بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Final 11

Metabolism of Eicosanoids

Written by : DST

Reviewed by : Shahad Alrawi

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الجامع لصفات العظمة والجلال والكبرياء، العظيم في ذاته وأسمائه وصفاته، فلا يستحق أحد من الخلق أن يعظّم كما يعظّم سبحانه بالقلب واللسان والجوارح.

الورود: ورد في القرآن (٩) مرات.

الشاهد: ﴿وَلَا يَـُٔودُهُۥ حِفْظُهُمَا ۚ وَهُوَ ٱلْعَلِىُّ ٱلْعَظِيمُ﴾ [البقرة:٢٥٥] .

اضغط هنا لشرحٍ أكثر تفصيلًا

Metabolism of Eicosanoids

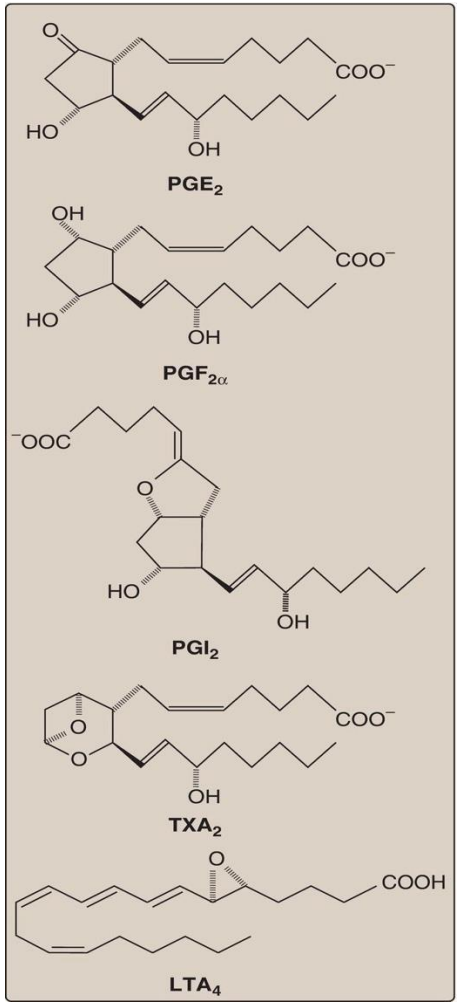
Dr. Diala Abu-Hassan

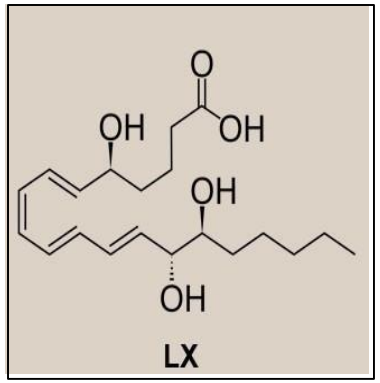
Eicosanoids are inflammatory mediators involved in both physiological and pathological responses (such as hypersensitivity).

✓ **They are derived from 20-carbon (Eicosa) polyunsaturated fatty acids, predominantly arachidonic acid, and are classified into four main groups based on their function as well as structure:**

- **Prostaglandins (PGs): Share a five-membered ring structure in addition to other changes on the 20 carbons of the arachidonic acid.**
- **Thromboxanes (TX): Contain a cyclic ether structure between different structures of TX.**
- **Leukotrienes (LT): Contain three conjugated double bonds (That's why they are trienes)**
- **Lipoxins (LX): Produced by lipoxygenase enzymes, they resolve inflammation rather than inducing it.**

- Eicosanoids are classified into four groups: (1) Prostaglandins (PG) and prostacyclins (PGI), and (2) thromboxanes (TX); (3) the leukotrienes (LT) and (4) lipoxins (LX).
- Prostaglandins, prostacyclins, and thromboxanes are known as prostanoids.

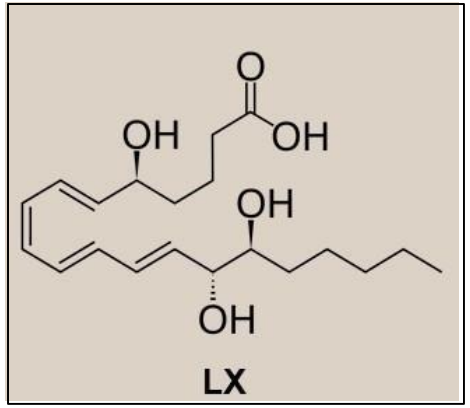


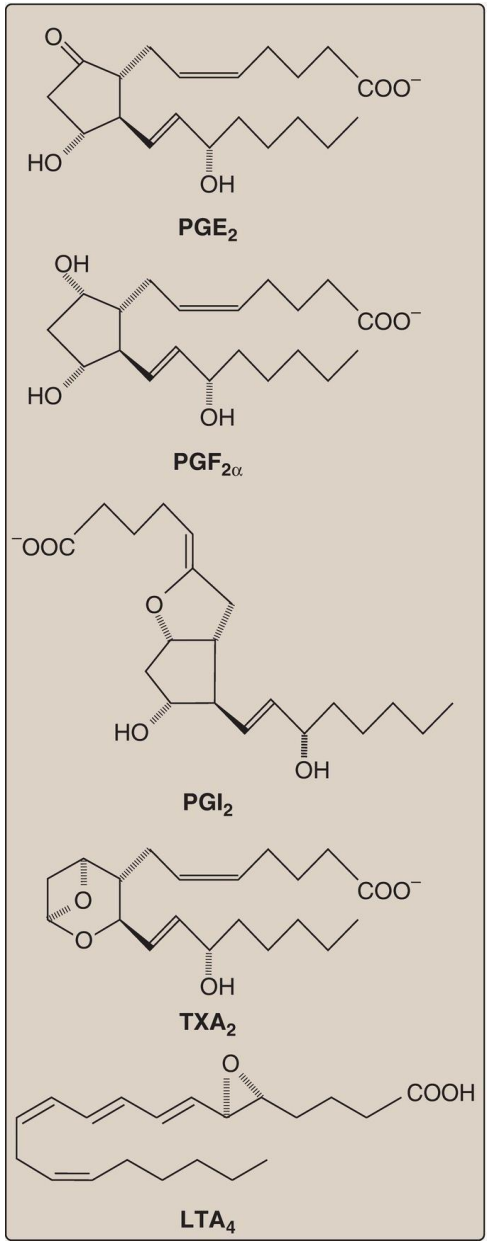


Overview of eicosanoids

Do not memorize the structures

- They are produced from ω-3 and ω-6 polyunsaturated FA with 20 carbons (eicosa = 20).
- They elicit physiologic (inflammatory) and pathologic (hypersensitivity) responses:
  - Gastric integrity, renal function, smooth muscle contraction (intestine and uterus), blood vessel diameter, and platelet homeostasis.
- They are not stored.
- They have a short half-life.(produced and released in a very short amount of time)
- They are rapidly metabolized to inactive products.
- They are not hormones but signaling molecules.





Reasons for naming

- Site of synthesis:
  - Prostaglandins were originally shown to originate from the prostate gland.
  - Thromboxanes from platelets (thrombocytes)(they are expressed in different locations)
  - Leukotrienes from leukocytes.
  - Lipoxins are inflammation resolving(rather than inducing inflammation)eicosanoids synthesized through **lipox**ygenase **in**teractions.(named after their enzyme that makes them)

