$p < 0.05$ versus IKK− #$p < 0.05$ versus IKK+ \$$p < 0.05$ versus IKK

Fig. 4. LA inhibits IKK2 in living cells.

a. HEK293 cells transfected with constitutively active mutant of IKK2, IKK2(EE), and a NF-κB reporter construct. 24 hours later, cells treated with LA for 24 h, and the activity was analyzed, n = 3.

b & c. HEK293 cells treated as in 'a' & expression of COX2 was analyzed, n = 3.

*$p < 0.05$ versus IKK2(EE)−/LA− #$p < 0.05$ versus IKK2(EE)+/LA−

Abstract Objective—α-Lipoic acid (LA) exerts beneficial effects in cardiovascular diseases though its antioxidant and/or anti-inflammatory functions. It is postulated that the anti-inflammatory function of LA results from its antioxidant function. In this study we tested whether inhibition of NF-κB by LA is dependent on its antioxidant function.

Methods—HUVECs were treated with TNFα in the presence of various antioxidants, including LA, tiron, apocynin, and tempol. The activation of the nuclear factor-κB (NF-κB) signaling pathway was then analyzed.

Results—LA, but not other tested antioxidants, inhibited TNFα-induced inhibitor-kappaB-α (IκBα) degradation and VCAM-1 and COX2 expression in HUVECs. Although LA activated the phosphatidylinositol-3-kinase (PI3-kinase)/Akt pathway in HUVECs, inhibition of Akt by LY294002 did not affect inhibition of TNFα-induced IκBα degradation by LA. In transient co-transfection assays of a constitutively active mutant of IκB kinase-2 (IKK2), IKK2(EE), and a NF-κB luciferase reporter construct, LA dose-dependently inhibited IKK2(EE)-induced NF-κB activation in addition to inhibiting IKK activity in in vitro assays. Consistent with the effect on luciferase expression, LA inhibited IKK2(EE)-induced cyclo-oxygenase-2 (COX2) expression, suggesting that IKK2 inhibition by LA may be a relevant mechanism that explains its anti-inflammatory effects.

Conclusions—LA inhibits NF-κB activation through antioxidant-independent and probably IKK-dependent mechanisms.

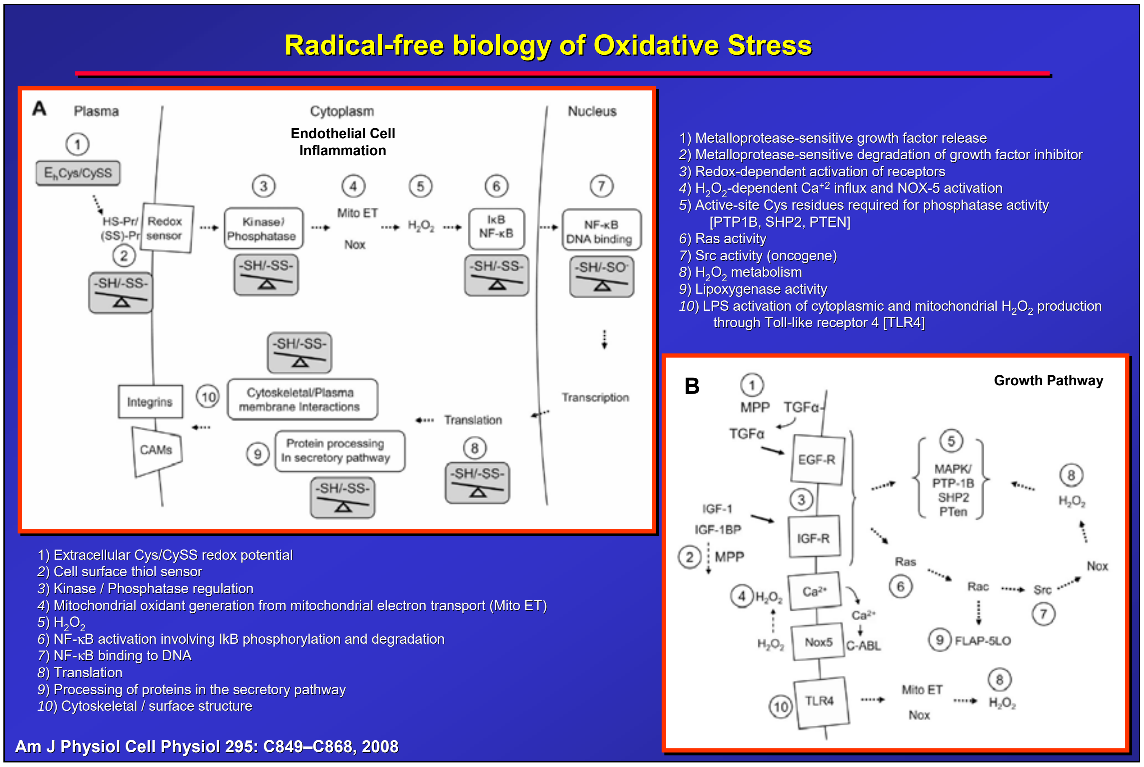


Figure 9. Redox-sensitive steps in hypothetical redox signaling pathways. Accumulated research from multiple investigators provides evidence for complex spatial and temporal redox events that can be combined in hypothetical signaling pathways with multiple redox sensitive steps. Evidence is collected from experiments using different cell lines and under different conditions so that the combined reactions should not be considered validated reaction pathways, but rather, as evidence that redox signaling involves so many redox-sensitive sites that the regulation is likely to have evolved a functional design that is coordinated through a smaller number of control nodes. Sites sensitive to changes in thiol-disulfide redox state are indicated by an "-SH/-SS-" balance.

***A*:** Early pro-inflammatory signaling in endothelial cells, redox-dependent signaling includes

1) Extracellular Cys/CySS redox potential

2) Cell surface thiol sensor

3) Kinase / Phosphatase regulation

4) Mitochondrial oxidant generation from mitochondrial electron transport (Mito ET)

5) H2O2

6) NF-B activation involving IkB phosphorylation and degradation

7) NF-B binding to DNA

8) Translation

9) Processing of proteins in the secretory pathway

10) Cytoskeletal/surface structure

***B*:** Receptor mediated signaling, redox-sensitive steps include

1) Metalloprotease-sensitive growth factor release

2) Metalloprotease-sensitive degradation of growth factor inhibitor

3) Redox-dependent activation of receptors

4) H2O2-dependent Ca2 influx and Nox-5 activation

5) Active-site Cys residues required for phosphatase activity [PTP1B, SHP2, PTen]

6) Ras activity

7) Src activity

8) H2O2 metabolism

9) Lipoxygenase activity

10) LPS activation of cytoplasmic and mitochondrial H2O2 production through Toll-like receptor 4 [TLR4]

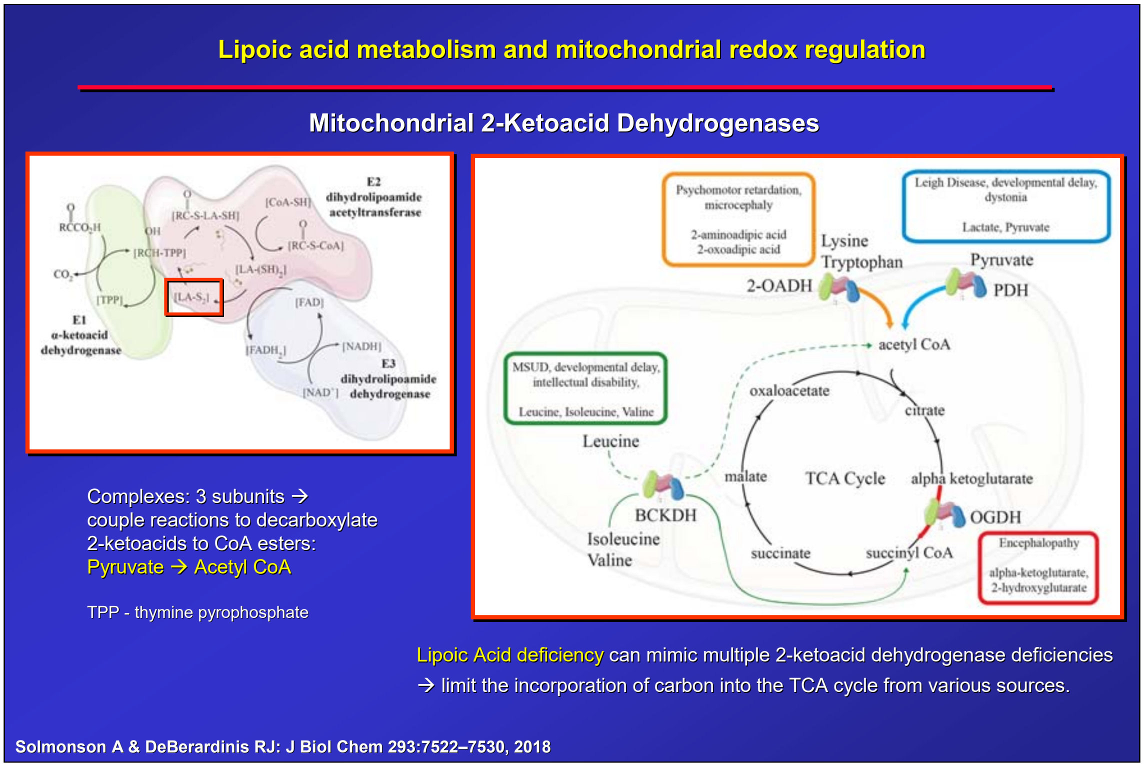
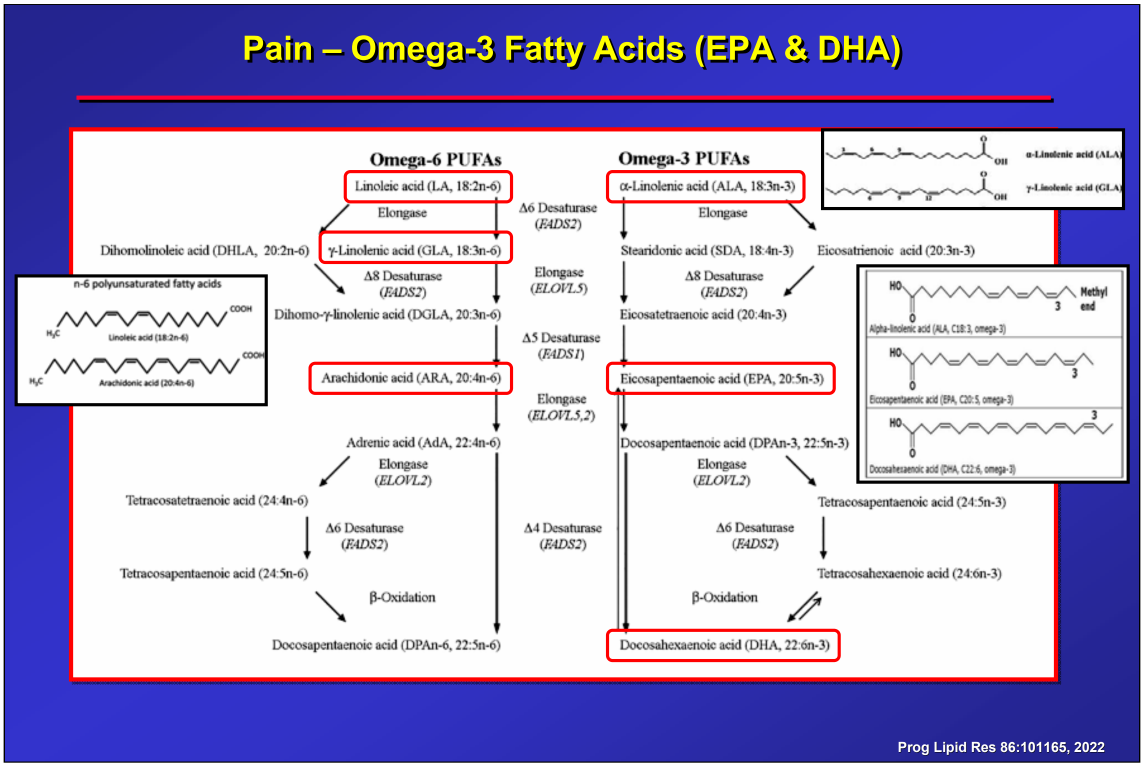


Figure 2. Reaction mechanisms of mitochondrial 2-ketoacid dehydrogenases and redox-dependent regulation.

2-Ketoacid dehydrogenase complexes consist of three enzyme subunits that use coupled reactions to decarboxylate a 2-ketoacid substrate and produce a CoA ester. The E1 subunit is a 2-ketoacid decarboxylase that uses a covalently bound thymine pyrophosphate (*TPP*) cofactor to decarboxylate the 2-ketoacid substrate followed by reductive acylation of a lipoyl moiety on the E2 subunit. The E2 subunit is a dihydrolipoamide acyltransferase that transfers the acyl intermediate from the E1 subunit to CoA generating an acyl-CoA and dihydrolipoamide. The E3 subunit is a dihydrolipoamide dehydrogenase that uses FAD to oxidize the lipoyl group on the E2 subunit for subsequent rounds of catalysis and generates NADH through coupled oxidation–reduction reactions of FADH2 and NAD.

Figure 3. Mitochondrial 2-ketoacid dehydrogenases and the TCA cycle.

Mitochondrial lipoylated enzymes individually contribute to pathways that generate products that can participate in the TCA cycle. Inborn errors in these dehydrogenases can be deleterious, with clinical symptoms including developmental delay (*PDH* and *BCKDH*), encephalopathy (*OGDH*), and microcephaly (*2-OADH*). Deficiencies in these enzymes can accumulate metabolites, including pyruvate and lactate (*PDH*), -ketoglutarate and 2-hydroxyglutarate, branched-chain amino acids and their corresponding 2-ketoacids, and 2-oxoadipic acid. Deficiencies in lipoic acid metabolism can phenocopy multiple simultaneous 2-ketoacid dehydrogenase deficiencies and can limit the incorporation of carbon into the TCA cycle from various sources.



Polyunsaturated fatty acids and fatty acid-derived lipid mediators: Recent advances in the understanding of their biosynthesis, structures, and functions ***Prog Lipid Res 86:101165, 2022***

Polyunsaturated fatty acids (PUFAs) are structural components of membrane phospholipids, and influence cellular function via effects on membrane properties, and also by acting as a precursor pool for lipid mediators. These lipid mediators are formed via activation of pathways involving at least one step of dioxygen-dependent oxidation, and are consequently called oxylipins. Their biosynthesis can be either enzymatically-dependent, utilizing the promiscuous cyclooxygenase, lipoxygenase, or cytochrome P450 mixed function oxidase pathways, or nonenzymatic via free radical-catalyzed pathways. The oxylipins include the classical eicosanoids, comprising prostaglandins, thromboxanes, and leukotrienes, and also more recently identified lipid mediators. With the advent of new technologies there is growing interest in identifying these different lipid mediators and characterising their roles in health and disease. This review brings together contributions from some of those at the forefront of research into lipid mediators, who provide brief introductions and summaries of current understanding of the structure and functions of the main classes of nonclassical oxylipins. The topics covered include omega-3 and omega-6 PUFA biosynthesis pathways, focusing on the roles of the different fatty acid desaturase enzymes, oxidized linoleic acid metabolites, omega-3 PUFA-derived specialized pro-resolving mediators, elovanoids, nonenzymatically oxidized PUFAs, and fatty acid esters of hydroxy fatty acids.

