



## Review

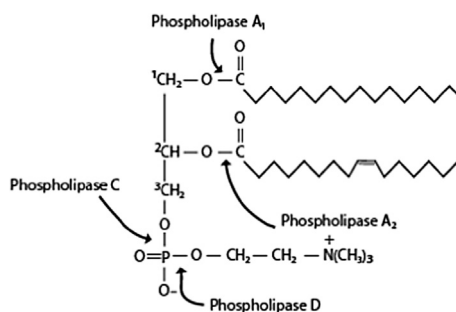
## Synopsis of arachidonic acid metabolism: A review

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## GRAPHICAL ABSTRACT



Sites of hydrolysis for each phospholipase (PLA1, PLA2, PLC and PLD).

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## ABSTRACT

Arachidonic acid (AA), a 20 carbon chain polyunsaturated fatty acid with 4 double bonds, is an integral constituent of biological cell membrane, conferring it with fluidity and flexibility. The four double bonds of AA predispose it to oxygenation that leads to a plethora of metabolites of considerable importance for the proper function of the immune system, promotion of allergies and inflammation, resolving of inflammation, mood, and appetite. The present review presents an illustrated synopsis of AA metabolism, corroborating the instrumental importance of AA derivatives for health and well-being. It provides a comprehensive outline on AA metabolic pathways, enzymes and signaling cascades, in order to develop new perspectives in disease treatment and diagnosis.

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## Introduction

Arachidonic acid (AA), all-*cis*-5, 8, 11, 14-eicosatetraenoic acid (where eicos or eikosi in Greek refers to the number 20), is an omega-6 polyunsaturated fatty acid (PUFA). Its chemical formula

is  $C_{20}H_{32}O_2$ , 20:4( $\omega$ -6), where 20:4 refers to its 20 carbon atom chain with four double bonds, and ( $\omega$ -6) refers to the position of the first double bond from the last, omega carbon atom. Arachidonic acid has an average mass of 304.467 g/mol and usually assumes a hairpin structure (Fig. 1). Due to the presence of its four double bonds in the *cis* position (which means that all hydrogen atoms are on the same side of the double bonds), the compound has a certain degree of flexibility for interaction with proteins [1]. Even at low temperature it helps in keeping the fluidity of cell

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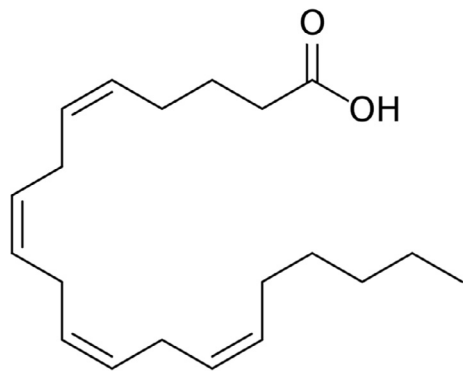


Fig. 1. Arachidonic acid hairpin conformation.

membranes. The four double bonds also enable interaction with molecular oxygen giving rise to bioactive oxygenated molecules including eicosanoids and isoprostanes via enzymatic and non-enzymatic mechanisms, respectively [2].

## Methodology

MEDLINE, PubMed, Google, and Google Scholar were used to collect data and references, searching by the following key words: arachidonic acid, phospholipases, cyclooxygenases, lipoxygenases, eicosanoids, isoprostanes, anandamides, lipoxins.

## Source

Arachidonic acid can be provided to humans and mammals by an exogenous source supplied either by the direct consumption of dietary food that contains high level of AA, whole eggs, salmon, tuna [3], a wide range of lean meat [4] and its visible meat fats [5], or through the parent molecule, linoleic acid (LA; 18:2n-6). LA is considered to be an essential fatty acid since humans and some mammals lack the enzymes required for its synthesis [6]. It is abundant in vegetable oils such as soya, corn, sunflower and safflower and also found in walnuts [7]. In human body, LA is subjected to series of desaturation enzymes (delta-6 fatty acid desaturase and delta-5 fatty acid desaturase), and elongation enzymes that carry out their action in the endoplasmic reticulum (ER) membrane [8]. Elongation of fatty acid consists of four steps. The first step involves a 3-keto-acyl-CoA synthase that can be encoded by seven different genes known as ELOVL1-7, responsible for elongation of long fatty acids. ELOVL5 is most likely to elongate 18:3 fatty acid through condensation of malonyl CoA with the fatty acid acyl CoA compound [9]. A reduction reaction is the second step via 3-keto-acyl-CoA reductase activity. This step requires NADPH as a co-factor. The third step, the resultant intermediate compound undergoes a dehydration action through 3-hydroxyacyl-CoA dehydrase. In the fourth and last step, another reduction reaction is carried out by trans 2,3- enoyl - CoA reductase [10,11] (Fig. 2). Proceeding with from the last desaturation reaction, AA in turn can be esterified with glycerol in the phosphatidylethanolamine, phosphatidylcholine, or phosphatidylinositides of the cell membrane. Beside the exogenous source, endocannabinoids such as *N*-arachidonoyl ethanolamine (anandamide) serve as an endogenous source of arachidonic acid. An integrated membrane protein enzyme, fatty acid amide hydrolase (FAAH), is responsible for the catalysis of anandamide into AA and ethanolamine to eliminate the anandamide signal in the nervous system [12].