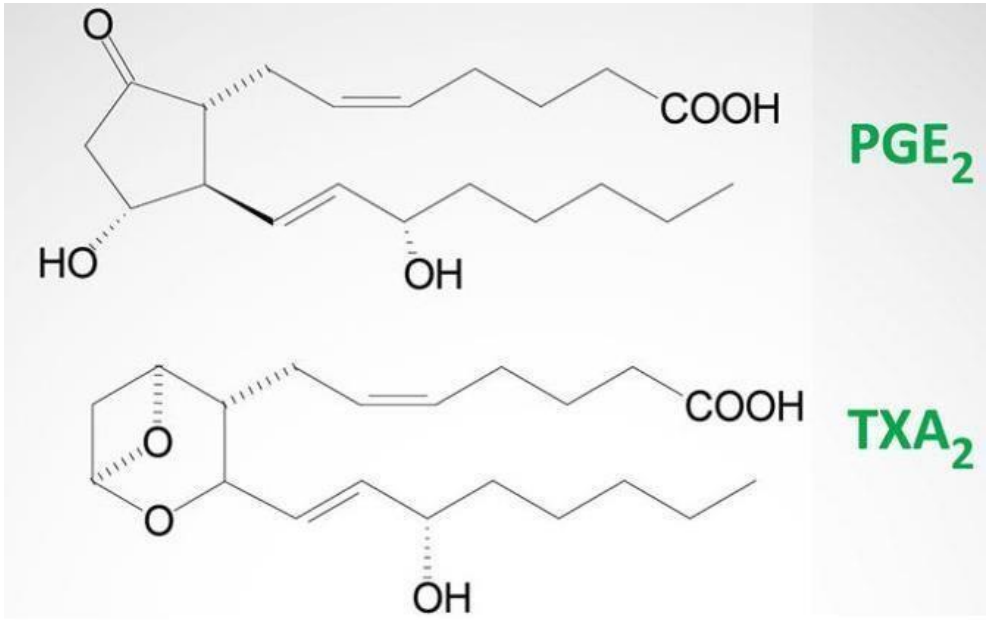
Prostaglandins and thromboxanes

TABLE 14.2: Salient features of prostaglandins

Name	*Substituent groups*
PGA	Keto group at C9; double bond C10 and 11
PGB	Keto group at C9; double bond C8 and 12
PGD	OH group at C9; keto group at C11
PGE	Keto group at C9; OH group at C11
PGF	OH groups at C9 and C11 (Fig.14.2)
PGG	Two oxygen atoms, interconnected to each other, and bonded at C9 and C11; hydroperoxide group at C15
PGH	Same ring as PGG; but C15 has OH group
PGI	Double ring. Oxygen attached to C6 and C9, to form another 5-membered ring. Hence called prostacyclin.

Do not memorize the table

- **Prostaglandins are classified based on modifications of the arachidonic acid structure:**

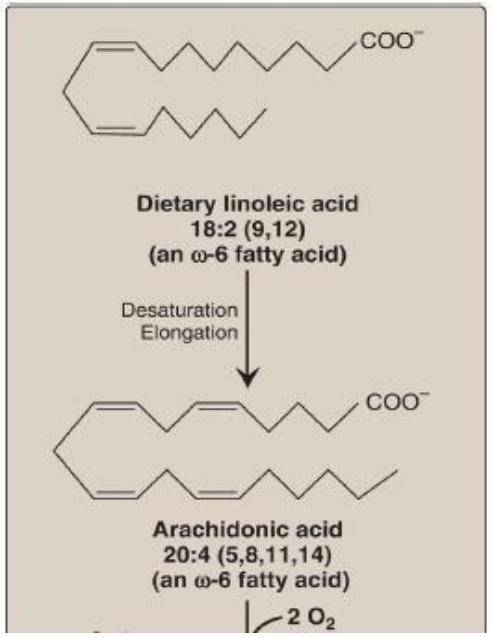


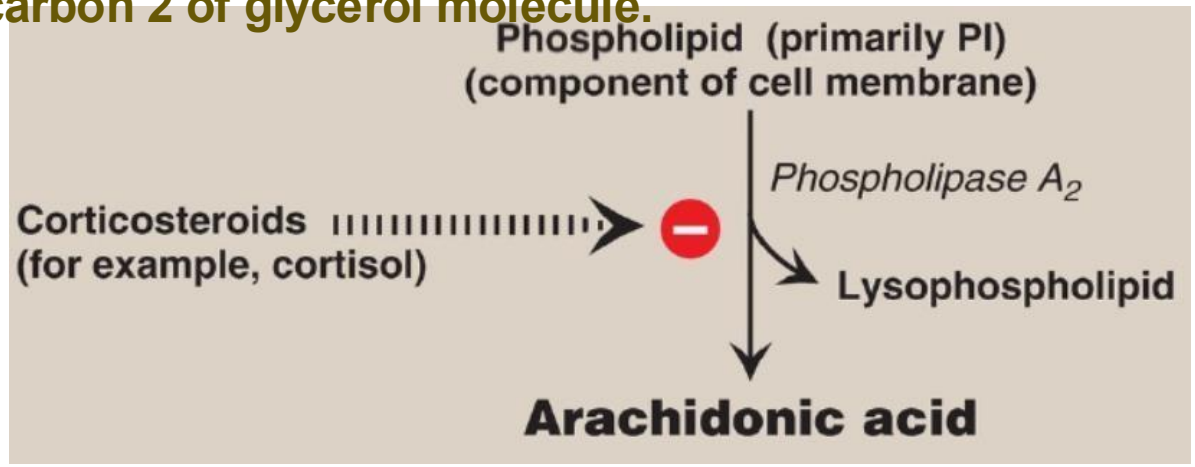
- Prostaglandins are produced by almost all nucleated cells in most tissues and organs.
- They have a cyclopentane ring.
  - They are designated by a letter that describes the ring modification followed by a number
  - that indicates the number of double bonds.
    - Series 1 PGs contain one double bond, series 2 has 2, and so on.
  - Thromboxanes have a 6-membered ring and cyclic ethers.

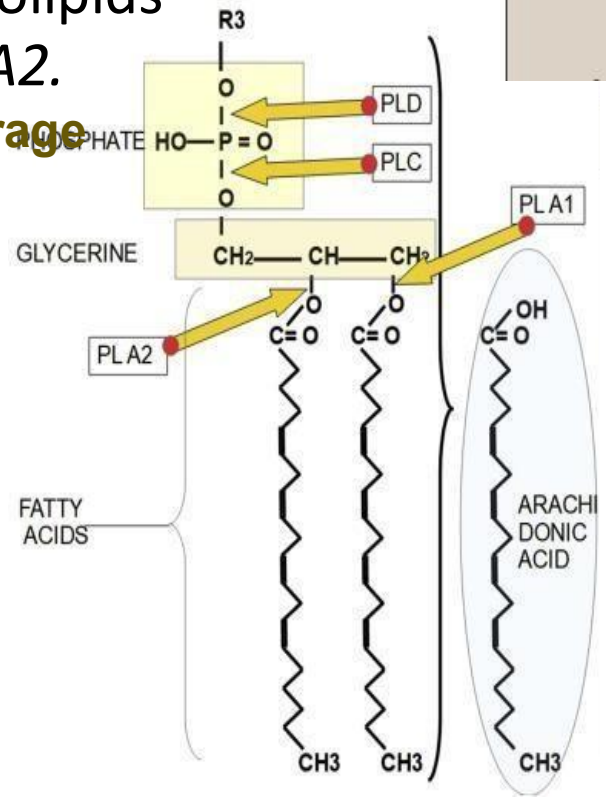
Synthesis from arachidonic acid

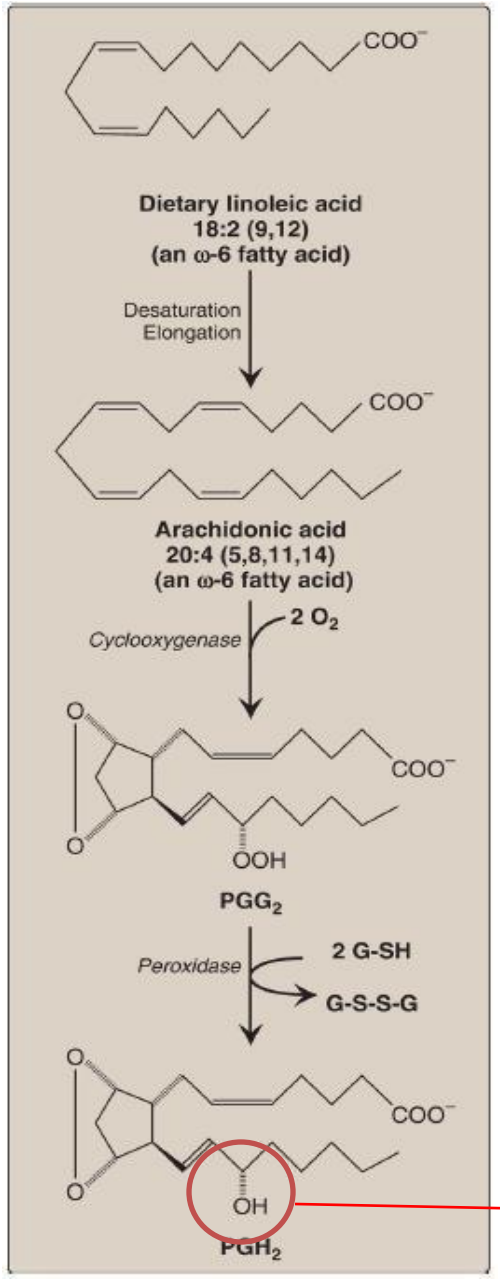
- Arachidonic acid (polyunsaturated fatty acid that has 4 double bond)(an eicosatetraenoic FA), is the immediate precursor of PG.
- Arachidonic acid is derived by the elongation and desaturation of the linoleic acid **(an omega-6 fatty acid (18 carbons, 2 double bonds).**
- Arachidonic acid is incorporated into membrane phospholipids (typically PI) at carbon 2 and released by *phospholipase A2.*

Specifically phosphatidylinositol (PI) that is considered as a storage For arachidonic acid and they are highly attached to arachidonic Acid at carbon 2 of glycerol molecule.