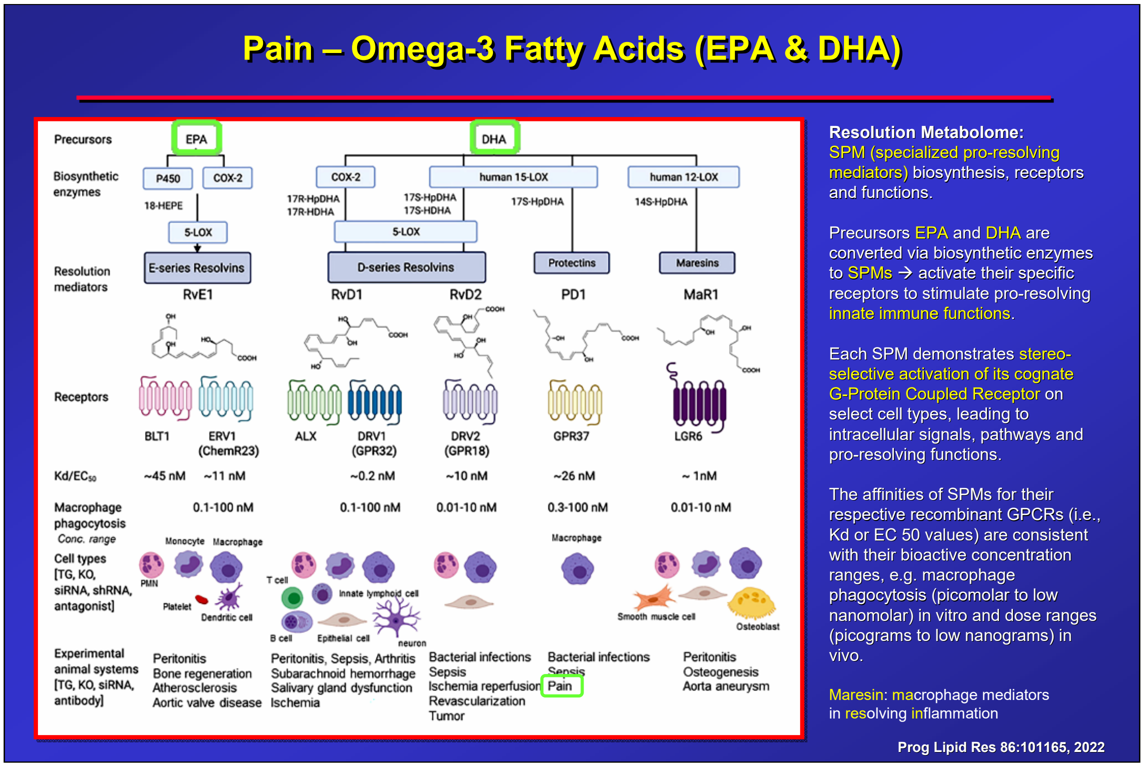


Fig. 2. Illustration of resolution metabolome: SPM biosynthesis, receptors and functions. Precursors EPA and DHA are converted via biosynthetic enzymes to SPMs, which in turn activate their specific receptors to stimulate pro-resolving innate immune functions. Each SPM demonstrates stereo-selective activation of its cognate GPCR on select cell types, leading to intracellular signals, pathways and pro-resolving functions. The affinities of SPMs for their respective recombinant GPCRs (i.e., Kd or EC 50 values) are consistent with their bioactive concentration ranges, e.g. macrophage phagocytosis (picomolar to low nanomolar) in vitro and dose ranges (picograms to low nanograms) in vivo. The in vivo functions of these SPM receptors were demonstrated using transgenic and/or knock-out mice, as well as specific blockage of the receptor.

4.2.3. SPM functions and receptors—Each SPM demonstrates potent stereo-selective actions (pico- to low nanomolar concentrations) via activation of specific G protein-coupled receptors (GPCR) on phagocytes and additional select cell types.

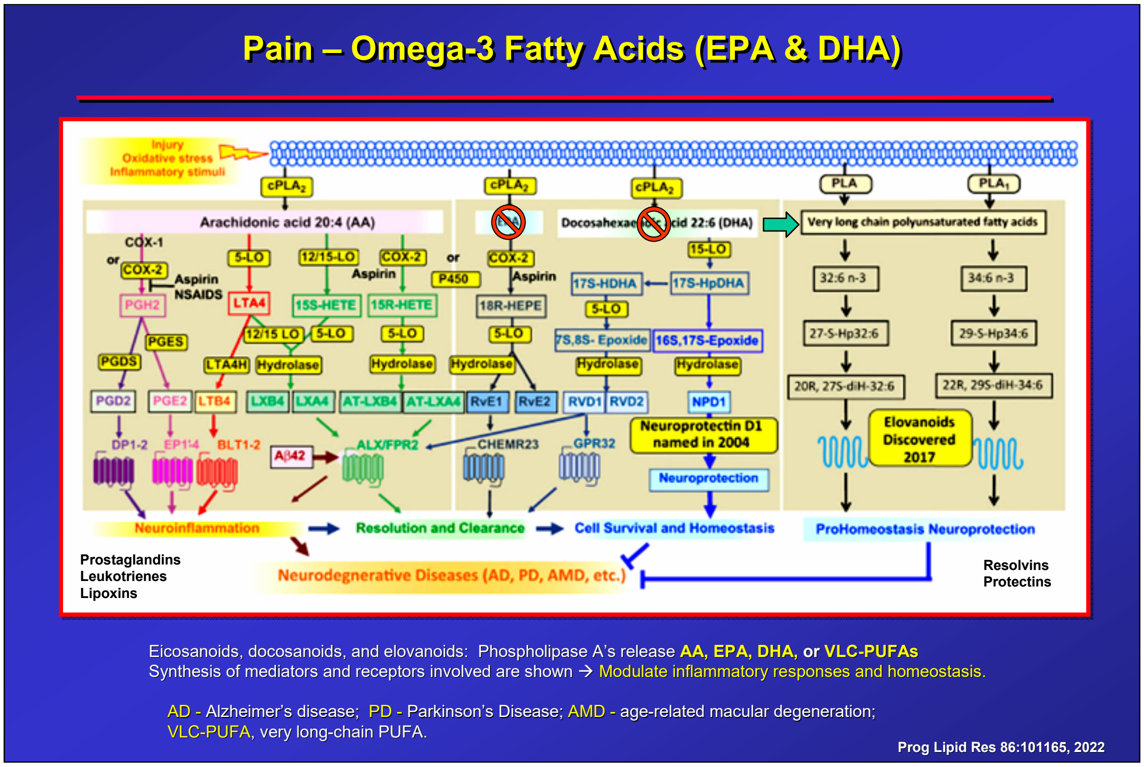
Resolvins: Resolution phase interaction products.

<u>E-series resolvins</u>: RvE1 was the first identified pro-resolving molecule derived from EPA. RvE1 via its receptor ERV1/ChemR23 (Kd ~11 nM) stimulates intracellular signals such as phosphorylation of S6 kinase (0.1–100 nM) (Fig. 2). RvD1, in vivo, controls vascular inflammation, protecting against atherosclerosis by modifying oxidized LDL uptake and enhancing macrophage phagocytosis. In aortic valve stenosis, targeted deletion of ChemR23 in mice heightens disease progression. Of interest, an agonist antibody to the RvE1 receptor confirms that activation of the endogenous resolution mechanisms can control both inflammation and cancer burdens in mouse models.

<u>D-series resolvins</u>: RvDs are biosynthesized from DHA; they are potent immuno-resolvents active in the picomolar to low nanomolar concentrations. RvD1 binds and activates human DRV1/GPR32 (Kd ~0.2 nM) to stimulate macrophage phagocytosis and efferocytosis (0.1–100 nM). **RvD2** (0.01–10 ng/mouse) **limits PMN infiltration in acute inflammation and controls bacterial sepsis** via its receptor DRV2/GPR18 in mice. RvD2 binds and activates human recombinant receptor DRV2/GPR18 (Kd ~10 nM) to stimulate macrophage phagocytosis and efferocytosis (0.01–10 nM). In human sepsis, survivors had a higher percentage of GPR18-positive peripheral blood neutrophils compared to non-survivors, suggesting that DRV2/GPR18 expression levels are associated with disease severity. In a more recent study, both DRV1 and DRV2 receptor expression were found to be higher on leukocytes from septic patients; both RvD1 and RvD2 partially reverse sepsis-induced leukocyte activation, and stimulate phagolysosome formation. **RvD2 suppresses tumor growth** and **enhances clearance of tumor cell debris**, while DRV2/GPR18-deficient mice display defective tumor clearance. In addition, RvDs are tissue/organ protective; RvD2 promotes **keratinocyte repair** in DRV2-dependent manner and stimulates **muscle regeneration**, as well as **limits tissue necrosis in burn wound**. RvD4 reduces thrombus burden and decreases the release of neutrophil extracellular traps (NETs), i.e. NETosis, a critical component for thrombosis development. These new roles of selective RvDs suggest that SPMs could provide an effective strategy in **controlling thrombo-inflammatory disease**. RvD5 and RvD1 controls E. coli and S aureus infection, by controlling phagocytosis and bacterial killing as well as inflammation arising from collateral tissue damage; together these lower the antibiotic requirements for bacterial clearance. RvD5 is the first SPM that shows sex dimorphism in pain, **inhibiting pain in male, but not female mice**.

<u>Protectins</u>: Protectin D1/Neuroprotectin D1 (PD1/NPD1) is also biosynthesized from DHA via 15-LOX-initiated mechanism in several human cell types, murine exudates, and brain tissues. In addition, PD1 is present in human exhaled breath condensates, and its levels are lower in subjects with asthma exacerbations. DHA is converted via 15-LOX to the 16S, 17S-epoxide intermediate, confirmed by epoxide trapping experiments. This epoxide intermediate is further converted to PD1 via enzymatic hydrolysis. The elicited bioactivity of this mediator in human retinal pigment epithelial cells led to coining its name as Neuroprotectin D1 (NPD1). This was strongly supported by the demonstration of its formation in the human brain and its **selective decrease in memory areas of the brains of Alzheimer's patients** and in experimental Alzheimer's disease models, as well as in **experimental ischemic stroke**. The complete stereochemical assignments enabled the demonstration of its **potent actions on human PMN** [1–100 nM] and **acute inflammation** in vivo [0.01–100 ng/mouse] as well as in many disease systems, confirmed and extended by many other investigators worldwide. Hence, while produced and functions in neural systems, the prefix (neuro)protectin D1 was introduced, and in the immune system, it is PD1. PD1/NPD1 displays **potent neuroprotective actions in brain**, retina and central nervous system, e.g. protecting from ischemic stroke, retina degenerative disease and traumatic brain injury. NPD1/PD1 activates recombinant and macrophage GPR37 [EC50 ~ 26 nM]. Mice lacking this NPD1/PD1 receptor display defects in macrophage phagocytic activity with delayed resolution of inflammatory pain. PD1's protective actions in multiple models of infections and sepsis are diminished in these Gpr37 receptor KO mice. PDX is a positional isomer of PD1, biosynthesized via two sequential lipoxygenations [95]. PDx [0.1–10 μM] inhibits platelet activation, improves insulin sensitivity and atherosclerosis in type-2 diabetes. Both PDx and PD1 at equal amounts suppress replication of influenza virus.

<u>Maresins</u>: The macrophage mediators in **resolving inflammation**. MaR1 was first identified in self-resolving inflammatory exudates and with human macrophages (MΦ) via 12-LOX-initiated mechanisms. **MaR1 is pro-regenerative, pro-repair and neuroprotective** in a wide range of tissues and organs across phyla. MaR1 activates LGR6 (leucine-rich repeat-containing G protein–coupled receptor 6), a cell surface G protein-coupled receptor [EC50 ~ 1 nM] and stimulates key pro-resolving functions of phagocytes in a LGR6-dependent manner [0.01–10 nM]. In addition, MaR1 inhibit smooth muscle cell activation and attenuate murine abdominal aortic aneurysm via LGR6 signaling. Further, LGR6 is **necessary for normal osteogenesis**, demonstrated using LGR6-deficient mice, and MaR1 activates LGR6 signaling in osteoblasts. With liver macrophages, MaR1 can also activate ROR-α (retinoic acid-related orphan receptor α), a nuclear receptor that might be relevant in liver pathology.



4.4. Elovanoids

In the following section N.G. Bazan details the discovery of the elovanoids (ELVs) and identification of their mechanisms of action.

The significance of polyunsaturated fatty acids (PUFAs) has evolved from the broad concepts of providing membrane structural plasticity and fluidity for proteins diffusion and rotation to a diverse universe of functions. For example, DHA is necessary for sight, and when administered, is beneficial in x-linked retinitis pigmentosa and other neurode-generative diseases [178]. DHA from the diet is packaged by the liver and targeted to the central nervous system (CNS), where it achieves the highest concentration in photoreceptors and synaptic membranes [179].

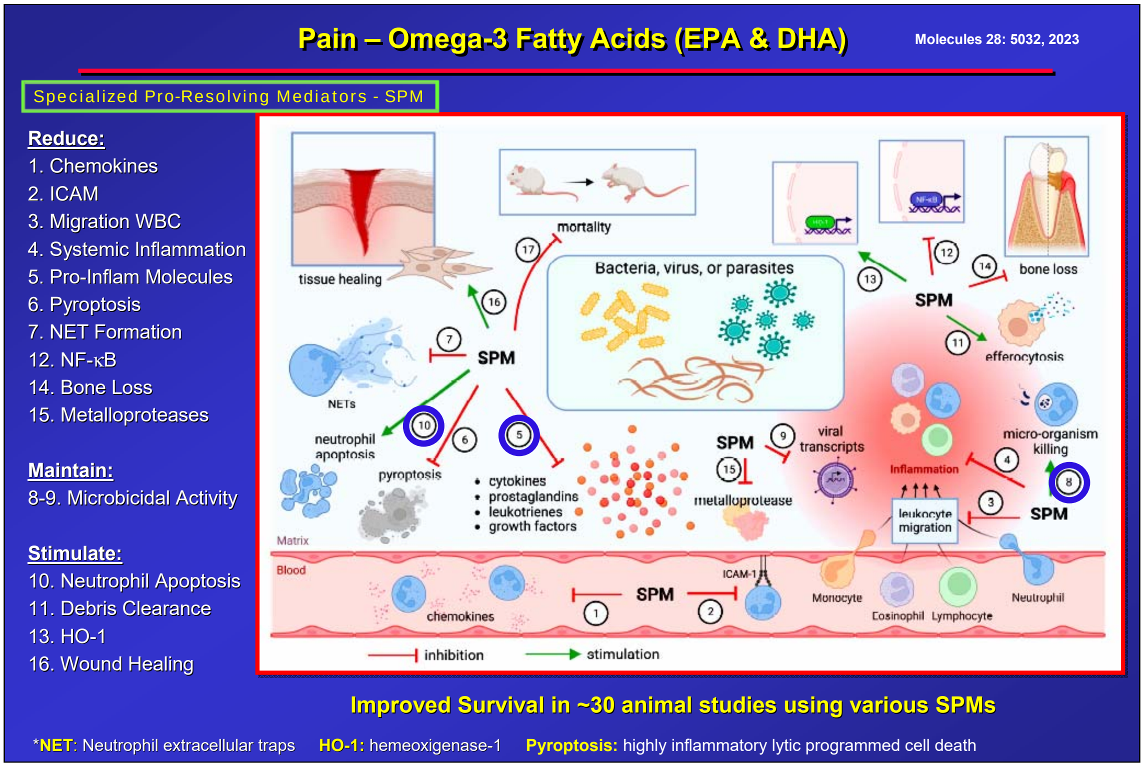
PUFAs, precursors of lipid mediators and components of membrane lipids, comprise a new multidisciplinary field at the boundary of biophysics, chemical biology, and molecular physiology. Thus, at least two important issues have emerged: the gene that encodes the enzyme that elongates PUFAs to chain length ≥ 28 carbons (ELOVL4) is critically important for cell function, and their products are precursors of the new family of lipid mediators, the elovanoids (ELVs).

4.4.1. ELOVL4—ELOVL4 catalyzes the rate-limiting condensation reaction for the synthesis of very long chain -saturated fatty acids (VLC-SFAs) and VLCPUFAs (chain length ≥ 28 carbons) [180]. This enzyme is expressed in brain neurons, photoreceptor cells, skin, testes, and meibomian glands [180]. In the skin, VLC-SFAs are components of sphingolipids, and these VLC-SFAs are necessary as a skin-permeability barrier [181]. ELOVL4 is selectively expressed in neurons and is evolutionarily conserved [182]. In photoreceptor cells, VLC-PUFAs are in phosphatidylcholines (PC) of the outer segment membranes, tightly bound to rhodopsin [183].

Mutation, loss, or down-regulation of ELOVL4 is linked to retinal degeneration. Studies of a large familial group with retinal degeneration revealed an autosomal dominant macular dystrophy phenotype which results from a 5-bp deletion, causing Stargardt-like macular dystrophy [184,185], and an STGD3 mouse Elovl4 mutation produces a C32-C36 PC deficiency [186], leading to the suggestion that loss or reduced VLC-PUFAs may cause loss of photoreceptors or functional perturbations [187], highlighting the importance of these molecules in the retina. Therefore, because of the inability to take up and incorporate DHA and the absence of VLC-PUFAs in the degenerating adiponectin receptor 1 (AdipoR1)−/− mouse retina, the synthesis of these molecules must rely on the presence of DHA. The occurrence of central geographic atrophy (CGA) and neovascular age-related macular degeneration (AMD) was found to be 30% less likely with high omega-3 LC-PUFA (e.g., DHA) intake [188], emphasizing the importance of maintaining adequate dietary amounts of DHA for retinal homeostasis.

Neuron-specific ELOVL4 is expressed in the CNS, including in hippocampal neurons of the dentate gyrus (DG) subgranular layer, a locus for medial temporal lobe epilepsy. Mutations in ELOVL4 lead to impaired neural development, mental retardation, neuronal dysfunction, hyperexcitability, and seizures [189].