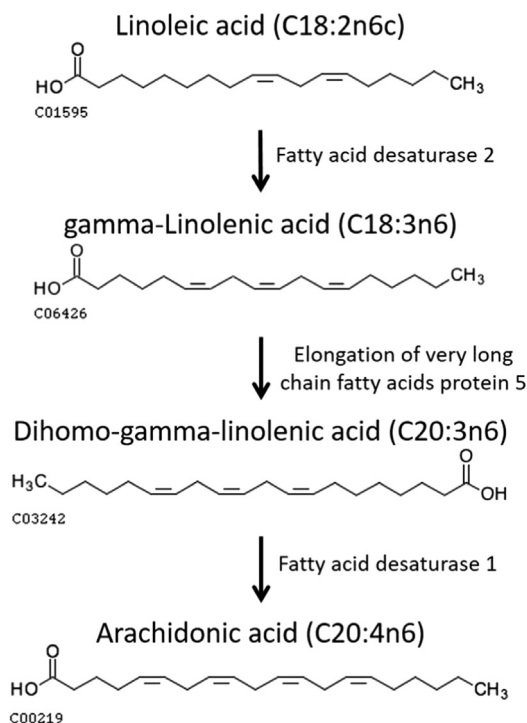


Fig. 2. Linoleic acid metabolism yielding arachidonic acid.

## Distribution

Arachidonic acid is naturally found incorporated in the structural phospholipids in the cell membrane in the body or stored within lipid bodies in immune cells [13]. It is particularly abundant in skeletal muscle, brain, liver, spleen and retina phospholipids [14]. Local levels of esterified AA in resting cells like platelets, for example, are around 5 mM. A concentration of 0.5 mM represents the diffusion of 10% of AA upon activation, and this percentage later on can be distributed between cellular uptake and albumin protein [15,16]. The concentration of free AA in the circulation is very low, owing the fact that in human plasma, albumin is highly abundant as its concentration reaches up to 35 mg/ml, which enables the binding of free fatty acids keeping their concentration below 0.1  $\mu\text{mol}$  [17,18].

## Overview on arachidonic acid metabolism

On a cellular level, three main phospholipases families can exert their action on phospholipids to liberate the esterified AA. The first enzyme is phospholipase A2 (PLA2), which mediates the hydrolysis of the sn-2 position on phospholipid backbone, yielding a free AA molecule directly in one single step [19]. The second and the third enzymes are phospholipase C (PLC) and phospholipase D (PLD) that may also generate free AA (Fig. 3). In two consecutive steps, PLC enzyme catalyzes phospholipids yielding AA through the generation of diacylglycerol (DAG) by the action of diacylglycerol lipase and lipid products containing arachidonate by the action of monoacylglycerol lipases [20]. It is true that PLD activity was described in plants for more than 30 years, but there is proof of PLD activity in higher eukaryotes such as humans as well [21]. Moreover, PLD was evidenced to liberate AA by the following reactions. Phosphatidylcholine is catalyzed by PLD generating phosphatic acid or DAG. The former can be further catalyzed by phosphatidate phosphohydrolase to form DAG. Then, DAG-lipase hydrolyzes DAG to generate AA [22].

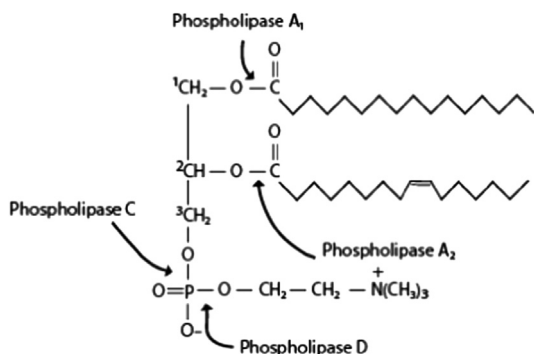


Fig. 3. Sites of hydrolysis for each phospholipase (PLA1, PLA2, PLC and PLD).

The expression and activation of PLA2 enzyme can be a response to a wide range of cellular activation signals from receptor dependent events requiring a G coupled transducing protein as Toll-like receptor 4 (TLR4), purinergic receptors and inflammation stimulation to calcium ionophores, melittin (bee venom) and tumor promoting agents [23,24]. Three fates wait for the liberated, free functional AA: it may diffuse to other cells, reincorporated into the phospholipids, or metabolized.

Furthermore, the activation of PLA2 enzyme can be through the binding of tumor necrosis factor alpha (TNF- $\alpha$ ) to its receptor, P75 and P55, inducing the release of AA from phosphatidylcholine and phosphatidylethanolamine. Free AA can have an important role in cell apoptosis as its accumulation that occurs as a result of arachidonyl CoA transferase inhibition, can promote the activation of sphingomyelinase, enzymes that trigger the degradation of sphingolipids (known to play an important role in cell regulation and cell cycle) to phosphocholine and ceramide [24,25].

Free AA can be metabolized via enzymatic reactions. Free AA can undergo four possible enzymatic pathways: Cyclooxygenase, Lipoxygenase, Cytochrome p450 (CYP 450) and Anandamide pathways to create bioactive oxygenated PUFA containing 20 C (eicosanoids) acting as local hormones and other compounds acting as signaling molecules. Enzymes involved in the cyclooxygenase pathway are COX-1 and COX-2 (also called prostaglandin H synthase), along with downstream enzymes that mediate the production of prostaglandins (PGH<sub>2</sub>, an unstable intermediate, PGE<sub>2</sub>, PGD<sub>2</sub> and PGF<sub>2</sub><sub>alpha</sub>), prostacyclins (PGI<sub>2</sub>), and thromboxanes (TXA<sub>2</sub>, TXB<sub>2</sub>). Lipoxygenase pathway consists of LOX-5, LOX-8, LOX-12, and LOX-15 enzymes and their products, leukotrienes (LTA<sub>4</sub>, an unstable intermediate, LTB<sub>4</sub>, LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>), lipoxins (LXA<sub>4</sub> and LXB<sub>4</sub> formed upon LXA<sub>4</sub> degradation) and 8-12-15-hydroperoxyeicosatetraenoic acid (HPETE). The CYP 450 pathway involves two enzymes, CYP450 epoxygenase and CYP450  $\omega$ -hydroxylase giving rise to epoxyeicosatrienoic acid (EETs) and 20-hydroxyeicosatetraenoic acid (20-HETE) respectively. Anandamide pathway comprises the FAAH (fatty acid amide hydrolase) to produce the endocannabinoid, anandamide [26–28].