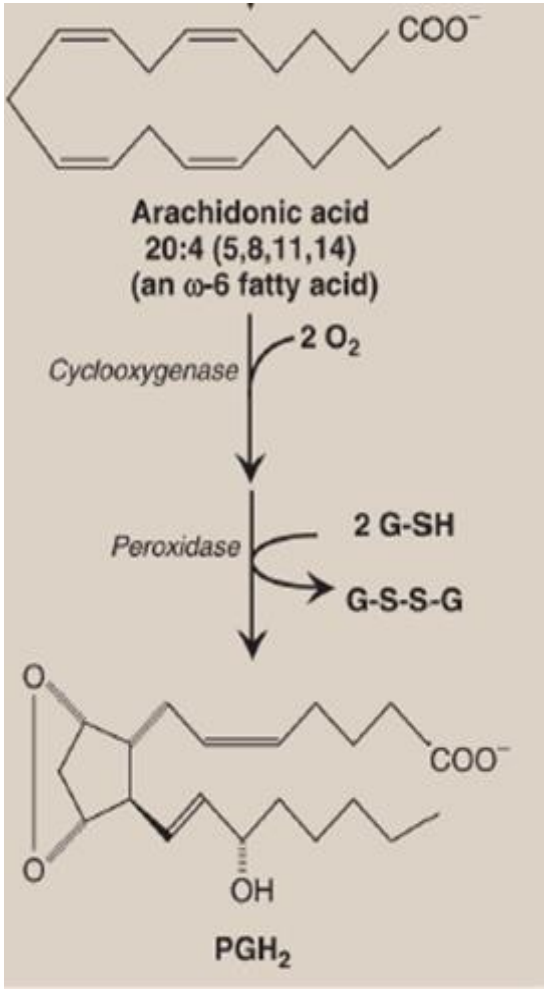
- ✓ **Arachidonic acid, which is stored in membrane phospholipids (like phosphatidylinositol), is released by the action of the enzyme phospholipase A2.**
- ✓ **cyclooxygenase (COX) incorporates oxygen into the arachidonic acid, The incorporation leads to the formation of a cyclic structure (a five-membered ring) within the 20-carbon chain, resulting in the formation of prostaglandin G2 (PGG2).**
- ✓ **A second enzyme activity, peroxidase, reduces the peroxide group present in PGG2. Peroxidase uses glutathione as a cofactor, and through an oxidation-reduction process, the glutathione is oxidized, forming a disulfide bond.**
- ✓ **This reduction step converts prostaglandin G2 to prostaglandin H2 (PGH2), which is considered the parent molecule for other prostaglandins and thromboxanes.**

Site of reduction

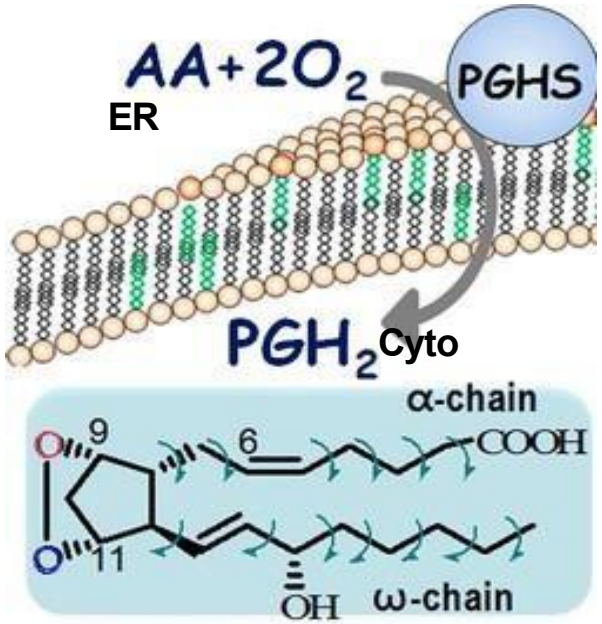
Prostaglandin H2 synthase

Cyclization & Oxidation-Reduction: These reactions are classified as oxidative cyclization. The process involves both oxidation-reduction reactions (with glutathione as a key cofactor) and cyclization (the formation of a ring structure).

- Synthesis of PGs and TXs starts by oxidative cyclization of arachidonic acid to yield PGH2 by PGH2 synthase (or, prostaglandin endoperoxide synthase).
- PGH2 synthase has two catalytic activities: a fatty acid cyclooxygenase (COX) and a peroxidase, which requires reduced glutathione.

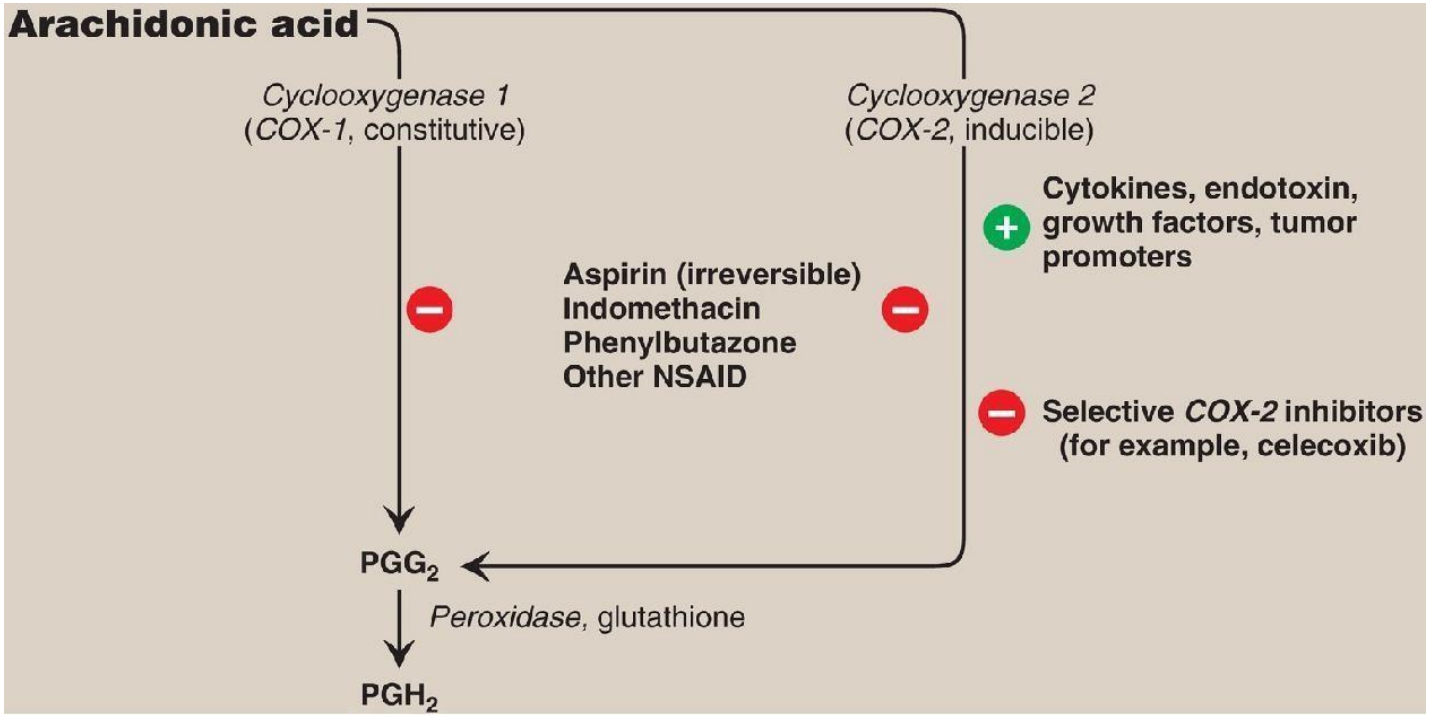


- **Cyclooxygenase activity:** The enzyme introduces an oxygen molecule into the arachidonic acid, forming a peroxide group. This process initiates the formation of a cyclic structure within the arachidonic acid, leading to the formation of prostaglandin G2 (PGG2).
- **Peroxidase activity:** The peroxidase activity of PGH2 synthase reduces the peroxide group in prostaglandin G2, converting it to prostaglandin H2 (PGH2). This reduction requires glutathione as a cofactor, which gets oxidized in the process and forms a disulfide bond.