4.4.2. Elovanoids are a new class of bioactive lipids synthesized from C32 or C34 FA precursors—In 2017, elovanoids (ELVs) were discovered and named [190–192]. This new class of endogenous lipid mediators is distinct from the widely known lipid mediators produced from PUFAs with C20 and C22, such as the classical eicosanoids and SPMs. ELV-N32 and ELV-N34 are stereo-specific di-hydroxylated derivatives of 32:6n-3 or 34:6n-3 (Fig. 5), respectively, made by the elongase ELOVL4 (elongation of VLC-FAs-4), which converts C26-derived FAs from EPA or DHA to VLC-PUFAs, ≥C28. These PUFAs are mainly esterified at the C1 (sn-1) position of PC that has DHA in the C2 (sn-2) position, and upon the appropriate stimulus (e. g., uncompensated oxidative stress), are released by phospholipase A1 (PLA1) and/or PLA2 for the formation of ELVs, NPD1, or other docosanoids (Fig. 6). Here, I describe key events in the discovery of ELVs and highlight some of their functions.



Specialized Pro-Resolving Lipid Mediators: Endogenous Roles and Pharmacological Activities in Infections
Molecules 28: 5032, 2023. https://doi.org/10.3390/molecules28135032

Figure 2. SPMs control infection by different mechanisms. SPMs limit chemokine release (1) and ICAM-1 expression (2), reducing migration of leukocytes (3) to the inflammatory site. Systemic inflammation (4) is also diminished, together with prominent reduction in pro-inflammatory molecules' release (5). SPMs also decrease pyroptosis (6) and NET formation (7), without compromising microbicidal activities (8 and 9). Neutrophil accumulation is avoided also by stimulating neutrophil apoptosis (10) and clearance of cell debris by macrophages (11). Inhibition of NF-B (12) and upregulation of HO-1 (13) favor inflammation control. As a result, less bone loss is observed (14), and tissue architecture is preserved by reducing the activity of metalloproteases (15) and stimulating tissue healing (16). Improvement of survival was also observed.

Abstract: During an infection, inflammation mobilizes immune cells to eliminate the pathogen and protect the host. However, inflammation can be detrimental when exacerbated and/or chronic. The resolution phase of the inflammatory process is actively orchestrated by the specialized pro-resolving lipid mediators (SPMs), generated from omega-3 and -6 polyunsaturated fatty acids (PUFAs) that bind to different G-protein coupled receptors to exert their activity. As immuno-resolvents, SPMs regulate the influx of leukocytes to the inflammatory site, reduce cytokine and chemokine levels, promote bacterial clearance, inhibit the export of viral transcripts, enhance efferocytosis, stimulate tissue healing, and lower antibiotic requirements. Metabolomic studies have evaluated SPM levels in patients and animals during infection, and temporal regulation of SPMs seems to be essential to properly coordinate a response against the microorganism. In this review, we summarize the current knowledge on SPM biosynthesis and classifications, endogenous production profiles and their effects in animal models of bacterial, viral and parasitic infections.

Pyroptosis is a highly inflammatory form of lytic programmed cell death

Hemeoxygenase 1 (**HO-1**) is an inducible enzyme responsible for the breakdown of heme but also exhibits numerous anti-inflammatory properties.

Pain – Omega-3 Fatty Acids (EPA & DHA)

Table 1. Consequences of having a long-chain omega-3 fatty acid deficiency in the brain (adapted from Logan) [63].

Nutrients 12:2333, 2020

20% reduction in 5′-nucleotidase activity (decrease membrane fluidity)
30% decrease in synaptic vesicle density in the hippocampus
30% decrease in glucose uptake by neurons
30–35% decrease in phosphatidylserine in brain cortex, brain mitochondria and olfactory bulb
40% decrease in cytochrome oxidase activity
40% reduction in Na-K-ATPase at nerve terminals
Decrease dopamine in vesicle pool, frontal cortex, olfactory bulb
90% decrease in tyramine-stimulated dopamine release from vesicle storage
Decreased dopamine release upon serotonin stimulation
Decreased cerebral microperfusion
Decreased hippocampal CA1 pyramidal neuron cell body size
Decreased vesicular monoamine transporter (VMAT2) which allows dopamine entry/storage in the vesicle)
Decreased pre and post-synaptic dopamine receptor (D2R) in the frontal cortex
Increased serotonin receptor (5HT2) density (indicating reduced serotonin function) similar to that found in those who have committed suicide
Animal studies have found that high levels of omega-3s associate with a 40% increase in frontal cortex dopamine levels including increased binding to the D2 receptor and inhibition of monoamine-oxidase B an enzyme that breaks down dopamine
Reduced nerve growth factor and neurite outgrowth
Decreased amino acid delivery across the blood-brain barrier
Increased proinflammatory cytokines and eicosanoids increased PDE4 activity and a possible reduction in brain-derived neurotrophic factor
Reduced phospholipid biosynthesis and increased phospholipid breakdown (increased brain atrophy)

Abstract: *Nutrients 12:2333, 2020*

Most of the global population is deficient in long-chain marine omega-3s. In particular, docosahexaenoic acid (DHA), a long-chain omega-3 fatty acid, is important for brain and eye development. Additionally, DHA plays a significant role in mental health throughout early childhood and even into adulthood. In the brain, DHA is important for cellular membrane fluidity, function and neurotransmitter release. Evidence indicates that a low intake of marine omega-3s increases the risk for numerous mental health issues, including Attention Deficit Hyperactivity Disorder (ADHD), autism, bipolar disorder, depression and suicidal ideation. Studies giving supplemental marine omega-3s have shown promise for improving numerous mental health conditions. This paper will review the evidence surrounding marine omega-3s and mental health conditions.

9. The Importance of Long-Chain Omega-3s and Brain Health

Approximately 20% of the dry weight of the brain is made up of polyunsaturated fatty acids and one out of every three fatty acids in the nervous system is a polyunsaturated fatty acid [63]. Docosahexaenoic acid (DHA) is particularly prevalent in the brain and can be retro-converted to eicosapentaenoic acid (EPA) serving as a generator of EPA. Thus, omega-3 polyunsaturated fatty acids are extremely important in brain function and can contribute to disorders of the brain including depression. Both EPA and DHA have been shown to be important in the treatment and prevention of depression, whereas only the latter is a major structural component of neuronal cell membrane phospholipids.

Marine omega-3s can improve neurotransmitter binding and signaling in the brain by maintaining an optimal membrane fluidity optimizing protein channel function in the lipid bi-layer [63]. Furthermore, neurological and metabolic benefits of consuming marine omega-3s may occur through activating G-protein coupled receptors (GPR40 and GPR120) as well as peroxisome proliferator-activated receptors (PPARs). Indeed, activation of PPAR-alpha can increase hepatic fatty acid oxidation and reduce triglyceride synthesis, PPAR-gamma activation can improve insulin sensitivity in adipose tissue and produce anti-inflammatory effects reducing inflammation and improving insulin sensitivity. GPR120 is highly expressed on adipocytes and inflammatory macrophages and DHA and EPA can promote GPR120-mediated gene activation inhibiting activation of nuclear factor kappa B and reducing inflammation. Furthermore, DHA promotes the translocation of the glucose transporter GLUT4 in adipocytes improving glucose uptake. All of these anti-inflammatory and metabolic effects may have a role in improving brain health. A low dietary intake of marine omega-3s reduces the concentration of omega-3s in cellular membranes, stiffening the membrane and creating a spring-like stress on protein channels which may affect their function. Indeed, low levels of long-chain omega-3 fatty acids in cellular membranes reduces the Na-K-ATPase in nerve terminals, which consumes around half the energy of the brain allowing for nerve transmission and communication [63]. Increasing long-chain omega-3s in the brain may reduce inflammatory cytokines, which may improve neurotransmitter function. There is also a reduction in dopamine and serotonin signaling with omega-3 deficiency in the brain [63]. A deficiency of omega-3 in the brain also reduces synaptic vesicle density in terminals of the hippocampus by 30%, phosphatidylserine levels in the brain by 30–35%, glucose uptake into neurons by 30%, and tyramine-stimulated dopamine release by 90% [63]. The overall consequences of having a long-chain omega-3 deficiency in the brain are summarized in Table 1 and the possible mechanisms for the benefits of omega-3s in depression are summarized in Table 2.

Optimizing Nutrition for Better Living

Effects of dietary supplements on PAIN:

Omega-3 Fish Oil:
1. Maintains bone mass & cartilage integrity
2. Diabetic Neuropathy:
   - Improves cold & vibratory perception
   - Improves sensory scores & pain
3. Rheumatoid Arthritis:
   - Reduces the number of tender & swollen joints
   - Improves clinical scores
   - Patients reduce their anti-rheumatoid medications in 3-4 months
   - Reduces morning stiffness & fatigue
   - Improves daily activity
   - Improves patient's global perception of their disease activity
   - Reduces pain in 3-4 months
4. Degenerative Arthritis:
   - Prevents the development of pain & stiffness
   - Maintains physical function
   - Allows increased minutes of exercise
5. Promotes weight loss
6. Reduces post-operative pain
7. Exercise:
   - Reduces post-exercise soreness, muscle edema, reduced range of motion
   - Reduces post-exercise fatigue & pain
   - Improves muscle power
   - Reduces muscle inflammation
8. Covid-19: Reduces inflammation, pain, & fatigue
9. Migraines may be reduced
10. Symptoms in Parkinson Disease & early Dementia improve & progression slowed
11. Depression, anxiety, & ADHD probably improve

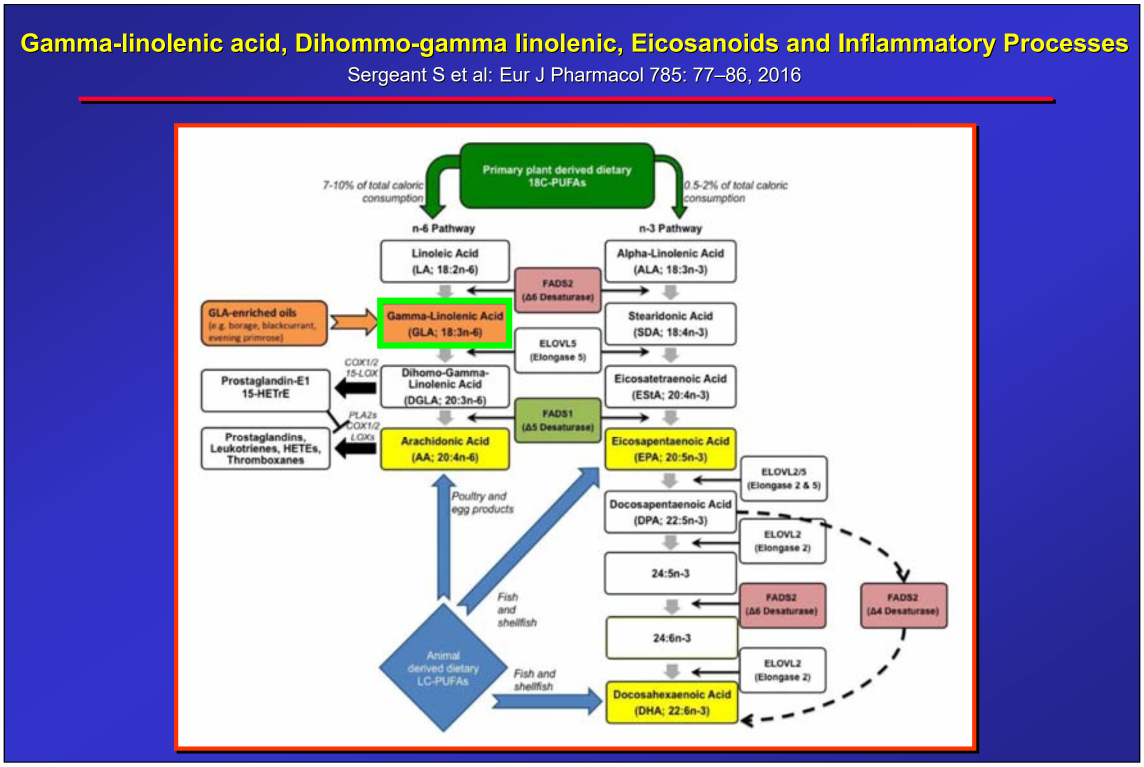
Alpha Lipoic Acid:
1. Diabetic Neuropathy: Improves nerve conduction, pain, & symptoms
2. Lumbo-Sacral Nerve Compression: Reduces pain & disability
3. Pain of Unknown Etiology (joint, nerve, or muscle): Reduced ~70% in 2 months
4. Treats Steato-Hepatitis
5. Scavenges free radicals & chelates heavy metals
6. Regenerates Vitamin C, Vitamin E, Glutathione, & anti-oxidant enzymes
7. Reactivates metabolic pathways by reducing the sulfur in their active sites
8. Critical co-factor for glucose metabolism & ATP production → reduces HgbA1c
9. Inhibits NF-κB → reduces inflammation
10. Generates H_2S → vasodilates, inhibits AS, stimulates Nrf2 (anti-oxidant enzymes)

Vitamin E:
1. Diabetic Neuropathy: Improves nerve conduction, numbness, & freezing pain
2. Degenerative Arthritis:
   - Reduces pain at rest & with movement
   - Reduces stiffness & improves function
   - Reduces inflammation in joint
3. Rheumatoid Arthritis: Reduces morning pain & pain with exercise

Vitamin C:
1. Surgery:
   - Reduces post-operative pain
   - Reduces "Complex Regional Pain Syndrome"
   - Improves outcomes after joint & back surgery

Alpha Lipoic Acid + Vitamin E + Vitamin C:
1. Improves muscle blood flow after exercise in older men

2025
What’s New



Abstract Gamma-linolenic acid (GLA, 18:3n-6) is an omega-6 (n-6), 18 carbon (18C-) polyunsaturated fatty acid (PUFA) found in human milk and several botanical seed oils and is typically consumed as part of a dietary supplement. While there have been numerous in vitro and in vivo animal models which illustrate that GLA-supplemented diets **attenuate inflammatory responses**, clinical studies utilizing GLA or GLA in combination with omega-3 (n-3) PUFAs have been much less conclusive. A central premise of this review is that there are **critical metabolic and genetic factors that affect the conversion of GLA to dihommo-gamma linolenic acid** (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6), which consequently affects the balance of DGLA- and AA-derived metabolites. As a result, these factors impact the clinical effectiveness of GLA or GLA/n-3 PUFA supplementations in treating inflammatory conditions. Specifically, these factors include: 1) the **capacity** for different human cells and tissues **to convert** GLA to DGLA and AA and to metabolize DGLA and AA to bioactive metabolites; 2) the opposing effects of DGLA and AA metabolites on inflammatory processes and diseases; and 3) the impact of **genetic variations** within the fatty acid **desaturase** (FADS) gene cluster, in particular, on AA/DGLA ratios and bioactive metabolites. We postulate that these factors influence the heterogeneity of results observed in GLA supplement-based clinical trials and suggest that "one-size fits all" approaches utilizing PUFA-based supplements may no longer be appropriate for the prevention and treatment of complex human diseases.

3.1 Differential Metabolism of GLA to DGLA and AA in Human Cells and Tissues Since metabolites of DGLA have predominantly anti-inflammatory effects and AA products generally enhance inflammation, it stands to reason that the balance of AA to DGLA (i.e. the ratio of AA/DGLA) in circulation, cells and tissues is a critical factor that impacts inflammatory processes. Several factors determine the levels of AA and DGLA and thus the ratio of AA metabolites and DGLA metabolites within cells and tissues. One is the differential capacities of cells or tissues to **elongate GLA to DGLA** and then to further **desaturate** it to AA. Differential expression of enzymatic activities is observed when comparing GLA metabolism within an inflammatory cell, such as the human neutrophil, and within a tissue bed or organ, such as the human liver. Both in vitro and in vivo studies demonstrate that human **neutrophils contain the elongase (ELOVL5) but not the Δ-5 desaturase** (i.e. FADS1) activity. In addition to human neutrophils, **skin**, murine peritoneal **macrophages** and **platelets** also appear to have high ELOVL5 elongase activity relative to FADS1 (Δ-5) desaturase activity. In contrast, several other tissues including **liver, kidney, testes, brain and intestine** appear to contain **both** activities.

4. The Impact of Genetic Variation in the Fatty Acid Desaturase (*FADS*) Gene Cluster on AA/DGLA Ratios and Eicosanoid Production These studies clearly support that associations between genetic variants and PUFA levels are strongly related to ethnicity. For example, **~80% of African Americans carry two copies of the alleles associated with increased levels of AA** and **DHA and reduced levels of DGLA**, compared to only ~45% of European Americans. Together these studies raise important questions of whether gene-PUFA interactions induced by a modern western diet are differentially driving the risk of diseases of inflammation in diverse populations, and are these interactions leading to health disparities.