Arachidonic acid may additionally undergo non-enzymatic reactions. Studies proved that the exposure of carbon tetrachloride (CCL<sub>4</sub>) to rats to mimic the oxidative stress and as an induction of lipid peroxidation state *in vivo*, leads to the formation of PGF<sub>2</sub>-like compounds called isoprostanes and other compounds such as nitroeicosatetraenoic acids. Arachidonic acid autoxidation by reactive oxygen species (ROS) and reactive nitrogen species (RNS) are also examples of non-enzymatic oxidative metabolism [29,30].

Arachidonic acid metabolism and enzymes expression usually vary from cell to cell and from tissue to another according to various factors; consequently, the level and type of biosynthesized eicosanoids will differ in each case. It was reported that bone

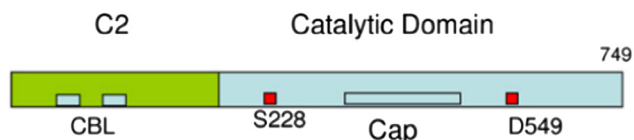
marrow macrophages differ from peritoneal macrophage responses regarding generated eicosanoids quantities and specificity. One more factor that causes this variation is the state of the cell whether it was stimulated or in resting phase. In normal cell state, eicosanoids are generated in very minute amounts and subsequent up regulation can only occur following an inflammatory stimulation [31].

The complexity of eicosanoid biosynthesis lies in the cell-cell interaction, where a donor cell has to transfer its unstable intermediate e.g. PGH<sub>2</sub>, LTA<sub>4</sub> to another recipient cell to trigger the latter for eicosanoids biosynthesis. The single donor cell should have all the necessary enzymes to produce eicosanoids while the recipient cell has not to have all the required enzymes for AA release. Hence, for initiation inflammation or tissue injury, at least two cells in the injured tissue must have the complete enzyme cassette to initiate eicosanoids production. Accordingly, eicosanoids are described, as mentioned above, as local hormones due to their autocrine and paracrine action. Adding to the complexity of the trans cellular interactions, the AA intermediate metabolites are lipophilic with short half-life time (90–100 s) and require some other mechanisms to be translocated [32]. These facts are in line with studies since 1985 by Dahinden et al. [33] who revealed that exogenous LTA<sub>4</sub> stabilized by albumin is uptaken by bone-marrow mast cells to produce sulfidopeptide LTC<sub>4</sub>. Later on, it was found by Dickinson et al. [34] that a group of special proteins called fatty acid binding proteins (FABP), specific for each cell type, are responsible for increase of AA intermediates export via their stabilization and lengthening their half-life time by up to 30 min at 4 °C.

Though eicosanoids have a short half-life time, they contribute to many biological activities in paracrine or autocrine manner. They have crucial dual role, regulating innate immunity and inflammation resolution through binding to G protein coupled receptors located on other cells. First, once tissues are inflamed or infected, beside pain and swelling, AA metabolites amplify the inflammatory signals to recruit leukocytes, pro-inflammatory cytokines, and immune cells to help in pathogens resistance and clearance. Second, they balance the induced inflammatory signals by producing resolving metabolites to act as host protection, since inflammation exerts a threatening action if it is not controlled in an appropriate time manner [23]. COX-1 induced eicosanoids were shown to be lethal when produced continuously. Von Moltke et al. [35] was able to prove that eicosanoids can be lethal, as COX-1-mediated prostaglandins generation were shown to be responsible for vascular leakage and mortality in mice model. Another example of homeostasis regulation, COX-1-mediated TXA<sub>2</sub> production regulates platelets aggregation while COX-2-mediated PGI<sub>2</sub> release inhibits platelets aggregation and promotes vasodilation. Imbalance between levels of PGI<sub>2</sub> and TXA<sub>2</sub> could elevate the risk of cardiovascular diseases. On these terms, physicians prescribe aspirin as a cardio protectant to inhibit COX-1 enzyme and subsequent inhibition of TXA<sub>2</sub> [23].

Lipidomics and lipid profile which can be described in eicosanoids' level assessment is an effective way to identify the severity of disease and its progress [36]. Heading us to personalized medicine to select the right treatment in case of disease-associated inflammation [23], it is important to take into consideration the case of influenza A virus where resolution is not for host benefit as PGE<sub>2</sub> causes apoptosis of macrophages which assist in virus replication [37].