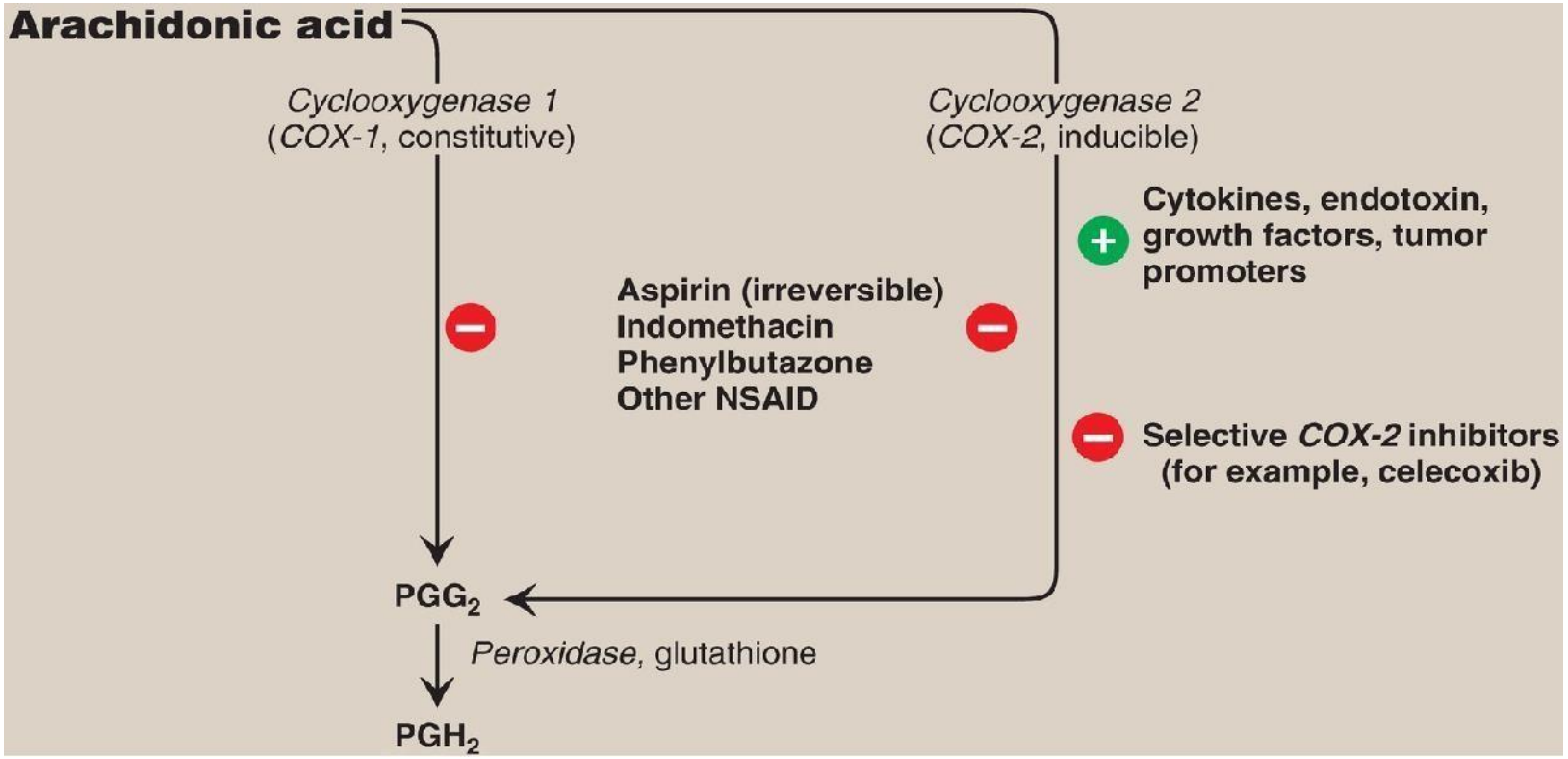
✓ PGH2 synthase is located in the endoplasmic reticulum (ER) membrane, where it performs these reactions

Cyclooxygenases



- ✓ **COX-1 is constitutively active and is activated under normal physiological conditions to perform normal functions, such as those related to gastric and renal tissues, maintaining homeostasis and normal function.**
- ✓ **COX-2, on the other hand, is inducible and activated by various stimuli, such as cytokines released during inflammation, endotoxins, growth factors, tumor promoters, etc. These stimuli mediate inflammatory and hypersensitivity reactions during infection and (abnormal)pathological conditions.**
- ✓ **Both COX-1 and COX-2 can produce prostaglandin G2, which is then converted or reduced to prostaglandin H2 by peroxidase in the presence of glutathione.**

Cyclooxygenases

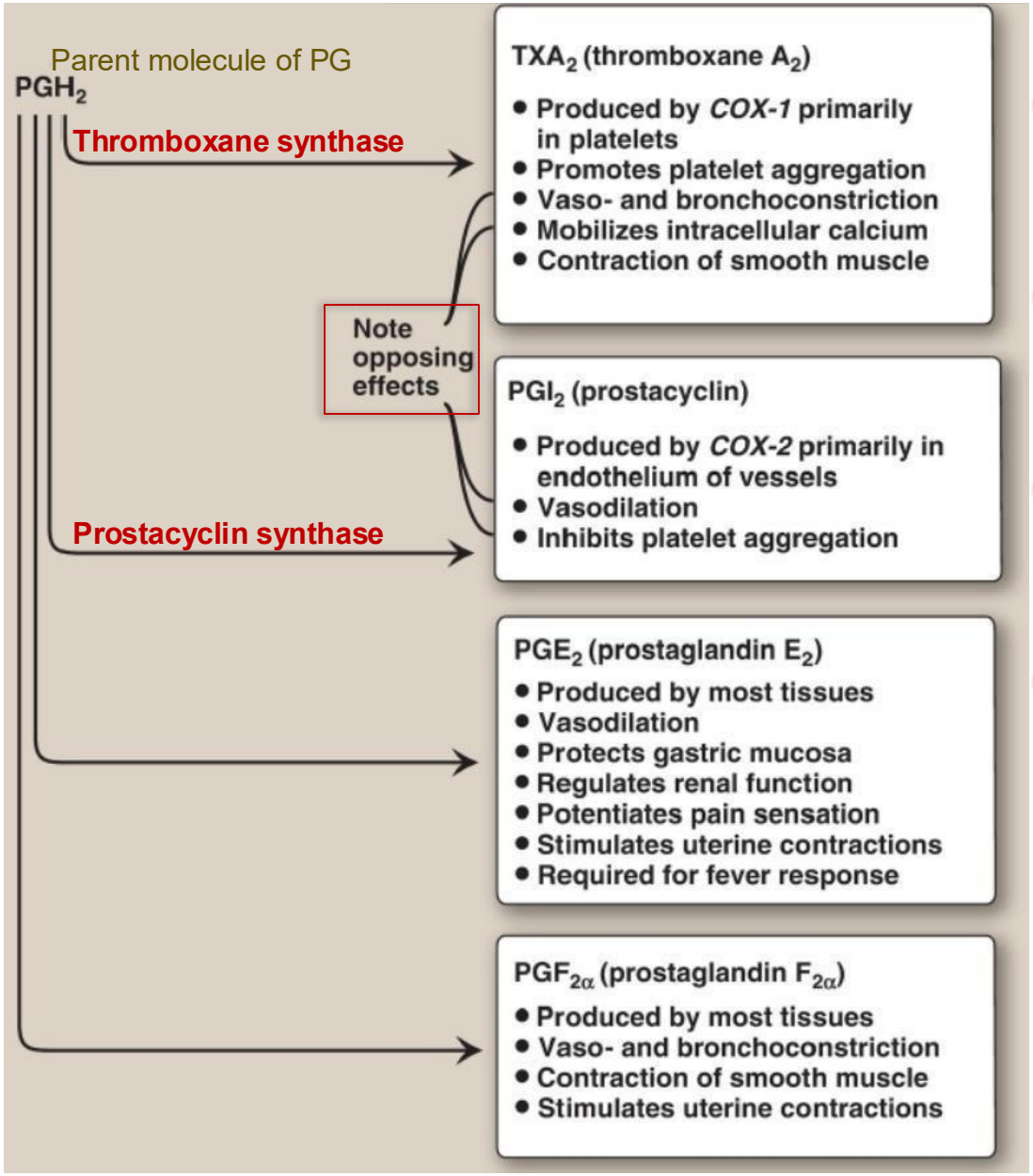


- There are two isozymes of PGH2 synthase: COX-1 and COX-2.
- COX-1 is made constitutively in most tissues and affects platelet aggregation and the functions of gastric renal tissues.
- COX-2 is inducible in specific tissues and mediates the pain, heat, redness, and swelling of inflammation and the fever of infection.
- Both COX-1 and COX-2 catalyze the two reactions.