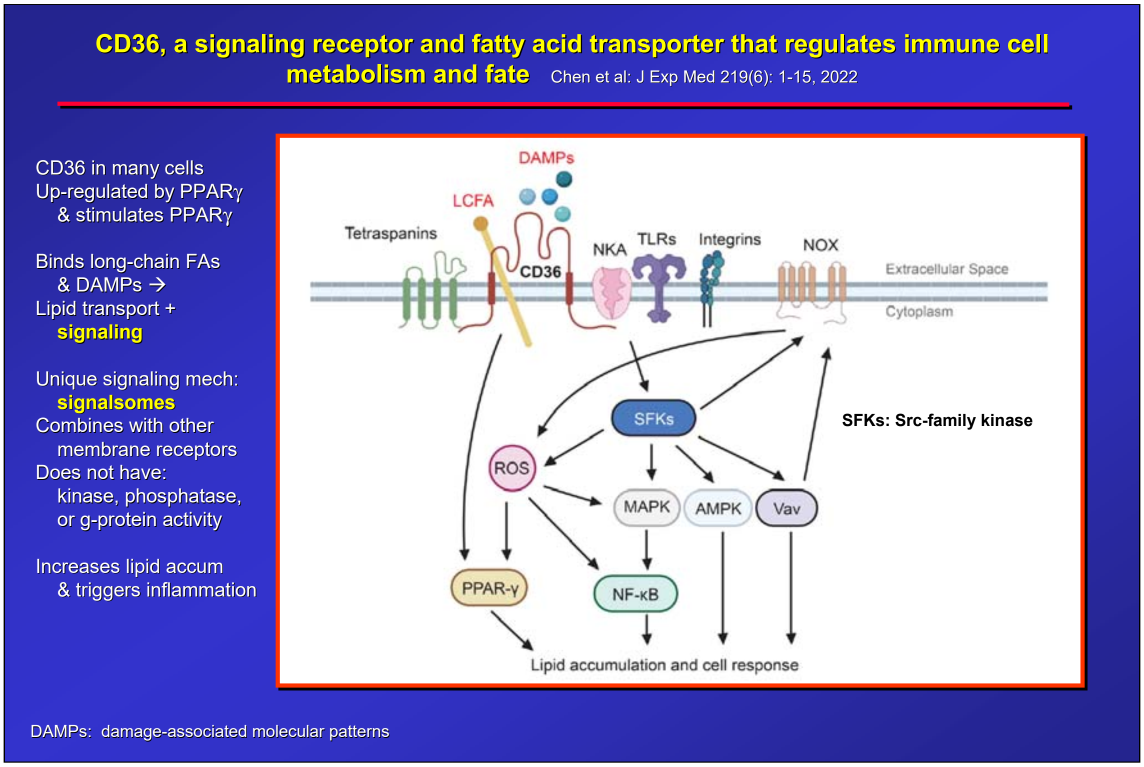


Figure 1. CD36 functions as both a signal transducer and fatty acid transporter. In response to extracellular signals such as DAMPs, CD36 assembles and interacts with other membrane receptors, including NKA, TLRs, integrins, and tetraspanins, forming distinct **signaling complexes (signalsomes)**. The signaling complexes subsequently relay the signals to various downstream effectors including SFKs (src-family kinase), MAPK, AMPK, guanine nucleotide exchange factor Vav, and the NOX family of nicotinamide adenine dinucleotide phosphate oxidases. Activation of these effectors leads to ROS production as well as transcription factor activation, including PPAR-γ and NF-κB. Meanwhile, CD36 also binds with LCFAs and facilitates their transport across the plasma membrane, which impacts fatty acid metabolism and may contribute to activation of the PPAR-γ pathway. The dual functions of CD36 eventually integrate, leading to different cellular responses such as migration, immune activation, differentiation, and cell growth/death.

ABSTRACT: CD36 is a type 2 cell surface scavenger receptor widely expressed in many immune and non-immune cells. It functions as both a signaling receptor responding to DAMPs and PAMPs, as well as a long chain free fatty acid transporter. Recent studies have indicated that CD36 can integrate cell signaling and metabolic pathways through its dual functions and thereby influence immune cell differentiation and activation, and ultimately help determine cell fate. Its expression along with its dual functions in both innate and adaptive immune cells contribute to pathogenesis of common diseases, including atherosclerosis and tumor progression, which makes CD36 and its downstream effectors potential therapeutic targets. This review comprehensively examines the dual functions of CD36 in a variety of immune cells, especially macrophages and T cells. We also briefly discuss CD36 function in non-immune cells, such as adipocytes and platelets, which impact the immune system via intercellular communication. Finally, outstanding questions in this field are provided for potential directions of future studies.

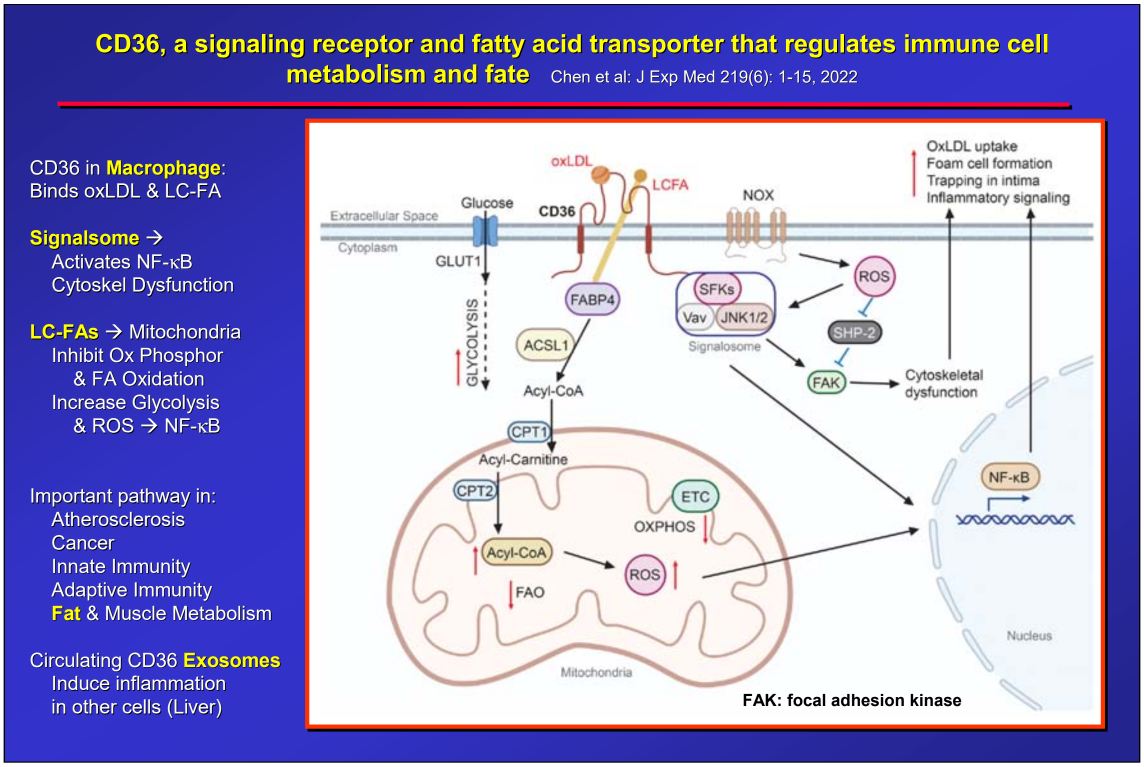
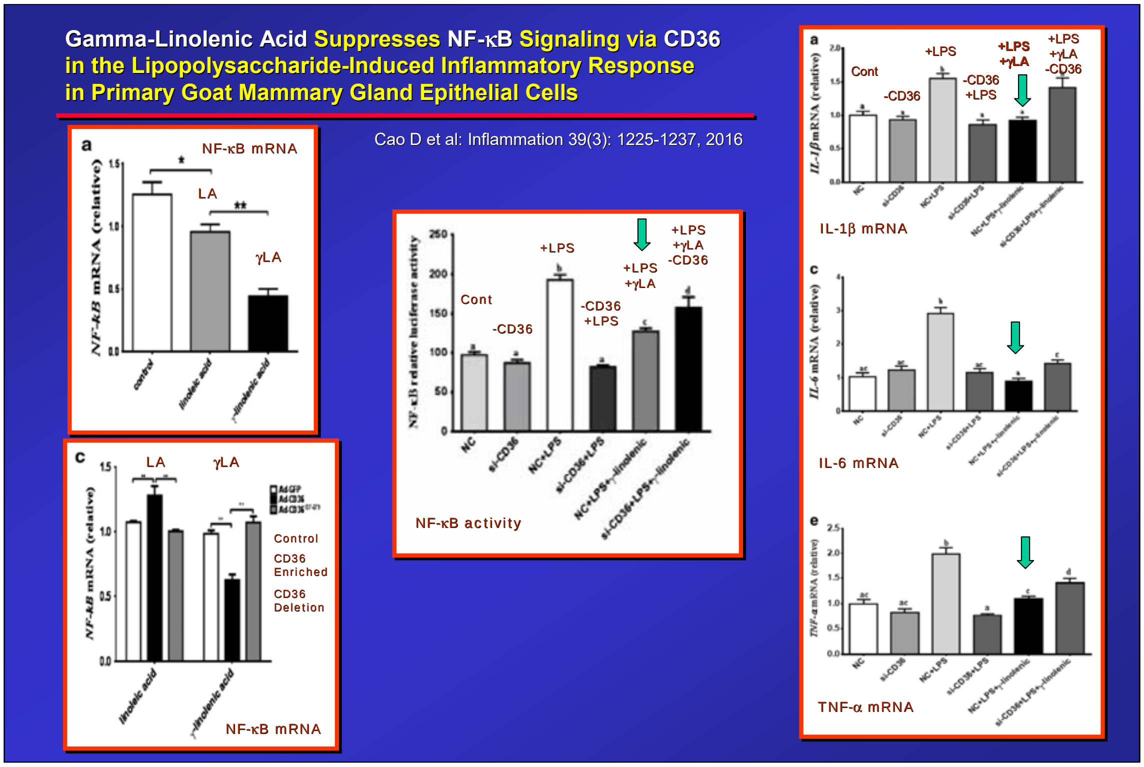


Figure 2. CD36 mediates macrophage activation. oxLDL binds to extracellular domains of CD36, which promotes both signaling cascades and lipid uptake. The CD36 cytosolic tail recruits a signalosome complex including SFKs, JNK1/2, and Vav, which is also enhanced by NOX-derived ROS. Both signalosome and ROS (by inhibition of SHP-2) activate focal adhesion kinase (FAK), which leads to dysregulated cytoskeletal dynamics. Meanwhile, extracellular LCFA is transported into the cell via CD36 and then into mitochondria matrix through a series of transport machinery interactions including FABP4, ACSL1, CPT1, and CPT2. LCFA influx to mitochondria facilitates a metabolic switch from OXPHOS to glycolysis, accompanied by a reduction in FAO and increase in ROS production. The signaling cascades and metabolic switch in combination results in NF-κB pathway activation and pro-atherogenic responses including pro-inflammatory activation, oxLDL uptake, foam cell formation, and trapping of macrophages in the neointima.

ABSTRACT: CD36 is a type 2 cell surface scavenger receptor widely expressed in many immune and non-immune cells. It functions as both a signaling receptor responding to DAMPs and PAMPs, as well as a long chain free fatty acid transporter. Recent studies have indicated that CD36 can integrate cell signaling and metabolic pathways through its dual functions and thereby influence immune cell differentiation and activation, and ultimately help determine cell fate. Its expression along with its dual functions in both innate and adaptive immune cells contribute to pathogenesis of common diseases, including atherosclerosis and tumor progression, which makes CD36 and its downstream effectors potential therapeutic targets. This review comprehensively examines the dual functions of CD36 in a variety of immune cells, especially macrophages and T cells. We also briefly discuss CD36 function in non-immune cells, such as adipocytes and platelets, which impact the immune system via intercellular communication. Finally, outstanding questions in this field are provided for potential directions of future studies.



Abstract: Gamma-linolenic acid (GLA) and linoleic acid (LA), which are both n-6 unsaturated fatty acids, play vital roles in lipopolysaccharide (LPS)-induced inflammation. The multi-functional protein scavenger receptor CD36 has also been shown to participate in inflammation. However, the molecular mechanisms underlying the interactions between CD36 and GLA or LA in LPS-induced inflammation remain unclear. We used small interfering RNA and adenoviral systems to manipulate CD36 expression in primary goat mammary gland **epithelial cells** (pGMECs), and the results showed that nuclear factor kappa B (NF-κB) levels were significantly decreased by CD36 receptor signaling following treatment with GLA but not LA. GLA inhibited NF-κB activation in LPS-induced pGMECs. However, silencing CD36 or deleting its fatty acid-binding domain blocked the anti-inflammatory effects of GLA, resulting in an increase in NF-κB activation and disrupting its localization during LPS-induced inflammation. The activity of the cytokines IL-1β, IL-6, and TNF-α, which act downstream of NF-κB, was also modulated when CD34 expression was manipulated by the addition of GLA in LPS-induced pGMECs. Our data suggest that **GLA, but not LA**, may interact with the **CD36 fatty acid-binding domain** to regulate the activation and localization of **NF-κB** in LPS-induced pGMECs.

Fig. 2. The effects of GLA and LA on NF-κB mRNA levels.

a, d: NF-κB mRNA levels and luciferase activity were measured in pGMECs that were treated with 100 μMGLA or LA for 12 hr.

b, e: The cells were pretreated with NC or si-CD36 for 24 h and then treated with 100 μMGLA or LA for 12 h.

c, f: The cells were infected with Ad-GFP, Ad-CD36, or Ad-CD36127–279 for 24 h and then treated with 100 μM GLA or LA for 12 h. The quantitative PCR data were normalized to GAPDH, UXT, and MRPL39.

The data shown represent means ± SEM from three experiments. $^*p< 0.05$; $^{**}p < 0.01$; $^{***}p< 0.001$, NS not significant.

Fig. 3. Effects of GLA on NF-κB activation.

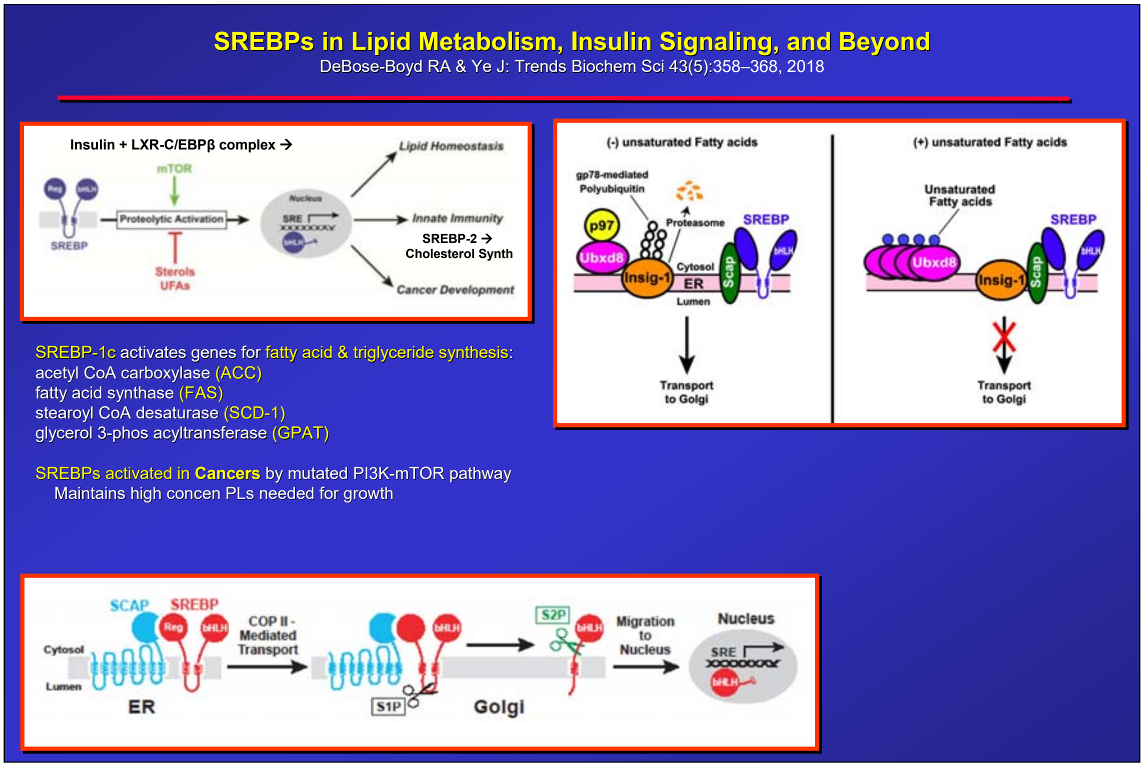
a After cells were pretreated with NC or si-CD36 for 24 h, NF-κB luciferase activity was measured in pGMECs that had been treated with 10 μg/mL LPS alone or with LPS and 100 μMGLA for 12 h.