PLA2 is up-regulated once triggered by same receptor that leads to inflammasome activation, prompting the class switching of eicosanoids from prostaglandins to lipoxins to reprogram cells from the pro-inflammation and inflammasome activation to resolution [38]. This mechanism can be enhanced by acetylated COX-2 and FLAP (5-lipoxygenase-activating protein, is a constitutively



**Fig. 4.** cPLA2 enzyme structure showing two domains: a catalytic domain (alpha/beta hydrolase embodying a cap region) with a catalysis pocket containing Ser-228 and Asp-549 and a C2 domain [embodying three calcium binding loops (CBL) forming an anion charge on the domain] [19].

expressed protein that serves the docking of AA with 5-LOX active site as well as the co-localization of both 5-LOX – cPLA2 to perinuclear membrane) to produce lipoxins [23,39].

#### PLA2 enzyme

Phospholipase A2 constitutes a superfamily of enzymes. Three members of this superfamily contribute in eicosanoids production: 1- Cytosolic calcium dependent group IV PLA2 (cPLA2 alpha) that catalyzes the hydrolysis of the phospholipid sn-2 ester bond, generating a free fatty acid and a lysophospholipid. 2- Secretory PLA2 (sPLA2) that is induced by the action of cPLA2, controls magnitude and duration of free AA release, acts in a paracrine manner propagating the inflammatory response to neighboring cells to amplify the signal as well. 3- The last member is cytosolic calcium independent PLA2 that plays a role of homeostasis for cellular function through generation of SPM (specialized pro-resolvins mediators) as well as the reacylation of the free AA in membranes [40].

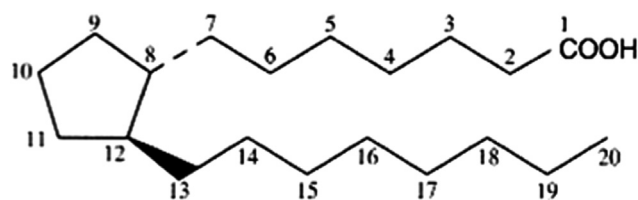
Focusing on the primary enzyme, cPLA2 alpha was mapped on chromosome 1q25 [41]. It has an average mass of 85 KDa and consists of 749 amino acids [42,43]. cPLA2 is composed of two domains, a catalytic domain (alpha/beta hydrolase embodying a cap region) with a catalysis pocket containing Ser-228 and Asp-549 and a C2 domain [embodying three calcium binding loops (CBL) forming an anion charge on the domain] shown in (Fig. 4 [19]) [44]. Upon stimulation, Gq protein coupled receptor activates phospholipase C that cleaves phosphatidylinositol 4,5 bisphosphate (PIP2) to inositol 1,4,5 triphosphate (IP3) and diacylglycerol (DAG). The presence of DAG and  $Ca^{++}$  activates protein kinase C and IP3 opens ER  $Ca^{++}$  channel [45]. The increase in intracellular  $Ca^{++}$  ions levels guides the PLA2 enzyme translocation to the perinuclear membrane through the binding of those ions to the enzyme C2 domain [19,46,47]. The significance of  $Ca^{++}$  ions lies not only in enzyme translocation but also in charge neutralization to ease the way for C2 domain to form hydrophobic interaction with phospholipid [44,48,49] and in conformational change of C2 domain, leading the catalytic domain to be stabilized for binding [50]. Prior to the phospholipase activity, a phosphorylation step is required, mediated by P42 MAP kinase enzyme [51,52]. The first to be discovered was the phosphorylation of Ser-505 following the demonstration of Ser-437, Ser-454, Ser-515, and Ser-727 phosphorylation [53].

### Arachidonic acid enzymatic and non-enzymatic pathways

#### Cyclooxygenase pathway

There are two isoforms of cyclooxygenase (COX) or prostaglandin G/H synthases reacting on free AA and producing ‘prostanoids’ a term used for prostaglandin and thromboxane products as their basic molecule is prostanic acid (Fig. 5).

The first isoform is COX-1, an enzyme constitutively expressed in all tissues that induces an acute inflammation in response to short exposure to lipopolysaccharide (LPS) stimulation and also directs the cell to promote or suppress the leukotrienes (LTs)



**Fig. 5.** Prostanic acid, the basic structure for prostanoids.

biosynthesis [54]. COX-1 prefers coupling and co-localization at perinuclear membrane or ER, with thromboxane synthase, prostaglandin F synthase, and two other prostaglandin D synthases isozymes [55], generating thromboxane A2 (TXA2), prostaglandin F2<sub>alpha</sub>, and prostaglandin D2, respectively. The second, COX-2, is an inducible isoform found in kidney and brain macrophages and is up regulated by inflammatory stimuli as bacterial endotoxin, cytokines, hormones, and growth factors after 3 to 24 h stimulation [56]. COX-2 prefers coupling with prostaglandin I synthase and three prostaglandin E synthases (cPGES, mPGES-1 and mPGES-2) [54] producing prostacyclin (PGI2) and prostaglandin E2, respectively. A new isoform, an enzymatically active splice variant of COX-1, COX-3, has been discovered in the brain and heart though its function is yet to be elucidated [57].

Both cyclooxygenases are structurally alike but COX-2 active site fits with large substrates as result of the substitution of isoleucine for valine at position 523 [58], and both introduce two O<sub>2</sub> molecules to AA to form a cyclic 9,11 endoperoxide, 15 hydroperoxide compound [59]. The structure thus formed, PGG2, is an unstable compound that quickly converts to PGH2 through a hydroperoxide glutathione-dependent reaction [60]. Further enzymatic sequences contribute in the generation of prostanoids (PGE2) (PGD2) (PGF 2<sub>alpha</sub>), prostacyclin (PGI2) and thromboxane (TXA2) as aforementioned, through prostaglandin synthases isomers. The production of prostanoids is dependent on the expression of these enzymes at site of inflammation. For example, platelets make primarily thromboxane A2 (TXA2) whereas endothelial cells generate prostacyclin (PGI2), according to the enzyme content of the tissue under consideration [61].