PGH2 is then converted to a variety of PG and TX

Enzyme	Thromboxane Synthase	Prostacyclin Synthase
Substrate	Prostaglandin H2	Prostaglandin H2
Product	Thromboxane A2 (TXA2)	Prostacyclin (PGI2)
Function	- Promotes platelet aggregation	- Inhibits platelet aggregation
	- Induces vasoconstriction	- Induces vasodilation
Effect on Platelets	Promotes aggregation (helps in clot formation)	Inhibits aggregation (prevents excessive clotting)
Effect on Blood Vessels	Induces vasoconstriction	Induces vasodilation
Sites of Action	Platelets, sites of vascular injury	Endothelial cells and other areas to prevent thrombus formation

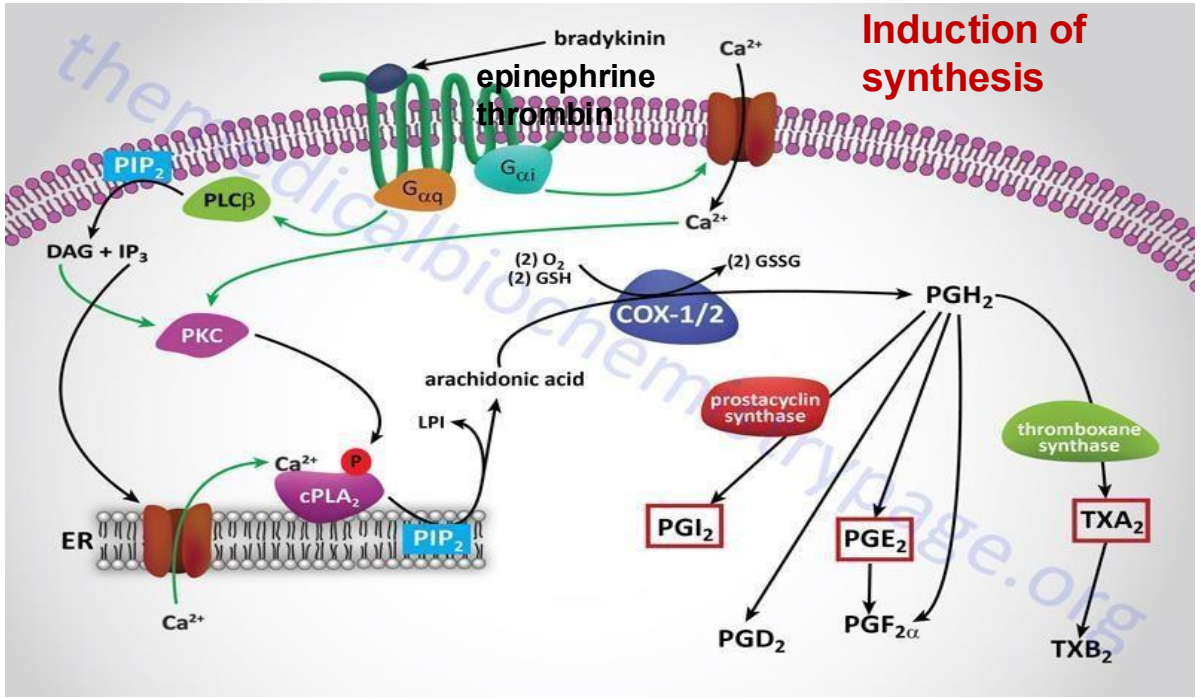
PGH2 is then converted to a variety of PG



- The opposing effects of thromboxane A2 (TXA2) and prostacyclin (PGI2) limit thrombi formation to sites of vascular injury.
- Aspirin has an antithrombogenic effect. It inhibits TXA2 synthesis by COX-1 in platelets and PGI2 synthesis by COX-2 in endothelial cells(reduce the formation of thrombi results in protective effect in old patients to reduce the risk of stroke, heart attack)
- COX-1 inhibition cannot be overcome in platelets because they cannot synthesize it anymore, but COX-2 inhibition can be overcome in endothelial cells.

  This is why low-dose aspirin is lowers the risk of stroke and heart attacks by decreasing the formation of thrombi.

Signals leading to synthesis of eicosanoids



1.signaling molecules (Epinephrine, Thrombin , Bradykinin) bind to G-protein-coupled receptors (GPCRs) on the cell membrane.

2. G-Protein Activation:

- **Binding of the ligands activates the alpha subunit of the G-protein. The activated G-protein activates phospholipase C, which cleaves PIP2 into (DAG) and IP3.**

3. Calcium Release:

- **IP3 opens calcium channels on the endoplasmic reticulum (ER), allowing calcium ions to flow into the cytosol.**

4. Protein Kinase C Activation:

- **DAG activates protein kinase C (PKC), which phosphorylates phospholipase A2.**
- **Phospholipase A2 cleaves arachidonic acid from the membrane phospholipids (like PIP2).**

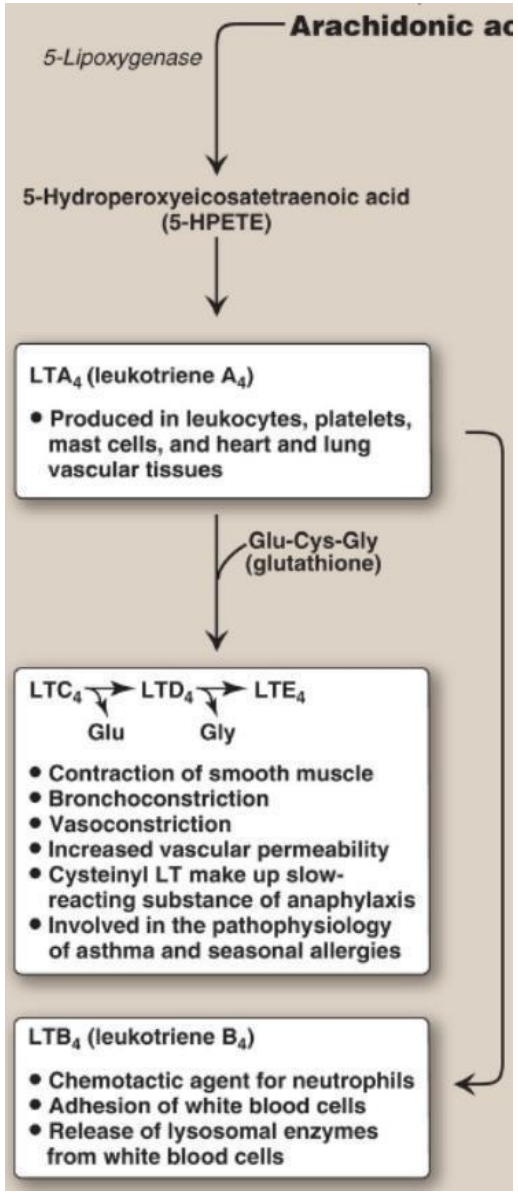
5. Eicosanoid Synthesis:

Arachidonic acid is then used by:

- **Cyclooxygenase 1and 2 (COX-1, COX-2) and the peroxidase activity in prostaglandin H2 synthase to form prostaglandin H2 (the parent molecule).**
- **Prostacyclin synthase to form prostacyclins.**
- **Thromboxane synthase to form thromboxanes.**

Leukotriene synthesis

Leukotrienes are eicosanoids that do not contain a ring structure. They are characterized by three conjugated double bonds.



1. Starting Point: Arachidonic Acid

- Derived mostly from PIP2 (phosphatidylinositol).

2. Conversion to 5-HPETE:(intermediate)

- 5-lipoxygenase converts arachidonic acid into 5-HPETE (5-hydroperoxyeicosatetraenoic acid).