Fig. 4. Effects of GLA on IL-1β, IL-6, and TNF-α mRNA expression levels.

a, c, e: After preincubation with NC or si-CD36 for 24 h, then RNA expression levels of IL-1β, IL-6, and TNF-α were measured in pGMECs that were treated with 10 μg/mL LPS alone or LPS and 100 μM GLA for 12

h. b, d, f: After infection with Ad-GFP, Ad-CD36, or Ad-CD36-127–279 for 24 h, the expression levels of IL-1β, IL-6, and TNF-α were measured in the pGMECs that were treated with 10 μg/mL LPS alone or LPS and 100 μM GLA for 12 h. The quantitative PCR data were normalized to GAPDH, UXT, and MRPL39. The data shown represent the means ± SEM from three experiments.

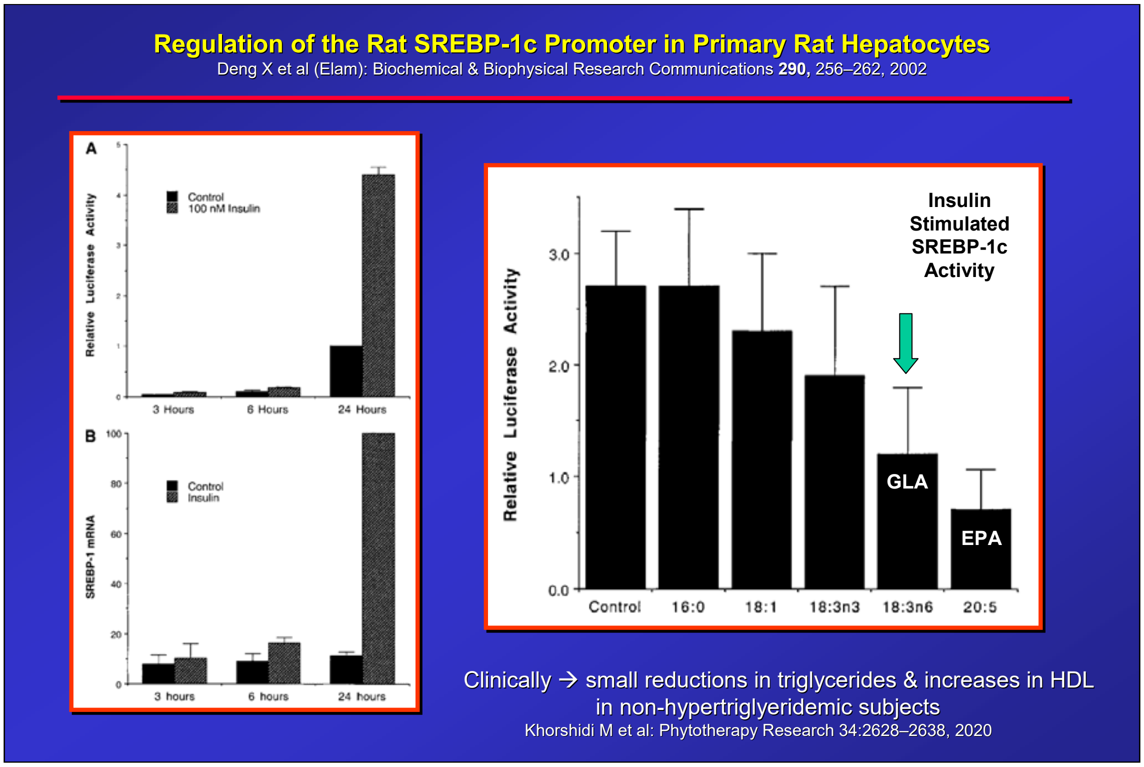
The different normal letters found in figures 4 a-f indicates significant difference among treatments at 0.05 level.



Abstract SREBPs are a family of membrane-bound transcription factors that activate genes encoding enzymes required for synthesis of cholesterol and unsaturated fatty acids. SREBPs are controlled by multiple mechanisms at the level of mRNA synthesis, proteolytic activation, and transcriptional activity. In this review, we summarize the recent findings that contribute to the current understanding of the regulation of SREBPs and their physiologic roles in maintenance of lipid homeostasis, insulin signaling, innate immunity, and cancer development.

Figure 1. Proteolytic activation of SREBPs Following synthesis on ER membranes, SREBP precursors bind to Scap through an interaction mediated by the C-terminal regulatory domain of the SREBP and the cytosolic C-terminal domain of Scap, which contains WD repeats. When cells are deprived of sterols, Scap escorts SREBPs from the ER to the Golgi by binding to the Sar1/Sec23/Sec24 complex of the COPII protein coat. In the Golgi, SREBP first encounters the Site-1 protease (S1P), which cleaves the SREBP in the lumenal loop, separating the protein into two membrane-bound halves. S1P-mediated cleavage renders the N-terminal half of SREBP a substrate for the Site-2 protease (S2P), which releases the bHLH-containing domain of SREBP by cleaving the intermediate near the cytosol-membrane boundary. Once released from membranes, transcriptionally active fragments of SREBPs migrate to the nucleus and bind to SREs in promotors of target genes to activate transcription.

Figure 3. UFA-mediated inhibition of ER-to-Golgi transport of Scap/SREBP In cells deprived of UFAs, Insig-1 is poly-ubiquitinated by gp78 and bound by the p97 adapter Ubxd8. Ubiquitinated Insig-1 is extracted from ER membranes through p97 and delivered to proteasomes for degradation. In the absence of Insig-1, the Scap escorts SREBP from the ER to the Golgi for proteolytic activation. Ubxd8 polymerizes upon binding to UFAs, resulting in its dissociation from Insig-1. As a result, Insig-1 becomes stabilized and blocks proteolytic activation of SREBPs by binding to Scap.



Abstract We have cloned 5 kb of genomic DNA encompassing 1.72 kb of 5'-regulatory sequence and exons 1-c and 2 of the rat SREBP-1c gene. A 1.5-kb segment upstream from the transcription start site was ligated ahead of the luciferase reporter gene and tested for promoter activity by transient transfection assays in primary rat hepatocytes. We discovered that insulin strongly activated the full-length promoter, regardless of whether 5 or 20 mM glucose was in the culture medium during treatment. Stimulation by insulin was blocked by dibutyryl-cAMP and by polyunsaturated fatty acids, such as a-linolenic acid, g-linolenic acid, or eicosapentaenoic acid; palmitic or oleic acids, however, had no inhibitory effect. A truncated promoter containing 149 bp of 5* flanking DNA, including proximal NF-Y, E-box, SRE, and Sp1 sites, retained most of the response. This is the first report that insulin, cAMP, and polyunsaturated fatty acids modulate the proximal SREBP-1c promoter in rat hepatocytes mirroring physiological regulation of SREBP-1c *invivo.*

FIG. 2. (A) Time course of the increased transcriptional activity of the SREBP-1c promoter produced by insulin. Freshly isolated hepatocytes were transfected overnight with 2 mg of pSREBP (21516/140) luc containing 1.5 kb of rat SREBP-1c 59-flanking DNA ligated upstream of *Photinus* luciferase reporter gene. Concurrent transfections with 1 mg of pRL-TK expressing *Renilla* luciferase were performed as a control for transfection efficiency. Cells were then incubated in medium with no additions or supplemented with 100 nM insulin. Samples were collected after 3, 6, or 24 h. Data, expressed as the ratio of *Photinus* to *Renilla* luciferase activity, from two hepatocyte preparations, with three plates in each preparation, are shown. (B) Time course of induction of SREBP-1 mRNA, determined by Northern analysis, by insulin in primary rat hepatocyte cultures. Hepatocytes were maintained for up to 24 h in medium containing glucose (20 mM) and dexamethasone (100 nM) with or without bovine insulin (100 nM). Data from 3 hepatocyte preparations are shown.

FIG. 5. Effect of fatty acids on transcription through the rat SREBP-1c promoter. Hepatocytes were transfected overnight with pSREBP (-372/+40)luc as described in the legend to Fig. 2 and subsequently incubated in medium containing 100 nM insulin plus 0.75% delipidated BSA and 0.5 mM palmitic acid, oleic acid, linolenic acid, g-linolenic acid, eicosapentaenoic acid, or no fatty acid. Data from 4 hepatocyte preparations (with three plates in each group) are shown.

Probing the intermolecular interactions of PPARγ-LBD with polyunsaturated fatty acids and their anti-inflammatory metabolites to infer most potential binding moieties

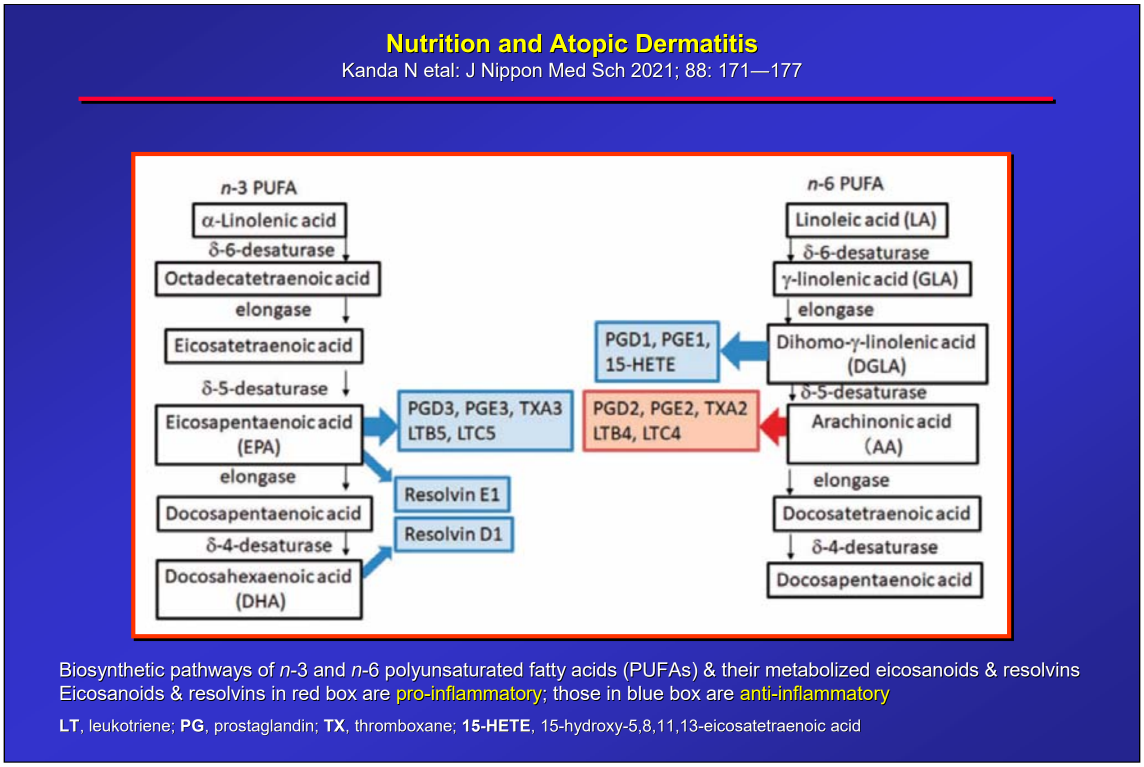
Muralikumar et al: Lipids in Health and Disease 16:17, 2017

LBD - ligand binding domain

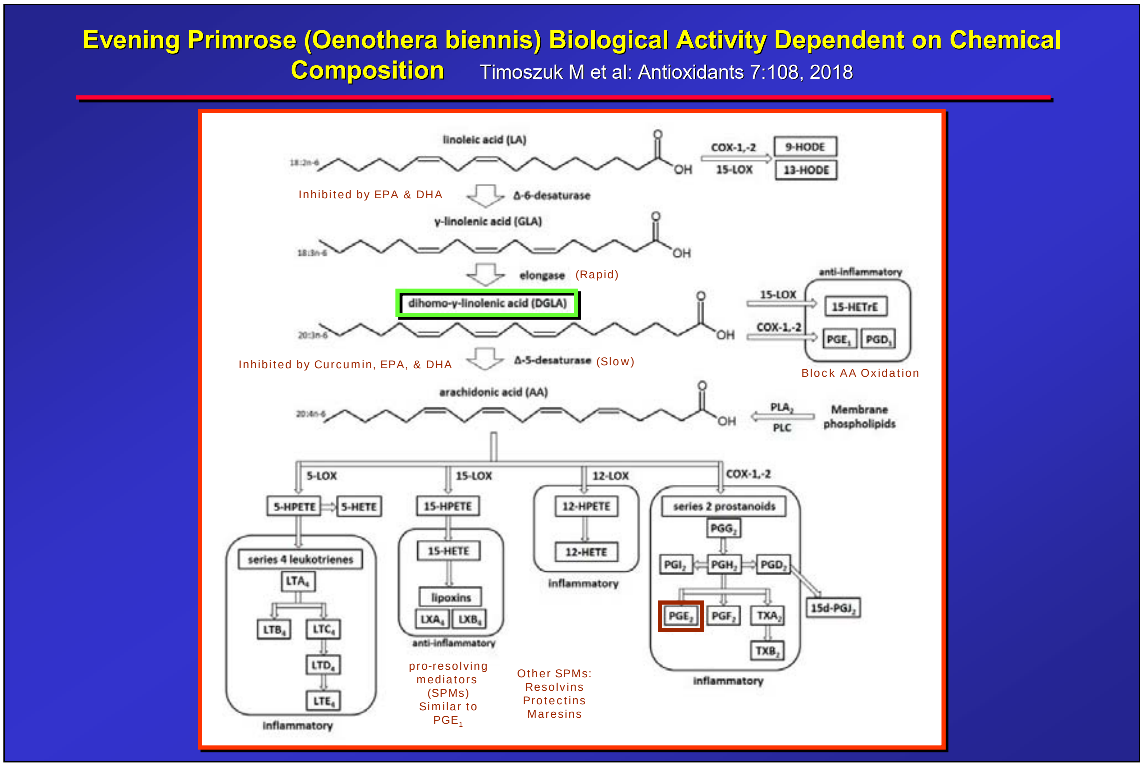
Table 1 Receptor-Ligand docking results with Glide XP score, H-bonds and MMGBSA

Compounds	XP Glide score kcal/mol	XH-bond Interactions: Donor	XH-bond Interactions: Acceptor	MMGBSA score kcal/mol
Rosiglitazone (BRL)	−6.833	Hie449:NH	BRL: O	−105.038
		Tyr473:OH	BRL:HN	
		Ser289:OH	BRL: O	
		Hie323:NH	BRL: O	
Resolvin E1 (RsvE1)	−9.900	Hie449:NH	REV1: O	−106.046
		Tyr327:HO	REV1: HO	
		Glu291: O	REV1: HO	
Neuroprotectin D1 (NPD1)	−9.664	Ser289:OH	NPD1: O^-	−102.57
		Ser289:OH	NPD1: OH	
Hydroxy-linoleic acid (H-LA)	−6.235	Tyr473:OH	HLA:HO	−95.027
		Ser289:OH	HLA: O^-	
Docosahexaenoic acid (DHA)	−7.925	Hie323:NH	AA: O	−89.785
Lipoxin A4 (LXA4)	−5.796	Tyr327:OH	LXA4: HO	−87.547
		Hie449:NH	LXA4: O	
Gamma-linoleic acid (GLA)	−5.068	Hie323:NH	GLA: O^-	−77.117
		Ser289:OH	GLA: O^-	
Arachidonic acid (AA)	−7.040	Hie323:NH	AA: O	−76.586
Alpha-linoleic acid (AL)	−5.174	Hie449:NH	AL: O^-	−73.657
Eicosapentaenoic acid (EPA)	−7.126	Hie323:NH	EPA: O	−71.147
		Ser289:OH	EPA: O	
Linoleic acid (LA)	−4.820	Glu343:NH	LA: O^-	−78.026

Compounds are ranked collectively based on XP glide score and MMGBSA score showing significance with reference ligand rosiglitazone (RsvE1 > NPD1 > HLA > DHA > LXA4 > GLA > AA > ALA > EPA > LA)