Cyclooxygenase knockout mice were important in elucidating COX isoforms’ specific functions and roles [62]. Knockout mice have indeed been instrumental in showing the definitive roles of prostaglandins (PG) in early pregnancy events, whereby COX-2, but not COX-1, was required for female fertility, positively affecting ovulation, fertilization, implantation, and decidualization [63]. Conversely, COX-2, but not COX-1, appeared to play a detrimental role in the LPS-induced septic shock syndrome via eliciting production of PGE2, a severe fever mediator [64]. COX-2-deficient mice also helped showing the role of the enzyme in promoting mammary neoplasia and explaining the reduced risk of breast cancer associated with regular use of nonsteroidal anti-inflammatory drugs [65]. Most importantly, COX-2 and PGE2 were implicated in neurodegeneration in several pathological settings, with COX-2 appearing to play an instrumental role in Parkinson disease neurodegeneration via formation of the oxidant species dopamine-quinone [66] and/or induction of c-Jun N-terminal kinases [67]. Recently, Rhai-Bhagal et al. [68] documented the uses and limitations of COX-1<sup>-/-</sup> and COX-2<sup>-/-</sup> animals as suitable model systems for studying brain disorders.

#### Prostanoids receptors

In 1989, TXA2 receptor from human blood platelets had been first isolated and was identified as a protein composed of 343 amino acids and a rhodopsin like seven transmembrane spanning G protein-coupled receptors (GPCRs) (Fig. 6 [69]).

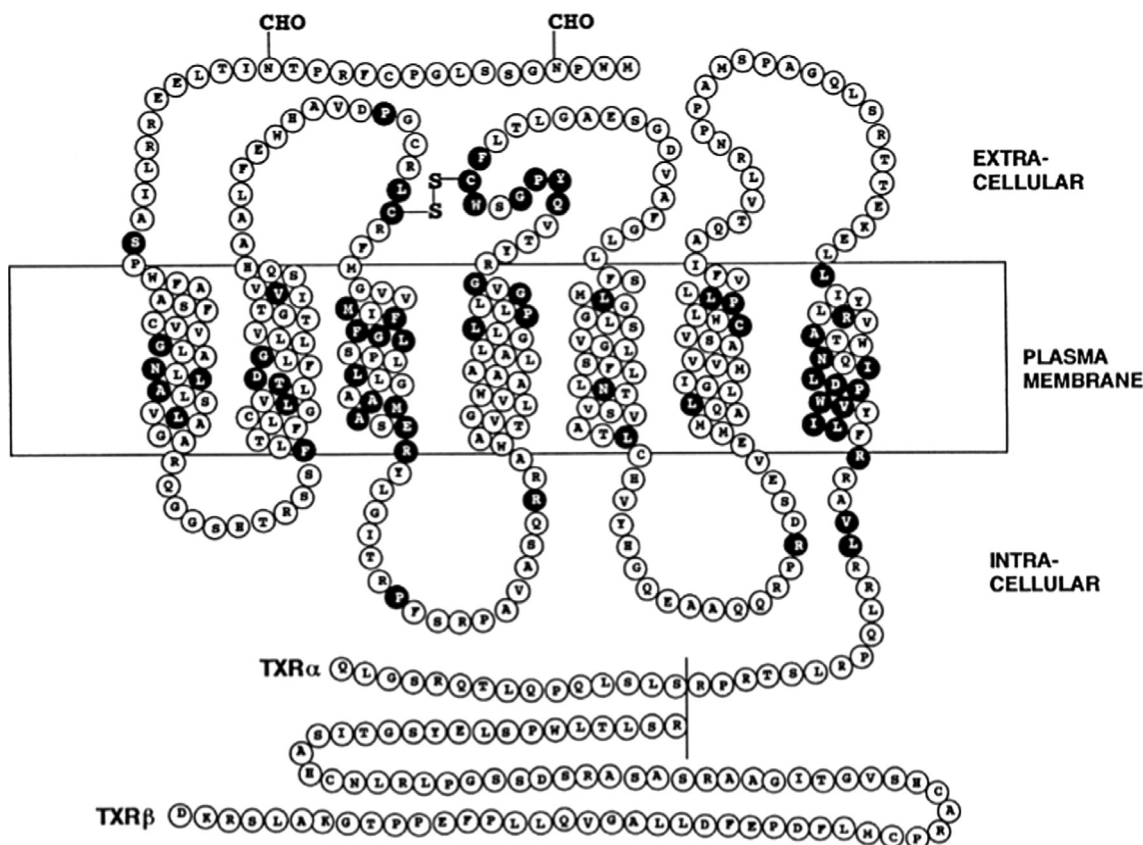


Fig. 6. The secondary structure of the seven transmembrane spanning G protein-coupled prostanoid receptor [69].

Subsequently, in 1991, TXA<sub>2</sub> receptor cDNA was cloned [70] and helped later in identifying the eight types and subtypes of prostanoid (TP) receptors through homology screening of cDNA libraries prepared from mouse and other mammals [71]. The amino acid sequence alignment of those eight types, subtypes and isoform (TP<sub>alpha,beta</sub>, IP, EP<sub>1,2,3,4</sub>, FP, and DP<sub>1</sub> in addition to another GPCR termed chemoattractant receptor-homologous molecule expressed on T helper 2 (TH2) cells (CRTH2 or DP<sub>2</sub>) that responds to PGD<sub>2</sub> but belongs to the family of chemokine receptors [72]) showed the presence of a total of 28 conserved amino acid residues. Aspartic acid (Asp or D) in the second transmembrane domain has been shown in other receptors to be involved in activation of the receptors, by coupling ligand binding to the activation of G proteins [73]. Two cysteine (Cys) residues, one in the first and the other in the second extracellular loop, are also conserved. They are responsible for shaping a disulfide bond for receptors structure stabilization and ligand binding [60].