3. Leukotriene A4 Production:

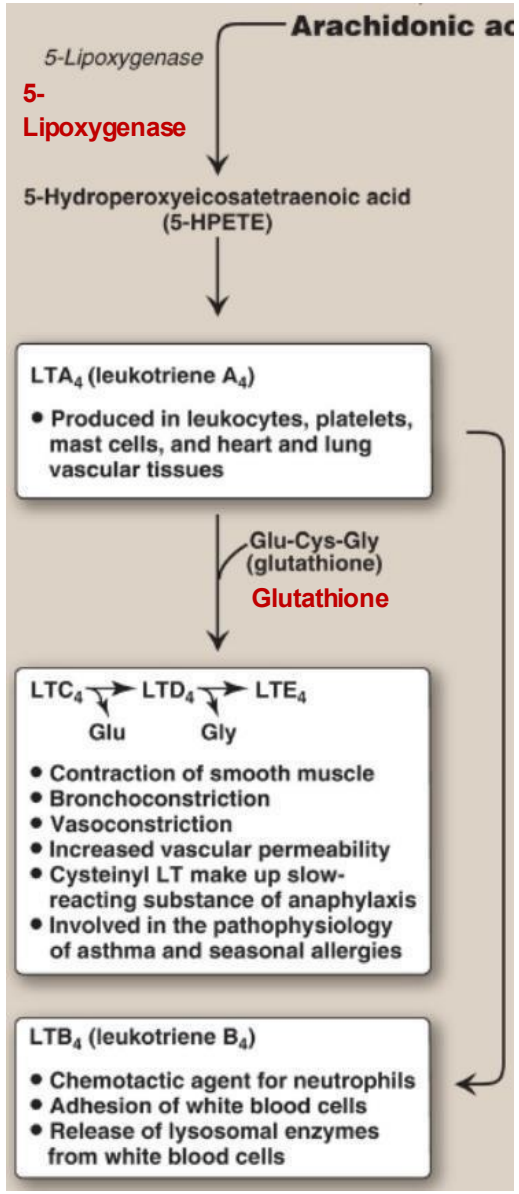
- 5-HPETE is converted to leukotriene A4

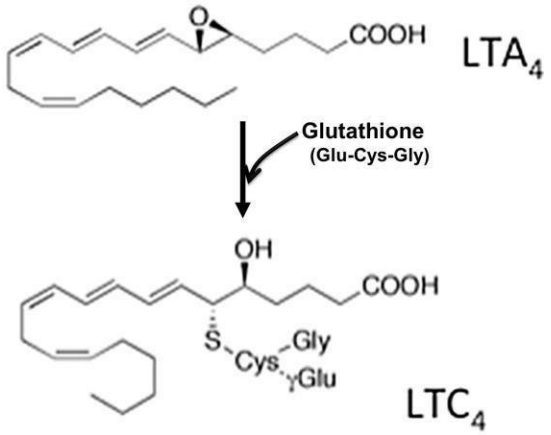
Leukotrienes are produced in various cells, including leukocytes, mast cells, platelets, and heart cells.

4. Further Conversion of Leukotriene A4:

- Glutathione is involved in the conversion of leukotriene A4 into other leukotrienes:
- Leukotriene C4 (LTC4)
- Leukotriene D4 (LTD4) by removing glutamate from LTC4.
- Leukotriene E4 (LTE4) by removing glycine from LTD4.
- Leukotriene B4

Leukotriene synthesis





- LT are mediators of allergic response and inflammation (they are synthesised in the mast cells). Inhibitors of 5-LOX and LT- receptor antagonists are used in the treatment of asthma.

. NSAIDs (Aspirin, ibuprofen , etc.) mainly inhibit the cyclooxygenase (COX) enzymes (COX-1and COX-2), which are involved in the synthesis of prostaglandins, but do not affect leukotriene synthesis. Therefore, NSAIDs do not reduce the concentration of leukotrienes in the body.

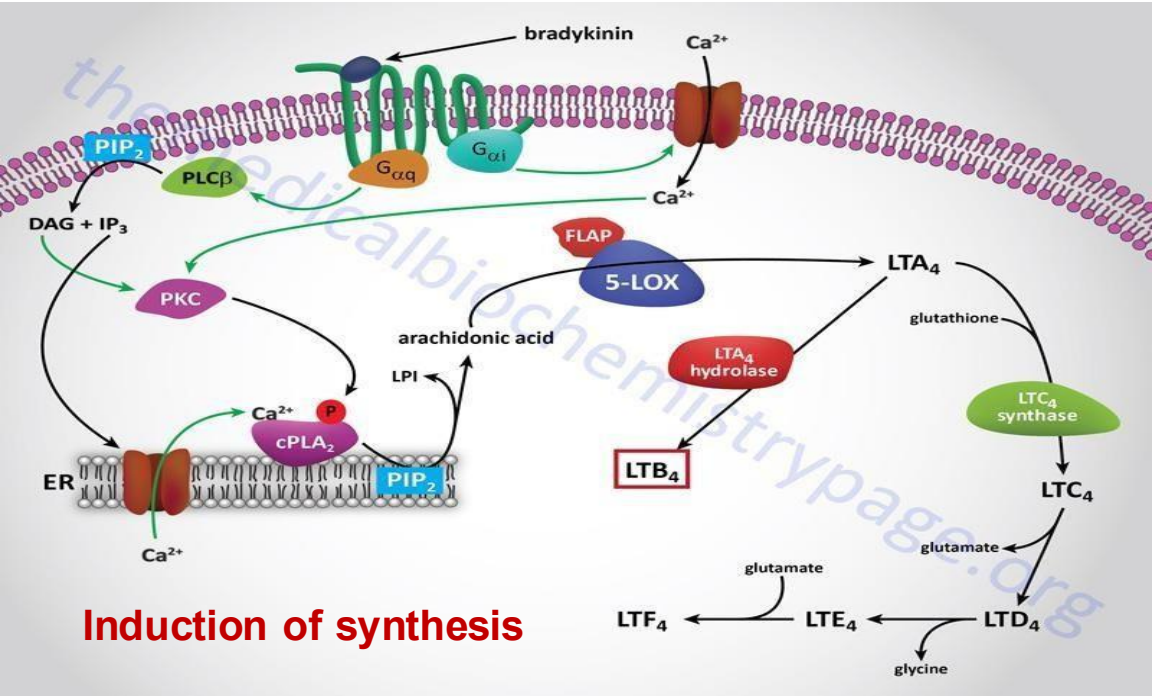
• Cortisol can inhibit the synthesis of leukotrienes by blocking 5-lipoxygenase, reducing leukotriene production.

• In inflammatory diseases or allergic diseases like asthma, leukotrienes play a significant role in mediating inflammation and airway constriction.

• Since NSAIDs do not affect leukotriene production, their use will not alleviate the inflammatory symptoms related to leukotrienes, potentially leading to exacerbated conditions, such as worsened asthma or other respiratory diseases.

- Note: LT synthesis is inhibited by cortisol and not by NSAID **((e.g., aspirin, ibuprofen)** Aspirin-exacerbated respiratory disease is a response to LT overproduction with NSAID use in ~10% of individuals with asthma.

Leukotriene synthesis



1. Signaling Pathway Activation:

- Bradykinin binds to GPCRs , This activates the alpha subunit of the G-protein, which then activates phospholipase C which cleaves PIP2 into two second messengers: DAG and IP3.

2. Calcium Release:

- IP3 opens calcium channels on the ER , allowing calcium ions to flow into the cytosol , The calcium ions bind to and activate phospholipase A2.

3. Activation of Phospholipase A2:

- The DAG also activates protein kinase C, which phosphorylates and further activates phospholipase A2 , Phospholipase A2 cleaves arachidonic acid from PIP2

4. Leukotriene A4 Synthesis:

- The arachidonic acid is then acted on by 5-lipoxygenase to produce the first leukotriene, leukotriene A4 (LTA4).