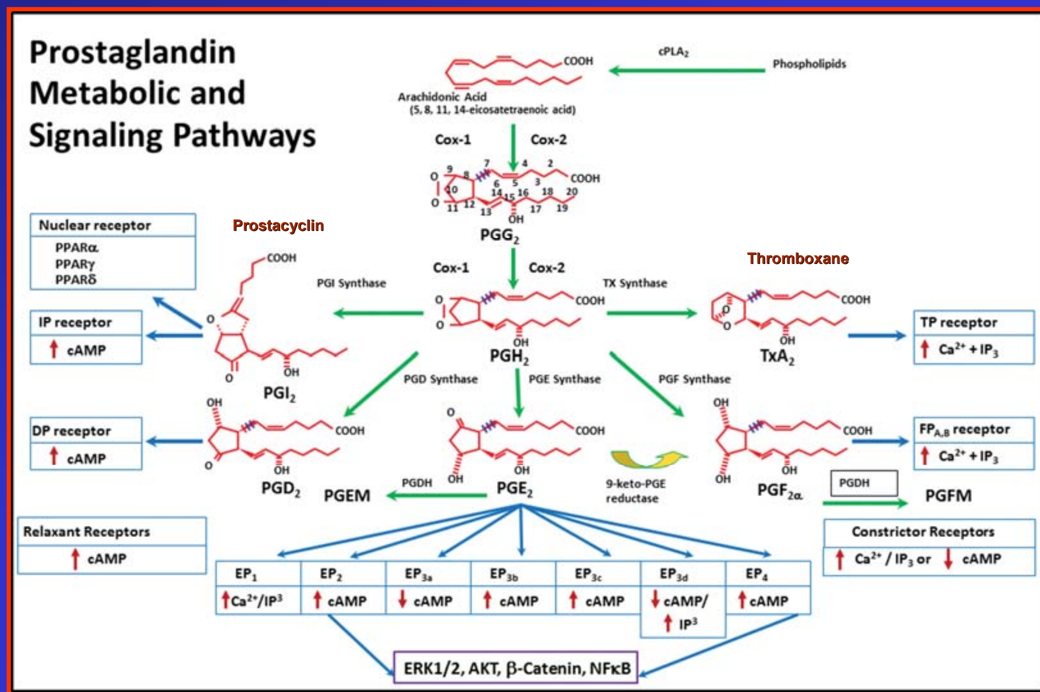
Atopic dermatitis (AD) is a chronic eczematous disease characterized by T helper 2 (Th2)-shifted allergic immunity, skin barrier impairment, and pruritus. Oral intake of certain nutrients might help regulate AD. Serum 25-hydroxyvitamin D levels are often low in patients with AD, and oral vitamin D supplementation improves AD. Vitamin D increases regulatory T (Treg) cells, which promote tolerance to allergens and prevent allergic inflammation by inducing expression of filaggrin and cathelicidin in keratinocytes. Vitamin A strengthens Treg cells by inducing expression of forkhead box P3 and inhibits mediator release from mast cells and eosinophils. Serum levels of γ-linolenic acid and its metabolite, dihomo-γ-linolenic acid, are low in patients with AD, and oral γ-linolenic acid improves AD through anti-inflammatory prostaglandin D1 and E1 derived from dihomo-γ-linolenic acid. Eicosapentaenoic acid and docosahexaenoic acid ameliorate AD by suppressing production of leukotriene B4, increasing ceramides in the stratum corneum, and through their metabolites, resolvin E1 and D1, which resolve inflammation. The probiotics *Lactobacillus* and *Bifidobacteria* improve the intestinal permeability barrier and induce Treg cells. Zinc levels in serum, hair, and erythrocytes are diminished in patients with AD. Zinc induces forkhead box P3 expression and increases Treg cells, and zinc-finger protein A20 suppresses nuclear factor-κB-dependent expression of inflammatory cytokines and cell-adhesion molecules. Oral supplementation of the above nutrients might have therapeutic or preventive roles in AD.



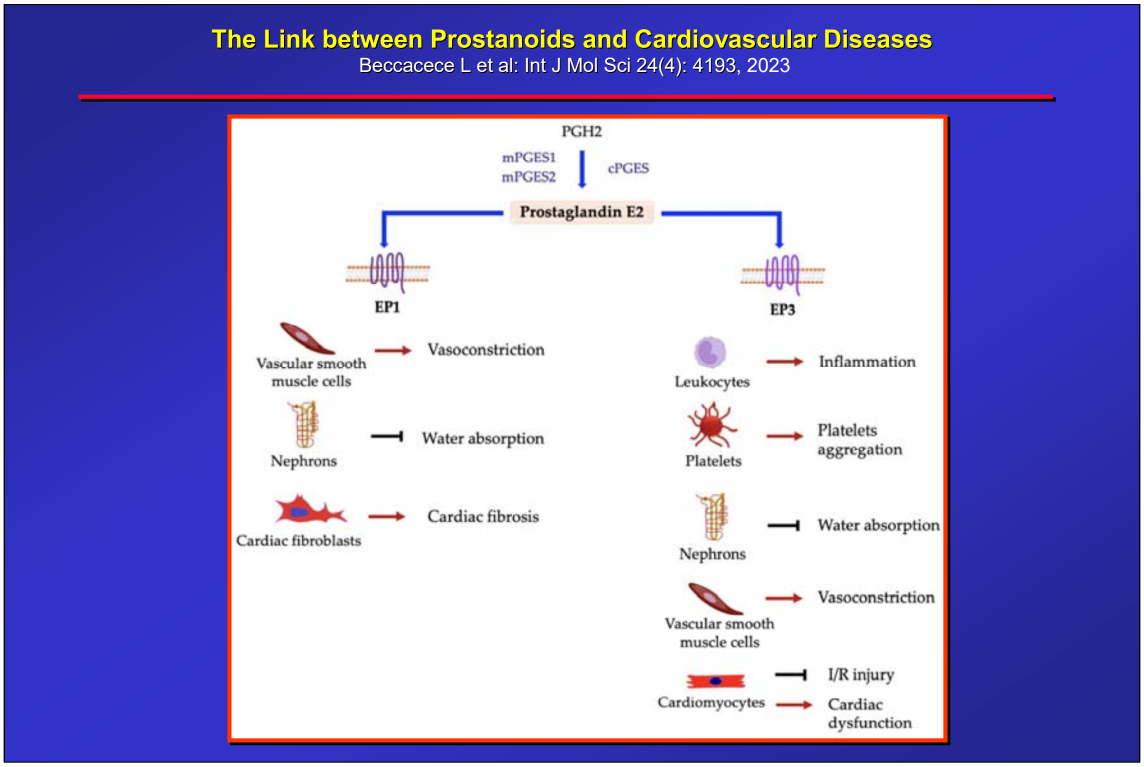
Abstract: Evening primrose (Oenothera L.) is a plant belonging to the family Onagraceae, in which the most numerous species is Oenothera biennis. Some plants belonging to the genus Oenothera L. are characterized by biological activity. Therefore, studies were conducted to determine the dependence of biological activity on the chemical composition of various parts of the evening primrose, mainly leaves, stems, and seeds. Common components of all parts of the Oenothera biennis plants are fatty acids, phenolic acids, and flavonoids. In contrast, primrose seeds also contain proteins, carbohydrates, minerals, and vitamins. Therefore, it is believed that the most interesting sources of biologically active compounds are the seeds and, above all, evening primrose seed oil. This oil contains mainly aliphatic alcohols, fatty acids, sterols, and polyphenols. Evening primrose oil (EPO) is **extremely high in linoleic acid** (LA) (70–74%) and **gamma-linolenic acid (GLA) (8–10%),** which may contribute to the proper functioning of human tissues because they are **precursors of anti-inflammatory eicosanoids**. EPO supplementation results in an increase in plasma levels of gamma-linolenic acid and its metabolite dihomo-linolenic acid (DGLA). This compound is oxidized by lipoxygenase (15-LOX) to 15-hydroxyeicosatrienoic acid (**15-HETrE**) or, under the influence of cyclooxygenase (COX), DGLA is metabolized to **series 1 prostaglandins**. These compounds have **anti-inflammatory and anti-proliferative** properties. Furthermore, **15-HETrE blocks the conversion of arachidonic acid (AA) to leukotriene A4 (LTA4)** by direct inhibition of 5-LOX. In addition, gamma-linolenic acid **suppresses** inflammation mediators such as interleukin 1 (**IL-1**), interleukin 6 (**IL-6**), and cytokine - tumor necrosis factor (**TNFa**). The beneficial effects of EPO have been demonstrated in the case of atopic dermatitis, psoriasis, Sjögren's syndrome, asthma, and anti-cancer therapy.

Figure 1. Linoleic acid (LA) metabolism.

9-HODE: 9-hydroxyoctadecadienoic acid	13-HODE: 13-hydroxyoctadecadienoic acid	15-HETrE: 15-hydroxyeicosatrienoic
5-HPETE: 5-hydroperoxyeicosatetraenoic acid	12-HPETE: 12-hydroperoxyeicosatetraenoic acid	15-HPETE: 15-hydroperoxyeicosatetraenoic acid
5-HETE: 5-hydroxyeicosatetraenoic acid;	12-HETE: 12-hydroxyeicosatetraenoic acid;	15-HETE: 15-hydroxyeicosatetraenoic acid;
PGE1: prostaglandin E1	PGD1: prostaglandin D1	PGG2: prostaglandin G2
PGH2: prostaglandin H2	PGI2: prostaglandin I2	PGD2: prostaglandin D2
PGE2: prostaglandin E2	PGF2: prostaglandin F2	15-d-PGJ2: 15-deoxy-delta-12,14-prostaglandin J2
LTA4: leukotriene A4	LTB4: leukotriene B4	LTC4: leukotriene C4
LTD4: leukotriene D4	LTE4: leukotriene E4	
LXA4: lipoxin A4	LXB4: lipoxin B4	
TXA2: thromboxane A2	TXB2: thromboxane B2	
PLA2: phospholipase A2	PLC: phospholipase C	



Arosh JA et al: J Dairy Science 99(7): P5926-5940, 2016



Prostaglandin E2 is maybe the best characterized prostaglandin. It is mainly known as an inflammation mediator and exerts its action via the interaction with four different receptors, E-type prostanoid receptors 1–4 (EP1-4), that are widely expressed in **platelets** and **VSMCs** [10,144,145]. PGE2 is synthesized in many cell types, including **endothelial cells, platelets, macrophages, and fibroblasts**, and is **rapidly inactivated** into 15-keto-PGE2 by 15-hydroxyprostaglandin dehydrogenase (15-PGDH or prostaglandin dehydrogenase 1) [146]. It is involved in many processes and in the pathogenesis of several diseases. Its synthesis is induced by both COX-1 and COX-2 activity, but in **pathological conditions** there is a correlation between **COX-2** and PGE2 synthesis [52]. Overall, PGE2 have different effects on various diseases depending on receptor interaction, with effects can ameliorate or worsen the pathological condition [147]. Figure 5 and Figure 6 show the effect of PGE2 on different cell types based on receptor interaction.

PGE2 and Inflammation

Many studies have displayed that it is **greatly expressed during inflammation**, promoting the synthesis and secretion of cytokines and giving rise to proinflammatory or anti-inflammatory responses. The different responses are mainly due to the interaction with distinct receptors and the timing of exposition [10,144,145].

During acute inflammation, caused by tissue damage or pathogen invasion, **COX-2 induction by pro-inflammatory stimuli** raises PGE2 expression [52], which mediates acute inflammatory responses (red flares, heat, and swelling reactions). Specifically, PGE2 triggers **vasodilatation, blood flow, and an increase in vascular permeability** by interacting with EP2, EP3, and EP4 receptors in vascular **smooth muscle cells** [144,148,149]. The interaction between PGE2 and EP3 on **mast cells** elicits the release of pro-inflammatory mediators, such as **histamine** and also interleukin-6 (**IL-6**). These mediators **augment vascular permeability** and promote consequent **leukocyte recruitment** and **edema**. Thus, the PGE2/EP3 axis is implicated in acute inflammation [150]. On the other side, EP2/EP4 stimulation of vascular smooth muscle cells and endothelial cells determines vasodilatation and the subsequent increase in blood flow [151].

Through EP2 and EP4 receptors, PGE2 regulates pro-inflammatory **interleukin-1β secretion** induced by inflammasome activation [152,153,154,155,156,157], a process that, if not properly regulated, leads to cell death and possibly the development of **autoimmune diseases** [158]. However, there are contrasting data points in this regard. Some studies have shown that PGE2, when interacting with the EP4 receptor, inhibits the NLRP3 inflammasome and IL-1β secretion on monocytes, exerting an anti-inflammatory role [152,153,154]. In contrast, other studies have discovered that this prostaglandin stimulates IL-1β synthesis and secretion [155,156,157] when it interacts not only with EP3 but even with EP2 and EP4 receptors. These differences are likely due to the different **timing of exposition to PGE2**, in addition to receptor binding.

PGE2 is also involved in chronic inflammation, which is characterized by the persistence of an inflammatory state due to the continuous production of pro-inflammatory cytokines, low efficiency of inflammation resolution, and protracted leukocyte recruitment. All of these events can result in **abnormal tissue remodelling and damage**, eventually leading to disease. Among chronic inflammatory conditions, PGE2 plays a role in cancer, diabetes mellitus, and cardiovascular diseases [8,159,160]. It has been proposed that this promotes chronic inflammation in a positive feedback loop. On one hand, the **PGE2/EP2** axis activates in endothelial cells the **NF-κB factor**, which in turn induces the **cytokine synthesis** and the indirectly triggered **activation of COX-2** triggered by the same cytokines [161]. On the other hand, the PGE2/EP4 axis **enhances** differentiation of T cells into Th1 (type 1 **T helper) cells** and Th17 expansion, due to the release of IL-12 and IL-23, increasing the inflammation [162].

Gamma-linolenic acid, Dihommo-gamma linolenic, Eicosanoids and Inflammatory Processes

Sergeant S et al: Eur J Pharmacol 785: 77–86; 2016

Effect of GLA supplementation on functional endpoints of immune cells

Reference	design	supplement	dose	duration	cells	*Functional Outcomes*
(Fletcher and Ziboh, 1990)	*In vivo* fatty acid supplementation	borage oil	3% oil (by weight) in diet	12wk	guinea pig neutrophil	~25% inhibition of fMLP-stimulated superoxide production (vs control diet; $p<0.005$) No effect on PMA-stimulated superoxide production (vs control diet)
(Iversen et al., 1991)	*In vitro* fatty acid Competition	LA and DGLA conc. Curve	0–100μM	10m	human neutrophil	50μM DGLA, 75% ↓ in LTB_4 generation 50μM LA, 60% ↓ in LTB_4 generation
(Iversen et al., 1992)	*In vitro* fatty acid treatment	DGLA conc. curve	0–100μM	10m	human PBMC [1]	50μM DGLA, 60% ↓ in LTB_4 generation 50μM DGLA, >7-fold ↑ in PGE1 generation 50μm DGLA, significant ↑ in 15-HETrE generation (>250ng/10mil cells)
(Ziboh and Fletcher, 1992)	*In vivo* fatty acid supplementation	borage oil	0.48g GLA/d	6wk	human neutrophil	50% ↓ LTB_4 generation (vs olive oil control), *ex vivo*
(Ziboh and Fletcher, 1992)	*In vivo* fatty acid supplementation	black currant oil	0.48g GLA/d	6wk	human neutrophil	50% ↓ LTB_4 generation (vs olive oil control), *ex vivo*
(Chapkin et al., 1988a)	*In vitro* treatment	15-HETrE	0–30μM	1hr	murine peritoneal macrophage	10μM 15-HETrE, 90% ↓ in LTB_4 generation
(Chilton- Lopez et al., 1996)	*In vitro* treatment	15-HETrE	0–20μM		human neutrophil	10μM 15-HETrE, 75% ↓ in LTB_4 generation
(Johnson et al., 1997)	*In vivo* fatty acid Supplementation	borage oil + controlled diet	3g GLA/day	21d	human neutrophil	58% ↓ LTB_4 generation (vs baseline), *ex vivo*
(Barham et al., 2000)	*In vivo* fatty acid supplementation	borage + fish oils + controlled diet	3g GLA + 3g EPA/day	21d	human neutrophil	30% ↓ LTB_4 generation (vs baseline), *ex vivo*
(Amagai et al., 2015)	*In vitro* fatty acid treatment	DGLA conc. curve	0–30μM	48hr	RBL-2H3 cells	30μM, significant (>30ng/ml) PGD_1 formation
(Amagai et al., 2015)	*In vivo* fatty acid supplementation	DGLA	11% of dietary fatty	5wk	NC/Tnd mouse skin	significant ↑ in PDG_1, PDE_1, PGD_2, 8-HETrE, 15-HETrE (vs control diet)

[1] PBMC, peripheral blood mononuclear cells

Abstract Gamma-linolenic acid (GLA, 18:3n-6) is an omega-6 (n-6), 18 carbon (18C-) polyunsaturated fatty acid (PUFA) found in human milk and several botanical seed oils and is typically consumed as part of a dietary supplement. While there have been numerous in vitro and in vivo animal models which illustrate that GLA-supplemented diets attenuate inflammatory responses, clinical studies utilizing GLA or GLA in combination with omega-3 (n-3) PUFAs have been much less conclusive. A central premise of this review is that there are critical metabolic and genetic factors that affect the conversion of GLA to dihommo-gamma linolenic acid (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6), which consequently affects the balance of DGLA- and AA- derived metabolites. As a result, these factors impact the clinical effectiveness of GLA or GLA/n-3 PUFA supplementations in treating inflammatory conditions. Specifically, these factors include: 1) the capacity for different human cells and tissues to convert GLA to DGLA and AA and to metabolize DGLA and AA to bioactive metabolites; 2) the opposing effects of DGLA and AA metabolites on inflammatory processes and diseases; and 3) the impact of genetic variations within the fatty acid desaturase (FADS) gene cluster, in particular, on AA/DGLA ratios and bioactive metabolites. We postulate that these factors influence the heterogeneity of results observed in GLA supplement-based clinical trials and suggest that "one-size fits all" approaches utilizing PUFA-based supplements may no longer be appropriate for the prevention and treatment of complex human diseases.