Thromboxanes (Tx), PGI, PGE, PGF, and PGD metabolites are considered to be ligands for TP, IP, EP, FP, and DP receptors, respectively. A further classification for TP receptor was suggested, including TP<sub>alpha</sub> and TP<sub>beta</sub> subtypes, as well as, EP receptor that was divided into four subtypes EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub>. The EP subtypes responding to the same agonist PGE<sub>2</sub> but differing in their effector function [74]. Each receptor is encoded by a distinct gene and some variants and isoforms are generated through alternative splicing of exons in their C-terminal tails region after the seventh transmembrane domain [60]. Chromosomal localization of the mouse and human prostanoid receptors genes has been reported. The genes encoding the mouse DP, EP<sub>1</sub>, EP<sub>3</sub>, EP<sub>4</sub>, FP, IP, and TP receptors were mapped to chromosomes 14, 8, 3, 15, 3, 7, and 10, respectively [75,76]. The genes encoding the human EP<sub>1</sub>, EP<sub>3</sub>, EP<sub>4</sub>, FP, IP, and TP receptors were mapped to chromosome bands

19p13.1, 1p31.2, 5p13.1, 1p31.1, 19q13.3, and 19p13.3, respectively [77,78].

According to the signal transduction and action of the G-coupled protein, these receptors can be classified into three groups: the relaxant receptors, the contractile receptors, and the inhibitory receptors [79]. The first group includes IP, DP, EP<sub>2</sub>, and EP<sub>4</sub> receptors, the relaxant receptors, which mediate increases in cAMP and induce smooth muscle relaxation. The second group comprises TP, FP, and EP<sub>1</sub> receptors, the contractile receptors, which mediate Ca<sup>++</sup> mobilization and induce smooth muscle contraction. The last group includes the EP<sub>3</sub> receptor, which is an inhibitory receptor that mediates decreases in cAMP and inhibits smooth muscle relaxation [80]. Table 1 details prostanoids major site of production, their cognate receptor(s) and their effector function.

#### Lipoxygenase pathway

The second possibility is for lipoxygenase (LOX) to act on free arachidonate. In this pathway, oxygenation can take place at many different AA positions, an oxygen atom is introduced at C-5, C-8, C-9, C-12 or C-15 through an array of lipoxygenases enzymes numbered according to the oxygen introduced to the carbon atom for example, 5-LOX, 8-LOX, 9-LOX etc., [107,108]. LOX-derived products are of hydroperoxyeicosatetraenoic acid (5, 8, 12, 15 HPETE). 5-HPETE and 15-HPETE are responsible for leukotrienes and lipoxins production. The latter have anti-inflammatory effect [109].

Leukotrienes production require 5-LOX enzyme to produce the 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is converted later to LTA<sub>4</sub> through leukotriene synthase (LT synthase). Two different enzymes exert their action on LTA<sub>4</sub>. One enzyme is LTA<sub>4</sub> hydrolase that uses water molecule to give diol, LTB<sub>4</sub> that induces

**Table 1**  
Prostanoids major site of production, their cognate receptor(s) and their effector function.

Ligand	Major site of production	Receptor	Effector function	Reference
PGD2	Mast cells [81] Central Nervous System [82]	DP1, CRTH2	<ul style="list-style-type: none"> <li>- Vasodilation accompanied by redness and swelling as they inhibit platelets aggregation</li> <li>- Mast cell maturation</li> <li>- Recruit basophils and eosinophil for allergic response</li> </ul>	[83–87]
PGE2	Kidney [88]	EP <sub>1</sub> , EP <sub>2</sub> , EP <sub>3</sub> and EP <sub>4</sub>	<ul style="list-style-type: none"> <li>- Decrease TNF-<math>\alpha</math> expression</li> <li>- Increase IL-10 in macrophage</li> <li>- Uterine contractions</li> <li>- Vasodilation, fever</li> <li>- Neutrophil eicosanoid class switching</li> <li>- Enhancement of neurotransmitter release</li> </ul>	[89–97]
PGI2	Heart and vascular endothelial cell [98]	IP	<ul style="list-style-type: none"> <li>- Inhibit platelets aggregation</li> <li>- Up-regulation IL-10, down regulation of TNF alpha</li> </ul>	[89,93,99–101]
PGF <sub>2</sub> $\alpha$	Reproductive system [82]	FP	<ul style="list-style-type: none"> <li>- Vasoconstriction, bronchoconstriction</li> <li>- Smooth muscle and uterine contraction</li> </ul>	[82,102]
TXA <sub>2</sub> , TXB <sub>2</sub>	Platelets [82]	TP <sub>alpha</sub> TP <sub>beta</sub>	<ul style="list-style-type: none"> <li>- Regulation platelets aggregation</li> <li>- Vasoconstriction, bronchoconstriction</li> </ul>	[103–106]

inflammation via its chemotactic and degranulating actions on polymorphonuclear lymphocytes (PMN). The other one is glutathione S-transferase enzyme that adds a glutathione molecule to generate LTC<sub>4</sub>. Further conversion occurs to LTC<sub>4</sub> with the addition of amino acids to produce peptidoleukotrienes LTD<sub>4</sub> and LTE<sub>4</sub> [60]. BLT1 and BLT2 receptors can recognize LTB<sub>4</sub> and it is mapped on 14q11.2 – q12 and on 14q12, respectively. CysLT1 and CysLT2 receptors can bind to peptidoleukotrienes and those receptors are located on Xq13.2–q21.1 and 13q14.2 [110].