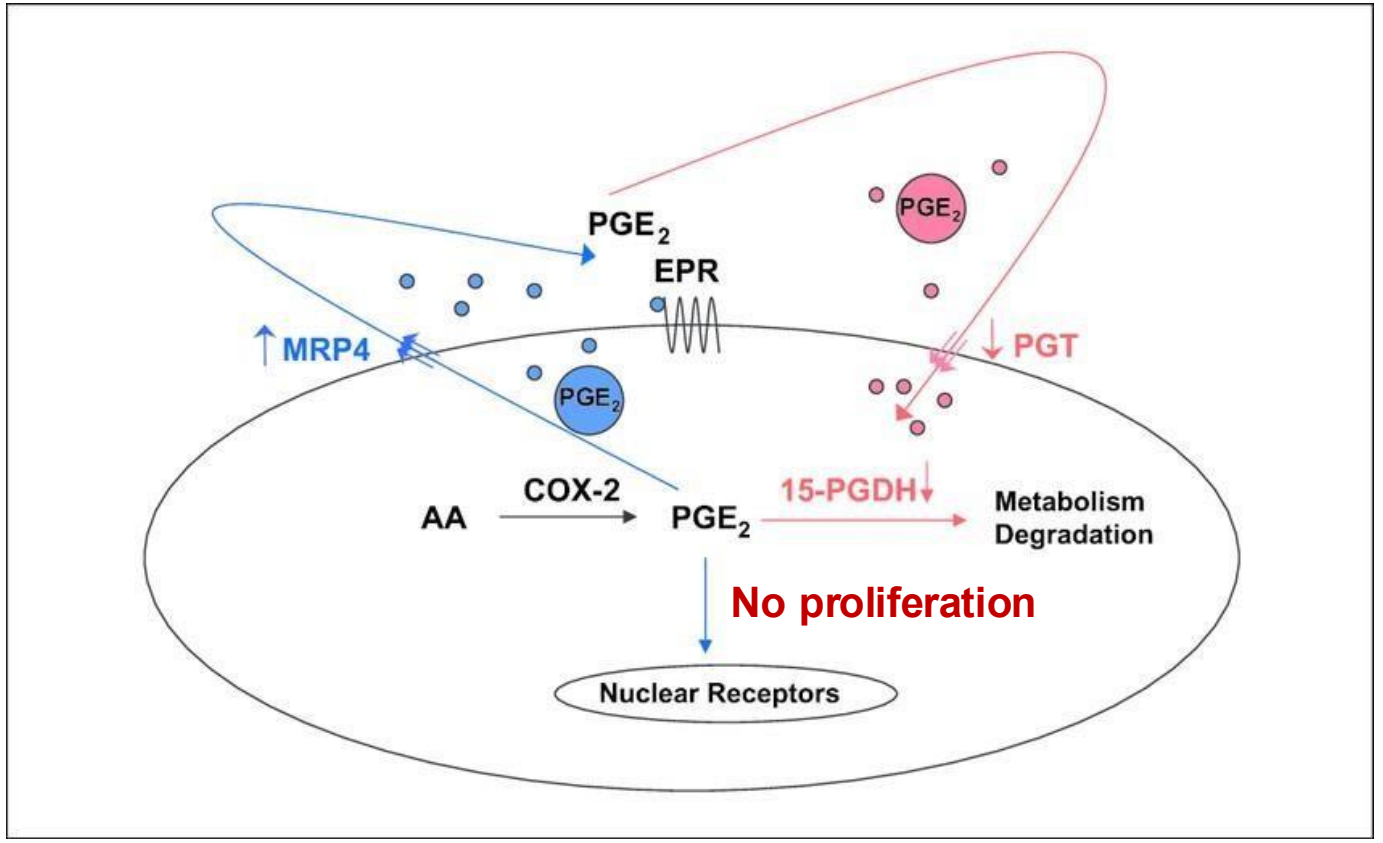
5. Further Leukotriene Conversion:

- LTA4 can be converted to other leukotrienes in the presence of glutathione and specific enzymes:
- LTC4 (Leukotriene C4) via LTC4 synthase.
- LTD4 (Leukotriene D4) by removing glutamate from LTC4.
- LTE4 (Leukotriene E4) by removing glycine.
- Leukotriene F4 can be formed by adding another glutamate.
- Leukotriene B4 (LTB4) can be formed by LTA4 hydrolase.
- So different leukotrienes can be formed one after another but the first one is leukotrienes A4

Catabolism of prostanoids (cyclic) (PGs, TXs, PCs)

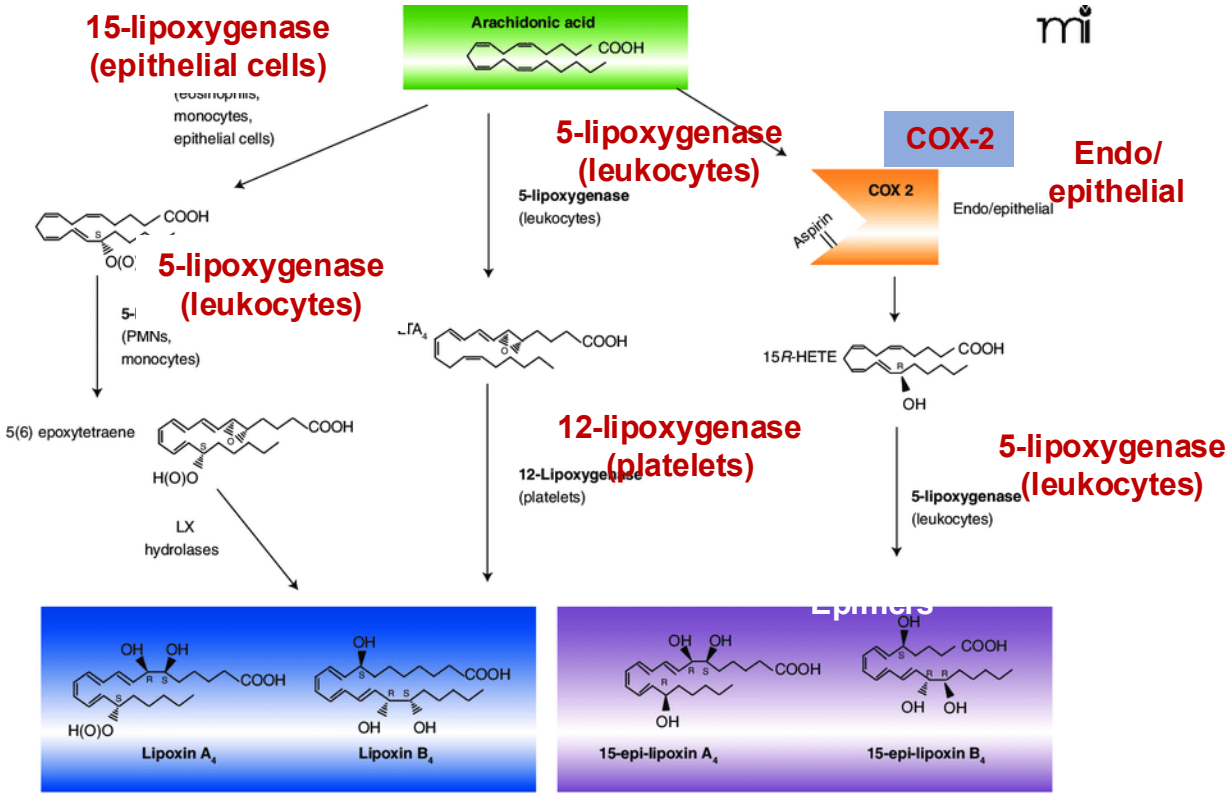
- Prostanoids are often deactivated quickly either spontaneously or enzymatically.
  - Half-lives of 30 seconds.(short)
- Prostanoids are first transported from the extracellular fluid to the cytoplasm by the prostaglandin transport protein (PGT) where they are converted into products that are either inactive or can inhibit cell proliferation.

Once the are degraded they are eliminated via the kidney into the urine.