GAMMA-LINOLENIC ACID (2.8 gms/D) TREATMENT OF RHEUMATOID ARTHRITIS
A Randomized, Placebo-Controlled Trial

Zurier RB et al: Arthritis & Rheumatism 39(11): 1808-1817, 1996

Table 2. Changes from baseline and comparison of clinical and laboratory measures of rheumatoid arthritis during months 1–6 of the study*

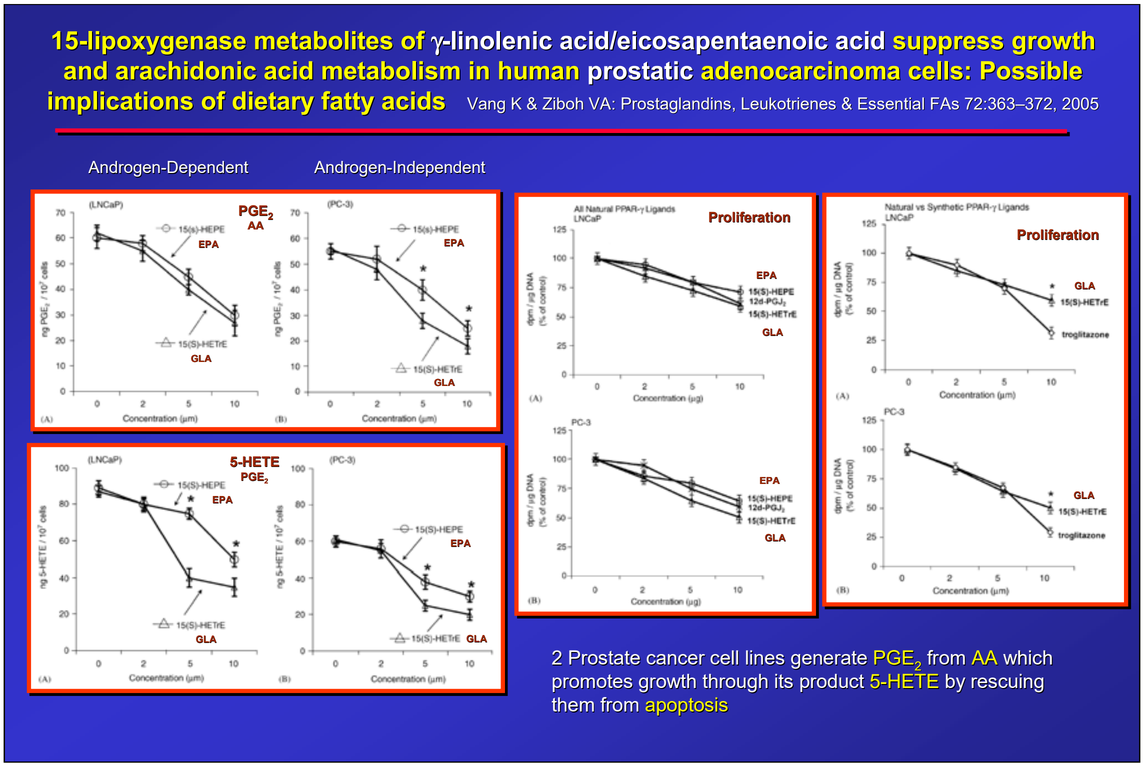
Variable	GLA group (n = 22)	Placebo group (n = 19)	P†
Swollen joint count, 0–66	−4.1‡	−1.82	0.034
% change	−20.9 (34.3)	−8.3 (22.1)	0.026
Swollen joint score, 0–3/joint	−6.1‡	−4.6	0.17
% change	−23.1 (31.2)	−16.1 (28.5)	0.101
Tender joint count, 0–68	−8.5‡	−2.4	0.006
% change	−35.2 (32.5)	−11.9 (36.8)	0.015
Tender joint score, 0–3/joint	−11.4‡	−2.8	0.021
% change	−37.5 (35.6)	−10.7 (45.4)	0.041
Morning stiffness, minutes	−67.5‡	10.6	0.109
% change	−55.4 (0.98)	7.9 (1.4)	0.092
Global assessment, 0–4			
Physician	0.045	−0.21	0.65
% change	2.1 (34.6)	−7.8 (29.3)	0.44
Patient	−0.45‡	−0.11	0.18
% change	−18.9 (35.6)	−4.5 (34.9)	0.2
Pain assessment, 0–4	−0.59‡	−0.37	0.44
% change	−24.1 (42.9)	−13.7 (41.5)	0.43
Visual analog scale, 1–10			
Global	−0.91‡	0.31	0.48
% change	−17.5 (38.0)	5.9 (33.4)	0.31
Pain	−1.36‡	0.09	0.004
% change	−26.8 (58.5)	1.6 (40.6)	0.082
HAQ score, 1–4	−0.192‡	0.082	0.027
% change	−11.0 (19.0)	4.75 (17.9)	0.041

* Values are the mean absolute change or the mean (SD) percentage of change from baseline. GLA = γ-linolenic acid; HAQ = Health Assessment Questionnaire.
† Determined by Mann-Whitney U test for between-group comparisons.
‡ $P < 0.05$ for intragroup change from baseline, by Wilcoxon sign rank test.

Table 3. Changes in clinical and laboratory measures of rheumatoid arthritis during months 6–12 of the study*

Variable	GLA group (n = 21)	Placebo/GLA group (n = 14)
Swollen joint count, 0–66	−3.55†	−8.00†
% change	−23.3 (41.8)	−38.2 (41.2)
Swollen joint score, 0–3/joint	−4.3†	−6.9†
% change	−23.8 (31.2)	−28.9 (42.5)
Tender joint count, 0–68	−2.75†	−1.64†
% change	−17.6 (68.3)	−9.2 (50.9)
Tender joint score, 0–3/joint	−3.8†	−4.6†
% change	−20.2 (87.9)	−19.3 (49.9)
Morning stiffness, minutes	−3.3	−69.9†
% change	−6.1 (90.1)	−47.0 (1.5)
Global assessment, 0–4		
Physician	−0.2	−0.36
% change	−10.2 (35.6)	−14.4 (34.0)
Patient	−0.20	−0.15
% change	−10.2 (35.6)	−6.4 (46.4)
Pain assessment, 0–4	0	−0.15
% change	0 (40.4)	−6.2 (41.2)
Visual analog scale, 1–10		
Global	−0.52	−0.34†
% change	−12.2 (54.2)	−6.2 (47.4)
Pain	0.15	−0.75
% change	4.0 (64.2)	−12.9 (49.4)
HAQ score, 1–4	−0.012	−0.064
% change	−0.8 (33.3)	−3.5 (24.5)

* Values are the mean absolute change or the mean (SD) percentage of change from the month-6 data. The placebo/GLA (γ-linolenic acid) group took GLA only during months 6–12. HAQ = Health Assessment Questionnaire.
† $P < 0.05$ versus month-6 data, by Wilcoxon sign rank test.



Abstract Although gammalinolenic acid (GLA) and eicosapentaenoic acid (EPA) have independently been reported to suppress growth of cancer cells, their relative potencies are unknown. To determine the possible attenuating efficacies of dietary GLA or EPA onprostate carcinogenesis, we hereby report the in vitro effects of GLA, EPA and their 15-lipoxygenase (15-LOX) metabolites: 15(S)-HETrE and 15(S)-HEPE, respectively, on growth and arachidonic acid (AA) metabolism in human androgen-dependent (LNCaP) and androgen-independent (PC-3) prostatic cancer cells in culture. Specifically, both cells were preincubated respectively with the above PUFAs. Growth was determined by [3H]thymidine uptake and AA metabolism by HPLC analysis of the extracted metabolites.

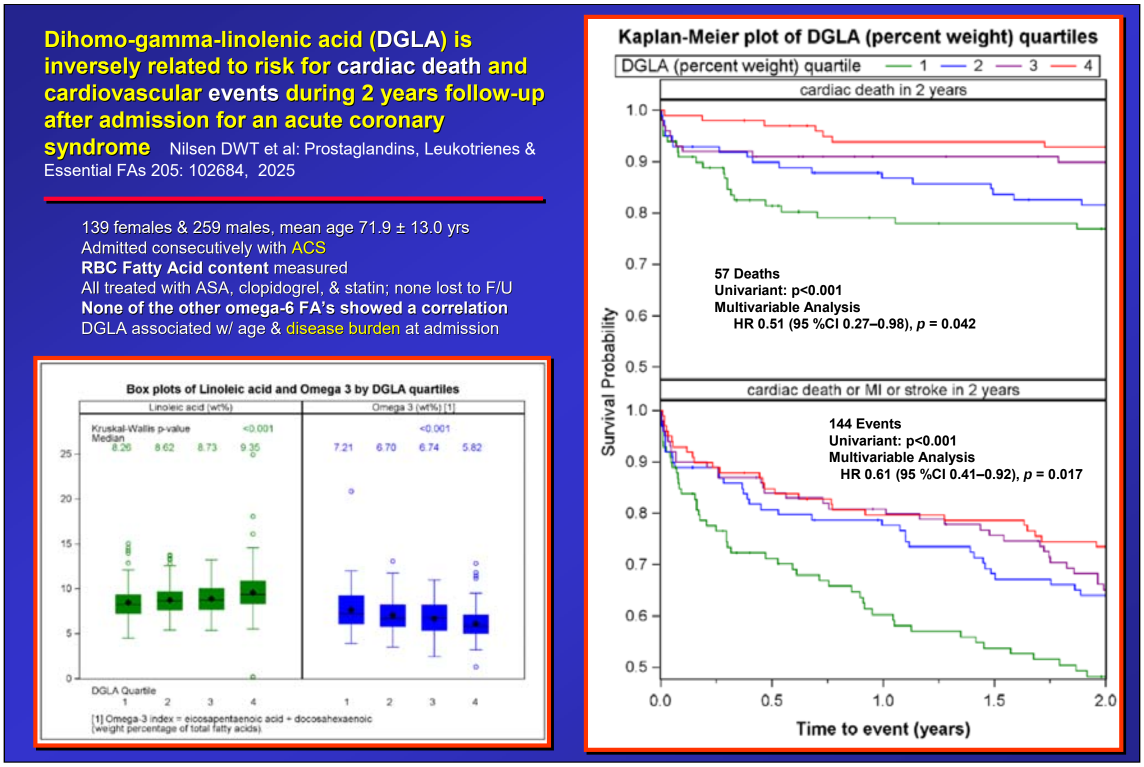
Our data revealed increased biosynthesis of prostaglandin E2 (PGE2) and 5-hydroxyeicosatetraenoic acid (5(S)-HETE) by both cells. Preincubation of the cells with 15(S)-HETrE or 15(S)-HEPE more markedly inhibited cellular growth and AA metabolism when compared to precursor PUFAs. Notably, 15(S)-HETrE exerted the greatest inhibitory effects. These findings therefore imply that dietary GLA rather than EPA should better attenuate prostate carcinogenesis via its in vivo generation of 15(S)-HETrE, thus warranting exploration.

Fig. 4. Concentration-dependent effects of 15(S)-HETrE and 15(S)-HEPE on PGE2 production in LNCaP and PC-3. The figure shows the effects of varying concentrations of 15(S)-HETrE and 15(S)-HEPE on calcium ionophore-induced PGE2 production in (A) LNCaP and (B) PC-3 cells. Each point represents the amount of PGE2 produced per 10 million cells±SEM from three separate experiments. The asterisk (*) indicates a significance between treatment groups, P<0:05

Fig. 5. Concentration-dependent effects of 15(S)-HETrE and 15(S)-HEPE on 5(S)-HETE production in LNCaP and PC-3. The figure shows the effects of varying concentrations of 15(S)-HETrE and 15(S)-HEPE on calcium ionophore-induced 5(S)-HETE production in (A) LNCaP and (B) PC- 3 cells. Each point represents the amount of 5(S)-HETE produced per 10 million cells±SEM from three separate experiments. The asterisk (*) indicates a significance between treatment groups, P<0:05

Fig. 8. Comparison of concentration-dependent effects between all naturally derived PPAR-g ligands in inhibiting DNA synthesis in LNCaP and PC-3. The figure shows the effects of varying concentrations of natural PPAR-g ligands: 15(S)-HETrE, 15(S)-HEPE and 12d-PGJ2 on [3H]thymidine uptake in (A) LNCaP and (B) PC-3. Each point represents percent of control±SEM from three separate experiments.

Fig. 9. Comparison of concentration-dependent effects between the naturally derived 15(S)-HETrE and synthetic troglitazone PPAR-g ligands in inhibiting DNA synthesis in LNCaP and PC-3. Comparison of varying concentrations of natural 15(S)-HETrE to the synthetic PPAR-g ligand troglitazone on [3H]thymidine uptake in (A) LNCaP and (B) PC-3. Each point represents percent of control±SEM from three separate experiments. The asterisk (*) indicates a significance between treatment groups, P<0:05



A B S T R A C T Background/Aim: Dihomo-gamma-linolenic acid (DGLA) is derived from linoleic acid. Its presence in red blood cell (RBC) membranes is mainly due to metabolism and not diet. RBC DGLA was negatively associated with all-cause mortality during 7 years follow-up in patients admitted with an acute coronary syndrome (ACS). We now present its 2-year cardiovascular prognostic utility compared to other n-6 fatty acids (FAs).

Methods: A total of 139 females and 259 males with a mean age of 71.9 ± 13.0 years were admitted consecutively in this study. Stepwise Cox regression models, applying continuous values of DGLA weight percent (wt%) and quartiles, were fitted for the biomarkers with cardiac death and a combined cardiovascular (CV) endpoint consisting of cardiac death or myocardial infarction (MI) or stroke as the dependent variables.

Results: Cardiac death was recorded in 57 patients, and the composite CV endpoint in 144 patients, respectively. DGLA was negatively associated with both endpoints, each with a p-value of <0.001 in univariate analysis. The hazard ratio (HR, per 1 wt% increase) remained significant after multivariable adjustment [cardiac death HR 0.51 (95 %CI 0.27–0.98), *p* = 0.042, and composite CV endpoint HR 0.61 (95 %CI 0.41–0.92), *p* = 0.017]. A similar pattern was obtained in ACS patients presenting with an acute MI at admission. No association with any outcome was found with the other n-6 FAs [linoleic acid, arachidonic acid and adrenic acid].

Conclusion: Higher RBC DGLA predicts lower risk for cardiac death and cardiovascular outcomes at 2 years follow-up in ACS patients, whereas other n-6 FAs do not.

Fig. 1. Showing median weight percent (wt%) increase in linoleic acid (LA) and median wt% decrease in the Omega-3 Index [eicoapentaenoic acid (EPA) + docosahexaenoic acid (DHA)] across increasing dihomo-gamma-linolenic acid (DGLA) quartiles.

Fig. 2. Kaplan-Meier curves showing the association of admission dihomogamma-linolenic acid (DGLA) with cardiac death and a composite endpoint including cardiac death or myocardial infarction (MI) or stroke during 2 years follow-up of 398 ACS patients.

Treatment of Diabetic Neuropathy With gamma-Linolenic Acid

Keen H et al: Diabetes Care 16(1): 8-15, 1993

- **111 patients** with mild neuropathy
- GLA 480 mg/day (EF4) for 1 year vs Placebo
- Mean Age 53 yrs & DM duration 13 yrs
- 30 women, 57 Type 1 DM
- Mean A1c 9.7%

Table 4—*Effects of treatment with EF4 or placebo on neurological parameters*

	Change with EF4	Change with placebo	Difference between treatments
Muscle strength (averaged × 10^2)			
Arm	+4.9 ± 2.7	−7.4 ± 2.9*	12.3† (4.5–20.1)
Leg	+5.4 ± 3.9	−9.2 ± 5.0	14.6 (2.2–27.1)
Tendon reflexes (averaged × 10^2)			
Arm	+2.9 ± 4.7	−12.0 ± 5.6	14.9* (0.4–29.4)
Leg	+17.7 ± 5.6†	+0.5 ± 6.8	17.1‡ (−0.2–34.4)
Sensation (averaged × 10^2)			
Arm	+6.9 ± 3.4	−7.3 ± 3.6‡	14.2* (4.3–24.1)
Leg	+15.0 ± 5.4*	−8.4 ± 3.9‡	23.4† (9.9–36.9)

Table 3—*Effects of treatment with EF4 or placebo on neurophysiological and thermal sensitivity parameters*

	Change with EF4	Change with placebo	Difference between treatments
MNCV (m/s)			
Peroneal	+2.23 ± 0.95*	−1.86 ± 0.99	4.09† (1.35–6.83)
Median	+2.37 ± 1.08*	−2.14 ± 1.02*	4.51† (1.54–7.49)
CMAP (mV)			
Extensor digitorum brevis	+1.22 ± 0.46‡	−0.62 ± 0.26*	1.84† (0.75–2.94)
Thenar	+1.44 ± 0.64*	−1.13 ± 0.52*	2.57† (0.90–4.24)
SNAP (μV)			
Sural	+1.68 ± 0.82*	−0.96 ± 0.65	2.64‡ (0.54–4.75)
Median	+2.41 ± 0.79†	−1.28 ± 0.58*	3.68§ (1.60–5.68)
Heat threshold (°C)			
Ankle	+0.54 ± 0.81	−0.15 ± 0.93	0.69 (−1.77–3.15)
Wrist	+0.84 ± 0.42*	−0.39 ± 0.22	1.23† (0.28–2.17)
Cold threshold (°C)			
Ankle	+1.79 ± 0.95	−0.42 ± 0.71	2.21 (−0.15–4.57)
Wrist	+0.82 ± 0.29†	−0.29 ± 0.20	1.11† (0.40–1.81)

Values are means ± SE, except values for the difference between treatments are means ± 95% confidence intervals. The last available values are shown. (+), improvement—EF4 better than placebo; (−), deterioration.