On the other hand, biosynthesis of lipoxins can occur through the following pathways. 1- lipoxins can be carried out by 5-LOX in leukocytes following the action of 12-LOX in platelets [111]. 2- In epithelial cells, lipoxins can be synthesized by the 15-LOX and followed by 5-LOX in leukocytes [112]. 3- Norris et al. [38] was able to demonstrate lipoxins production upon inflammation signals mediated by TLR4 and P2X7 receptor in macrophages. Subsequent to toll-like receptor (TLR4) activation, 1 to 3 percent of oxygenated AA converts to 15-HETE, mediated by the peroxide active site. The generated compound is either secreted and diffused from cells or incorporated in membrane phospholipids via fatty acyl COA ligase. The incorporated 15-HETE can be hydrolyzed by the action of 5-LOX enzyme coupled with cPLA2 and with the assistance of arachidonate 5-lipoxygenase-activating protein (FLAP) necessary for LXA<sub>4</sub> and epi-LXA<sub>4</sub> (lipoxins) production. The last pathway usually is enhanced by the action of aspirin to inhibit COX-2 [113]. Lipoxins act as ligands for G-protein coupled receptor (GPCR) known as ALXR. It was identified as one of formyl

peptide receptors (FPR) family that recognizes the N-formyl peptides derived from bacterial degradation [114]. Its gene is mapped on chromosome 19 [115]. Table 2 details lipoxygenase ligands, major site of production, their cognate receptor(s) and their effector function.

Knockout lipoxygenase-5 mice revealed that not only COX-2 [62] but also LOX-5 is critical for development of the toxic shock symptoms as it contributes to organ dysfunction and failure via leukotriene-mediated up-regulation of adhesion molecules and, consequently, accumulation of inflammatory leukocytes [124]. Data generated using knockout mice additionally indicate a role for 12/15-lipoxygenase in the pathogenesis of atherosclerosis [125] and diabetes [126,127].

#### Cytochrome p450 pathway

Cytochrome p450 (CYP) enzymes contain a heme iron, are expressed mainly in liver and other tissues, and used to eliminate toxins [23]. CYP 450 pathway consists of 2 enzymes cytochrome P450 epoxygenase and P450  $\omega$  hydroxylase [28]. Epoxide derivatives occur by the insertion of an oxygen atom on a carbon attached to one of the double bonds of AA by CYP 450 epoxygenase enzyme. It produces four epoxyeicosatrienoic acid (EET) regioisomers, 5,6-, 8,9-, 11,12-, and 14,15-EET, that function as autocrine and paracrine mediators. Hydroxylation reaction by wp450 hydroxylase produces 16-,17-,18-,19-,20-HETE [28]. Table 3 details

**Table 2**  
Lipoxygenase ligands, major site of production, their cognate receptor(s) and their effector function.

Ligand	Major site of production	Receptor	Effector function	Reference
LTB <sub>4</sub>	Mast cell [116]	BLT1, BLT2, PPAR <sub>alpha</sub>	<ul style="list-style-type: none"> <li>- Neutrophil chemotaxis, vascular permeability, T-cell proliferation</li> <li>- With PPAR<sub>alpha</sub> receptor, inhibit LTB<sub>4</sub> synthesis as negative feedback</li> </ul>	[23,117–120]
LTC <sub>4</sub> LTD <sub>4</sub> LTE <sub>4</sub>	Alveolar macrophage [116]	CysLT1, CysLT2	<ul style="list-style-type: none"> <li>- Respond to inflammation recruiting leukocyte to site of injury</li> <li>- Stimulate bronchoconstriction</li> <li>- Involved in asthma and anaphylaxis</li> <li>- Promote mucus secretion in airway and gut</li> </ul>	[117,121,122]
LXA <sub>4</sub> LXB <sub>4</sub>		ALX	<ul style="list-style-type: none"> <li>- Promote bacterial clearance</li> <li>- Promote wound healing macrophage</li> <li>- Phagocytosis</li> <li>- Efferocytosis</li> </ul>	[123]
8HPETE 12HPETE 15HPETE		PPAR <sub>alpha</sub> PPAR <sub>Gamma</sub>	<ul style="list-style-type: none"> <li>- Binds to peroxisome proliferator-activating receptor to operate as homeostasis for inflammation</li> <li>- Regulate cholesterol levels in liver</li> </ul>	[23,120]

**Table 3**  
CYP450 ligands, major site of production, their cognate receptor(s) and their effector function.

Ligand	Major site of production	Receptor	Effector function	Reference
EETs	Kidney [28]	PPAR <sub>alpha</sub> PPAR <sub>Gamma</sub>	– Vasodilator – Anti-inflammatory and angiogenic functions – Inhibit Na <sup>+</sup> transport	[23,128,129]
HETE	Kidney [28]		– Vasoconstrictor	[130]

**Table 4**  
Anandamide major site of production, cognate receptor(s) and effector function.

Ligand	Major site of production	Receptor	Effector function	References
Anandamide	Brain [136]	CB1 CB2	– Neural generation of pleasure and motivation – Regulates eating and sleeping patterns – Act as pain relief	[137–138]

CYP450 ligands, major site of production, their cognate receptor(s) and their effector function.