Synthesis of lipoxins

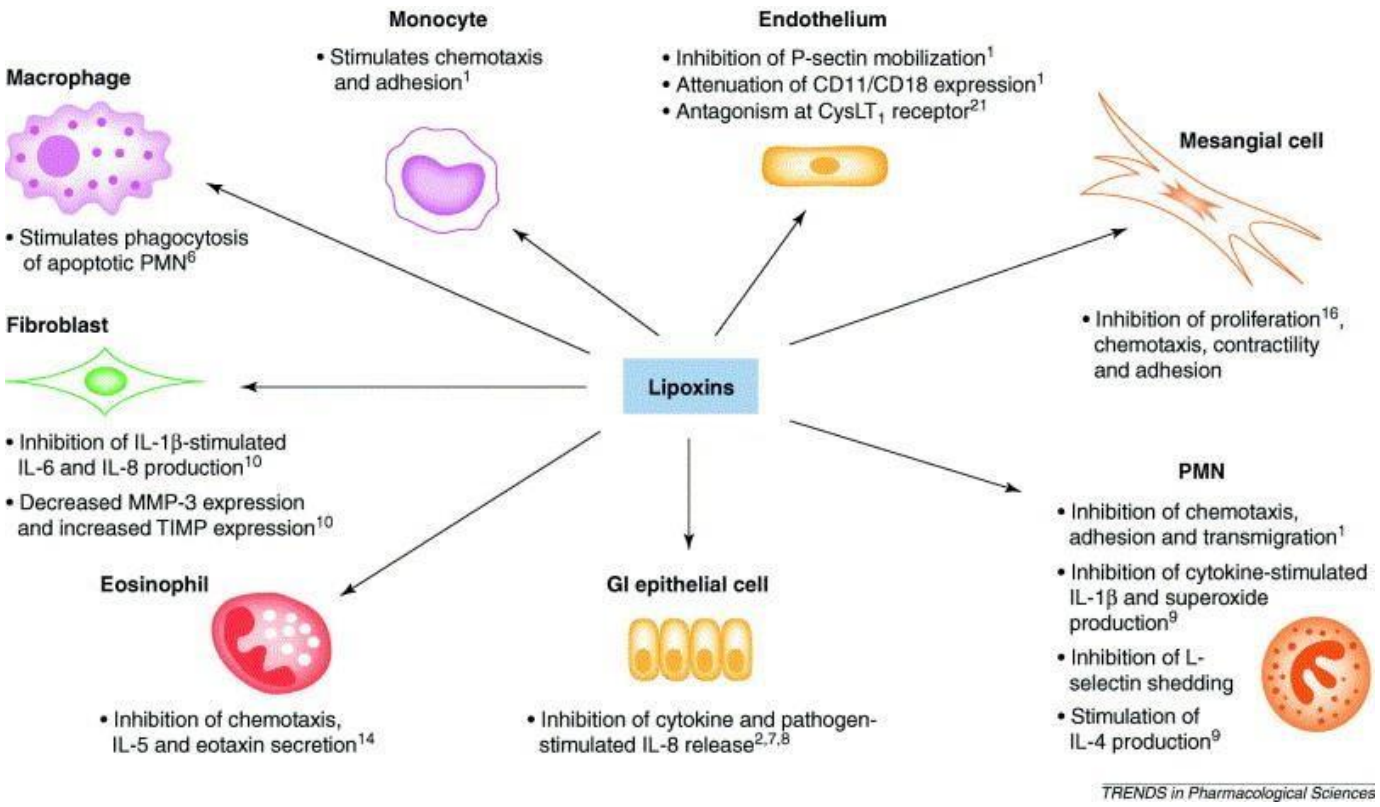
- The lipoxins are anti-inflammatory since they inhibit the actions of the leukotrienes.
- Synthetic pathways of lipoxins:
  - The “classic” pathway: 5-lipoxygenase (5-LOX) in leukocytes followed by 12-LOX in platelets.
  - 15-LOX in epithelial cell, such as airway cells, followed by 5-LOX action in leukocytes.
  - Aspirin-mediated acetylation of COX-2.
    - Aspirin-induced acetylation of COX-2 alters the enzyme such that it converts arachidonic acid to biologically active LXs in monocytes and leukocytes by 5-lipoxygenase (5-LOX).



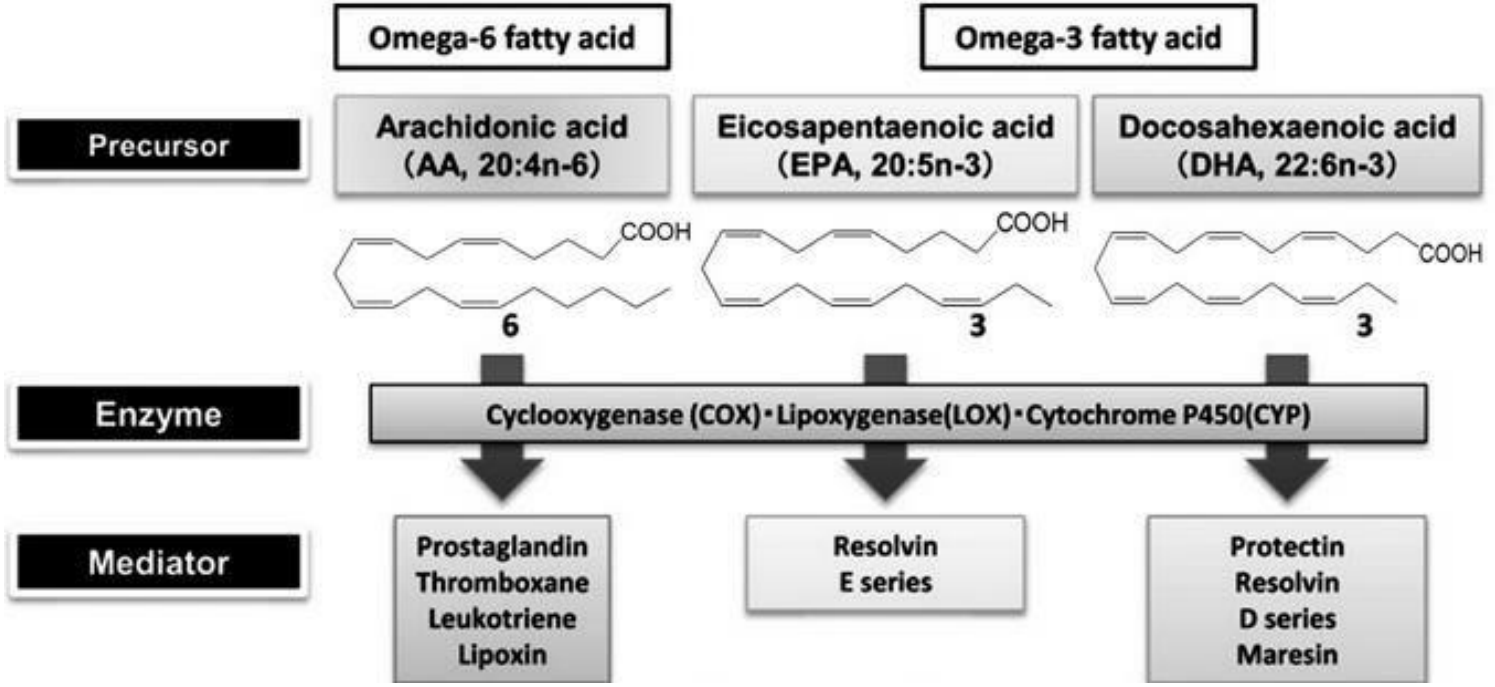
The functions of lipoxins

Do not memorize.
Just understand the concept

- The lipoxins LXA4 and 15 epi-LXA4 function through lipoxin A4 receptor (ALXR), a G protein-coupled receptor (GPCR) to:
  - Increase the production of prostacyclin (PGI_2) and nitric oxide (NO),
  - promote the relaxation of the vasculature,
  - inhibit polymorphonuclear leukocyte (PMN)-mediated increases in vasopermeability, and PMN chemotaxis, adhesion and migration through the endothelium,
  - stimulate phagocytosis of apoptotic PMNs by macrophages (the resolution phase of inflammatory events),
  - blocking the expression of the pro-inflammatory IL-8 by macrophages and endothelial,
  - regulate the actions of histamine leading to a reduction in edema.



The specialized pro- resolving mediators (SPM)



- Resolvins (Rv), protectins (PD), and maresins (MaR) are anti-inflammatory lipids that are derived from the omega-3 EPA- and DHA by lipoxygenases.
- Aspirin triggers their synthesis.
- They stimulate the resolution of the inflammatory responses through G protein-coupled receptors via diverse action like:
  - limiting further neutrophil recruitment to the site of inflammation
  - promoting macrophage clearance of debris, apoptotic cells and bacteria.

Resources

- Lippincott's Biochemistry
- Eicosanoid Metabolism: Prostaglandins, Thromboxanes, Leukotrienes, and Lipoxins (https://themedicalbiochemistrypage.org/eicosanoid-metabolism-prostaglandins-thromboxanes-leukotrienes-and-lipoxins/)
- Bioactive Lipid Mediators of Inflammation (https://themedicalbiochemistrypage.org/bioactive-lipid-mediators-of-inflammation/)

رسالة من الفريق العلمي:

"أطنان من التعب ؛
تمحُوها فكرة مطمئنة .
«وأنَّ سعيه سوف يُرى»

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1	Slides **20**,**21** & **22** were added		
V1 → V2			