Table 6—*Clinical results in the seven centers*

Uniform Results

	GLA better	No difference	Placebo better
Muscle weakness			
Arm	5	2	0
Leg	4	1	2
Tendon reflexes			
Arm	5	1	1
Leg	5	0	2
Sensation			
Arm	7	0	0
Leg	7	0	0
Totals	33	4	5

HgbA1c >10% → no significant change

OBJECTIVE — To compare the effects of placebo and GL A on the course of mild diabetic neuropathy over 1 yr.

RESEARCH DESIGN AND METHODS— We entered **111 patients** with mild diabetic neuropathy from seven centers into a randomized, double-blind, placebo - controlled parallel study of GLA at a dose of 480 mg/day. MNCV, SNAP, CM AP, hot and cold thresholds, sensation, tendon reflexes, and muscle strength were assessed by standard tests in upper and lower limbs.

RESULTS— For all 16 parameters, the change over 1 yr in response to GLA was more favorable than the change with placebo, and for 13 parameters, the difference was statistically significant. Sex, age, and type of diabetes did not influence the result, but treatment was more effective in relatively well-controlled than in poorly-controlled diabetic patients.

CONCLUSIONS — GLA had a beneficial effect on the course of diabetic neuropathy.

Impact of evening primrose oil consumption on psychological symptoms of postmenopausal women: a randomized double-blinded placebo-controlled clinical trial

Sharif SN & Darsareh F: Menopause 27(2): 194-198, 2020

189 women treated with 1,000 mg E. Primrose Oil (10% GLA) or Placebo for 8 weeks

TABLE 3. *Comparison of psychological scores of postmenopausal women between groups (mean ± standard deviation)*

200 women started study	Intervention (n=94)	Control (n=95)	*P*
Baseline			
Depressive mood	1.81 ± 1.42	2.02 ± 1.76	0.13
Irritability	2.03 ± 1.79	2.38 ± 1.69	0.41
Anxiety	2.73 ± 2.09	2.87 ± 2.12	0.95
Mental exhaustion	2.57 ± 2.21	2.38 ± 1.98	0.97
Total Score	9.14 ± 2.17	9.65 ± 2.01	0.98
End of study			
Depressive mood	1.32 ± 0.98	2.12 ± 1.75	0.05
Irritability	1.12 ± 1.01	2.16 ± 1.86	0.05
Anxiety	1.08 ± 0.87	2.63 ± 2.19	0.01
Mental exhaustion	1.51 ± 1.88	2.07 ± 1.32	0.05
Total score	5.03 ± 1.79	8.98 ± 1.99	0.01

Based on independent sample *t* test.

Abstract **Objective:** The purpose of this study was to determine the efficacy and safety of evening primrose oil on women's psychological symptoms during menopause.

Methods: A double-blinded randomized placebo-controlled trial carried out from September 2018 to February 2019 in Bandar Abbas, Iran. Eligible women randomly received either 1,000 mg of evening primrose oil capsules daily or matching placebo for 8 weeks. The Main outcome measures were psychological symptoms based on the psychological subscale of the Menopause Rating Scale. Independent samples t test was used for intergroup comparisons and paired samples t test for pre- and post-treatment comparisons. P<0.05 was considered statistically significant.

Results: The 8-week treatment was completed by 189 women. The mean baseline psychological score did not differ among the two groups. After intervention, the psychological score, however, differed significantly among groups (P<0.01). To distinguish the effect of evening primrose oil, we compared the reduction in the psychological score in each group. Regarding mean differences of the psychological score in both groups, there was a prominent alleviation in the intervention group mean difference: 3.44 (95% confidence interval of difference: 4.01 to 1.20) (P<0.01). In addition, only one patient reported gastric upset in the intervention group.

Conclusions: This study could provide evidence regarding the potential benefits of evening primrose oil for the psychological symptoms of postmenopausal women. Longer trials are necessary to make more reliable decisions about the use of evening primrose oil and its safety in clinical practice.

Evening primrose oil is effective in atopic dermatitis: A randomized placebo-controlled trial

Senapati S, Banerjee S, & Gangopadhyay DN: Indian J Dermatol Venereol Leprol 74(5): 447-452, 2008

50 Patients randomized to EPO or Placebo for 5 months → improvements in 24 vs 8
Doses: 500 to 6000 mg depending on age (~10% GLA)

Table 2: The scores of study cases at baseline and at five monthly evaluations

Score 0-3		Base-line	1 month	2 months	3 months	4 months	5 months
				Mean ± Standard deviation *P* value (unpaired t test)			
Extent	EPO	1.92±0.7	1.68±0.69	1.44±0.58 *P*<0.005	1.04±0.45 *P*<0.000001	0.88±0.44 *P*<0.00001	0.68±0.47 *P*<0.00001
	Control	1.84±0.62	1.84±0.55	1.56±0.65	1.52±0.65 *P*<0.05	1.52±0.77	1.76±0.66
Intensity	EPO	2.12±0.6	1.6±0.5 *P*<0.0001	1.08±0.4 *P*<0.00001	0.92±0.27 *P*<0.00001	0.88±0.44 *P*<0.00001	0.72±0.54 *P*<0.00001
	Control	1.48±0.5	1.44±0.5	1.28±0.45	1.32±0.55	1.2±0.5 *P*<0.025	1.24±0.43 *P*<0.05
Itching	EPO	1.76±0.59	1.12±0.52 *P*<0.00003	1±0.57 *P*<0.0003	0.56±0.5 *P*<0.00001	0.48±0.58 *P*<0.00001	0.32±0.47 *P*<0.00001
	Control	1.76±0.59	1.56±0.58	1.44±0.5 *P*<0.05	1.48±0.65	1.6±0.76	1.6±0.64
Dryness	EPO	1.2±0.57	1.2±0.57	0.88±0.66 *P*<0.05	0.88±0.66 *P*<0.05	0.56±0.58 *P*<0.001	0.52±0.5 *P*<0.00003
	Control	1.4±0.64	1.4±0.64	1.28±0.67	1.2±0.64	1.12±0.72	1.16±0.74
Total score	EPO	7±1.75	5.96±1.61 *P*<0.0228	4.4±1.55 *P*<0.00001	3.4±1.38 *P*<0.00001	2.8±1.47 *P*<0.00001	2.24±1.45 *P*<0.00001
	Control	6.48±1.44	6.24±1.5	5.66±1.5 *P*<0.0228	5.52±1.7 *P*<0.022	5.4±2.1 *P*<0.0228	5.76±1.78

ABSTRACT Background: Atopic dermatitis (AD) is a chronic, relapsing, itchy dermatosis of multifactorial origin, which commonly starts in childhood. Defective metabolism of essential fatty acids leading to relative dominance of pro-inflammatory prostaglandins (PGE2 and PGF2) has been reported as an important factor in the pathogenesis of AD. Evening primrose oil (EPO) as a source of gamma-linolenic acid (GLA) has been of interest in the management of AD.

Aim: To evaluate the efficacy and safety of EPO in atopic dermatitis in our patients.

Methods: Consecutive new out-patient department (OPD) patients of a referral hospital in Kolkata clinically diagnosed as having AD were randomly allocated to two groups. To the first group, evening primrose oil was supplied as 500-mg oval clear unmarked capsules, while placebo capsules identical in appearance and containing 300 mg of sunflower oil were given to the other group. Treatment continued for a period of 5 months. With pre-designed scoring system (based on four major parameters: extent, intensity, itching, and dryness), clinical evaluation was done at baseline and subsequent monthly visits. Data of the first 25 patients from each group who completed the 5 months of trial were compiled and analyzed.

Results: At the end of the fifth month, 24 (96%) patients of EPO group and 8 (32%) patients of placebo group showed improvement. There was significant difference in outcome of treatment between two groups ($P < 0.00001$). No significant adverse effect was reported by any patient/guardian at any point of assessment.

Conclusion: Evening primrose oil is a safe and effective medicine in management of AD. However, since not all researchers across the world have found the same good result, further large trials on Indian patients are needed.

Prostaglandin E_1 Analogues

Misoprostol
- Prevents gastric irritation caused by NSAIDS
  (FDA-Approved Indication)
- Inhibits histamine-stimulated gastric acid secretion
- Stimulates mucin & bicarb secretion into stomach
- Increases mucosal blood flow
- Labor induction
- Blocks PGE_2

Alprostadil improves erectile dysfunction
(FDA-Approved Indication)

Limaprost
- Causes vasodilatation & anti-thrombosis
- Improves erectile dysfunction
- Improves neuropathy
  (FDA approved: thromboangiitis obliterans, lumbar spinal canal stenosis, & other ischemic conditions)

Lubiprostone (Amitiza) treats Constipation (FDA-Approved Indication)
- Activates Chloride Channels

Prostaglandin E_1

Contrast-induced nephropathy:

Incidence 11.1% → 3.7% (163 patients) Li WH et al: Int Urol Nephrol 46:781–786, 2014

Creatinine increases 0.72 vs 0.30 mg/dl (130 patients) Koch JA et al: Nephrol Dial Transplant 15:43-49, 2000

Decompensated CHF:

Greater GFR recovery 41% vs 5% (286 patients) Hou ZQ et al: Heart Vessels 28:589-595, 2013

Reduced Pulm BP, wedge press, systemic & pulm resistance; Increased CO & renal blood flow

Wimmer A et al: Jpn Heart J 40:321-334, 1999

Pulm Arterial Hypertension: (49 patients)

Reduced Pulm BP 14%, Resistance 26%; Improved LV EF 10%, CO 23%, Exercise 250%

Shen J et al: Chest 128:714-719, 2005

Improves Pulm BP prior to cardiac transplant

Ischemic Heart Disease: (14 explanted hearts)

Induces angiogenesis & improves hypoxia in ischemic myocardium by increasing CD34 & VEGF

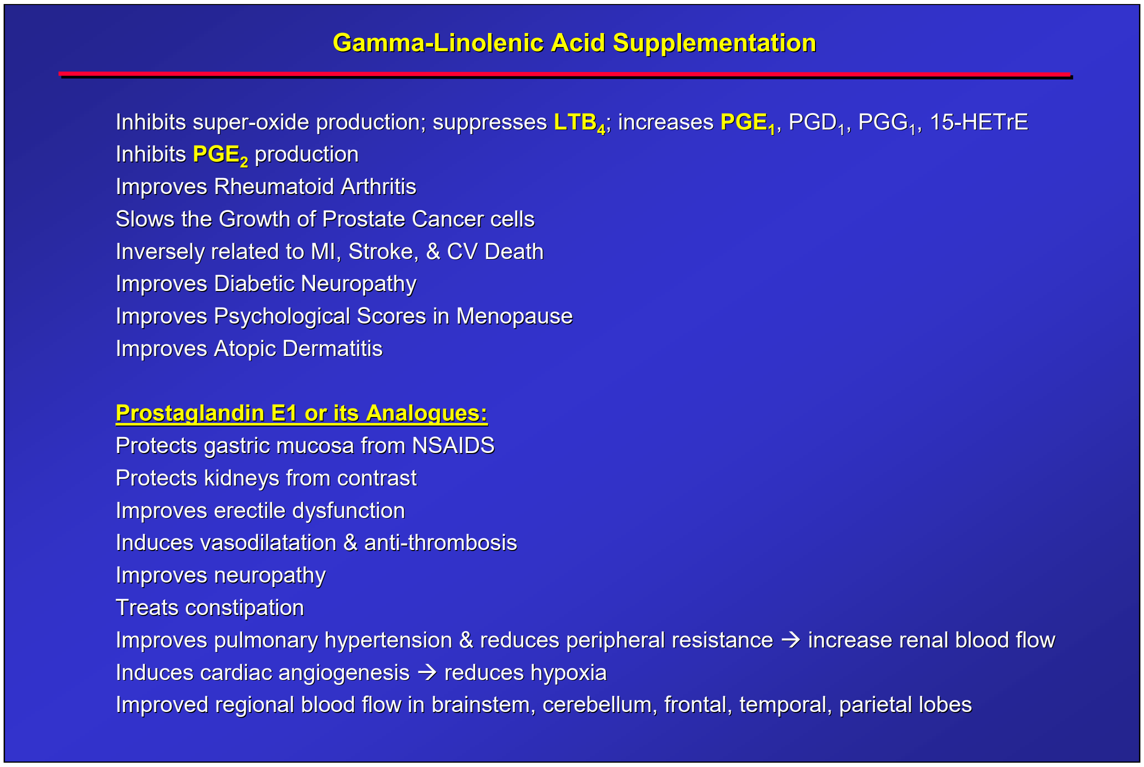
Mehrabi MR et al: Biomedicine & Pharmacotherapy 57:173–178, 2003

Cerebral Infarction:

Improved regional blood flow in brainstem, cerebellum, frontal, temporal, parietal lobes

Improved flow to non-infarcted area adjacent to infarct → no steal

Komaba Y et al: Internal Medicine 37: 841-846, 1998



Abstract Gamma-linolenic acid (GLA, 18:3n-6) is an omega-6 (n-6), 18 carbon (18C-) polyunsaturated fatty acid (PUFA) found in human milk and several botanical seed oils and is typically consumed as part of a dietary supplement. While there have been numerous in vitro and in vivo animal models which illustrate that GLA-supplemented diets attenuate inflammatory responses, clinical studies utilizing GLA or GLA in combination with omega-3 (n-3) PUFAs have been much less conclusive. A central premise of this review is that there are critical metabolic and genetic factors that affect the conversion of GLA to dihommo-gamma linolenic acid (DGLA, 20:3n-6) and arachidonic acid (AA, 20:4n-6), which consequently affects the balance of DGLA- and AA- derived metabolites. As a result, these factors impact the clinical effectiveness of GLA or GLA/n-3 PUFA supplementations in treating inflammatory conditions. Specifically, these factors include: 1) the capacity for different human cells and tissues to convert GLA to DGLA and AA and to metabolize DGLA and AA to bioactive metabolites; 2) the opposing effects of DGLA and AA metabolites on inflammatory processes and diseases; and 3) the impact of genetic variations within the fatty acid desaturase (FADS) gene cluster, in particular, on AA/DGLA ratios and bioactive metabolites. We postulate that these factors influence the heterogeneity of results observed in GLA supplement-based clinical trials and suggest that "one-size fits all" approaches utilizing PUFA-based supplements may no longer be appropriate for the prevention and treatment of complex human diseases.

Optimizing Nutrition for Better Living

Adequate Protein intake – body weight in pounds x 0.6
Calcium intake ~1,000 mg daily
Vitamin D: 25-VitD serum level 40 → 100 ng/ml
Reduce carbohydrate intake to maintain normal body weight
Exercise Daily

Daily Supplements:
Multivitamin
Anti-Oxidant Team:
- Vitamin C 1,000 mg
- Vitamin E 800-1,000 iU
- Selenium 200 mcg
- alpha Lipoic Acid 600 mg twice daily

Fatty Acids:
- Omega-3 (EPA + DHA) ~4 to 9 grams
- Gamma Linolenic Acid (borage oil) 2 to 6 caps daily

Creatine: 5-10 grams (stamina & myopathy)
Biotin: 10,000 mcg (hair, skin, & nails)

Others: (Vinik formula - to be determined)
Vitamin B1 (thiamine) 300 mg daily
Vitamin B6 (pyridoxine) 50 mg daily
Vitamin B12 1,000 mcg daily
Folic Acid 1 to 5 mg daily

Daily Cost:
90 grams Protein: $5.25
Supplements: $1.60
Walmart.com
Delivered to your door free
$205.50 / month
Add Veggies & Nuts