#### Anandamide pathway

Is considered the last enzymatic pathway where the biosynthesis of anandamide occurs through enzymatic routes that involve

the presence of high levels of AA and ethanolamine [131,132]. The first route to be discovered is a reversible reaction that is catalyzed by fatty acid amide hydrolase enzyme whose natural reactant is anandamide to produce AA and ethanolamine. In case of tissue damage for example liver hepatectomy, levels of AA and ethanolamine increase dramatically that lead to reverse the action of the fatty acid amide hydrolase to generate anandamide [133]. The latter is considered as a ligand for CB1 and CB2 (cannabinoids receptor) which are responsible for liver regeneration and cell proliferation [134] (Fig. 7 [133]).

The second route of anandamide biosynthesis, where AA is esterified at 1-position instead of the usual 2-position by phosphodiesterase to generate a precursor storing form of anandamide called N-arachidonyl phosphatidylethanolamine [135]. Table 4 shows anandamide major site of production, cognate receptor(s) and effector function.

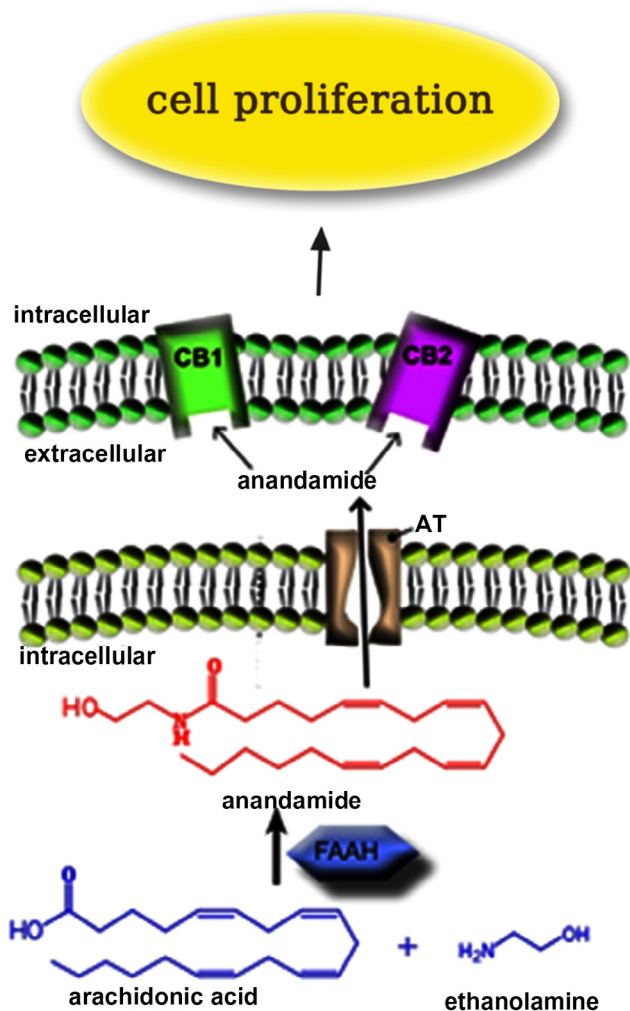
#### Non-enzymatic pathway

As mentioned above, the isoprostanes are derived from the reduction of the endoperoxide intermediate following lipid peroxidation events. Isoprostanes are involved in platelet aggregation, vasoconstriction and contribute in smooth muscles proliferation. Examples of Isoprostanes are: 15-F<sub>2t</sub> Isop, 15-E<sub>2t</sub> Isop and 15-F<sub>2c</sub> Isop. The latter isoprostane is proven to stimulate PGF<sub>2α</sub> receptor to endanger hypertrophy cardiac smooth muscle cells [139].

Also, nitrogen dioxide is able to interact with AA to generate mono-nitrated nitroalkenes such as 9-nitro-eicosa-5,8,11,14-tetraenoic acid (9-AANO<sub>2</sub>), 12-nitro-eicosa-5,8,11,14-tetraenoic acid (12-AANO<sub>2</sub>), 14-nitro-eicosa-5,8,11,14-tetraenoic acid (14-AANO<sub>2</sub>), and 15-nitro-eicosa-5,8,11,14-tetraenoic acid (15-AANO<sub>2</sub>) [140]. Those products are considered to be as marker for oxidative stress and can be used to estimate the role of free radicals in human diseases.

#### Conclusions

This review provides an outline on signals that trigger AA metabolism through enzymatic and non-enzymatic routes yielding a plethora of mediators. Arachidonic acid metabolites play a wide range of physiological roles in human health and diseases, aiding in cell proliferation and tissue regeneration and in the diagnosis of disease progression as well. The review, thus, is a foundation for opening the road to new strategies in disease treatment and diagnosis. It has actually promoted the establishment of plans and procedures to recommend an adequate AA intake to achieve an equilibrium between its inflammatory and resolution effects, supporting healthy immune functions. Also, it has promoted



**Fig. 7.** The biosynthesis of anandamide occurs through enzymatic routes that involve the presence of high levels of AA and ethanolamine, catalyzed by FAAH fatty acid amide hydrolase enzyme. The anandamide is considered as a ligand for CB1 and CB2 (cannabinoids receptor) which are responsible for liver regeneration and cell proliferation [133].

promising projects towards the production of AA at the commercial level for the first time in Egypt, the Middle East and Africa.

### Conflict of Interest

The authors have declared no conflict of interest.

### Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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