I. Introduction
Eicosanoids (from the Greek “eicosa”, for twenty) contain twenty carbon atoms, and are important signaling molecules. They are derived from twenty carbon polyunsaturated fatty acids that are present in the diet, or that are synthesized from essential fatty acids present in the diet. Arachidonic acid (5,8,11,14-all cis eicosatetraenoic acid) is the predominant eicosanoic acid in humans. Almost all eicosanoic acids are esterified at the second position of membrane phospholipids (see figure, page 7), so a major aspect of eicosanoid metabolism is the regulation of cellular phospholipases that catalyze the hydrolytic release of these fatty acid eicosanoid precursors. Once released from their “stored” esterified (membrane phospholipid) state, the intracellular free eicosanoic acids can be converted to a wide variety of eicosanoids, with particular tissue distributions determined primarily by the specificity and quantity of the converting enzymes. The eicosanoid products are released from the cells in which they are synthesized and bind to and activate highly specific receptors on these same cells (autocrine signaling) or on neighboring cells (paracrine signaling). The activated receptors (members of the heterotrimeric G protein coupled family, which also includes glucagon and epinephrine receptors) then stimulate particular cellular processes depending on the nature of the G proteins and their effectors.

A. Nomenclature of eicosanoids
1) Compounds to be considered in some detail
   Prostaglandins (PGs), Prostacyclins (PGIs), and Thromboxanes (TXs)
   Compounds to be mentioned briefly
   Hydroperoxyeicosatetraenoic acids (HPETEs), Hydroxyeicosatetraenoic acids (HETEs), and Leukotrienes (LTs).
2) PGs and PGIs are derivatives of prostanoic acid; TXs are rearranged derivatives of prostanoic acid.
3) Structures of prostanoic acid, PGs, PGIs, and TXs (Figure 1). The abbreviated nomenclature of the prostaglandins (PGs), prostacyclins (PGIs) and thromboxanes (TXs) is based on the fact that they are derivatives of prostanoic acid. PG and TX stand for prostaglandin and thromboxane, additional capital letters symbolize specific substituents or modifications of the prostanoic acid backbone, and numerical subscripts indicate the number of carbon-carbon double bonds in the R₁ plus R₂ side chains. The further subscript “α” in PGF family members indicates that the hydroxyl at position nine is on the same side of the five-member ring as R₁. As shown in the discussion of biosynthetic pathways below, since arachidonic acid is the predominant eicosanoic acid in humans, the overwhelming majority of PGs and TXs in humans are of the “two” series, such as PGF₂α, PGI₂, and TXA₂.

B. Some biologic functions (paracrine and autocrine)
1) PGE₂: Vasodilation; smooth muscle relaxation
2) PGF₂α: Vasoconstriction; smooth muscle contraction
3) PGI₂: Vasodilation; stimulation of cAMP production; inhibition of platelet aggregation
4) TXA₂: Vasoconstriction; reduction of cAMP production; stimulation of platelet aggregation
5) LTC₄, LTD₄, LTE₄: Bronchoconstriction; smooth muscle contraction.
Many eicosanoids, including some listed here, mediate fever, pain, and inflammation. Inflammation is a complex process whose overall function is to destroy, and to repair the damage caused by, invading organisms. The process includes increased leukocyte-endothelial cell (blood vessel) adhesion, increased blood vessel permeability, vasodilation, leukocyte chemoattraction, leukocyte activation, and stimulation of leukocyte production.

II. General Biosynthetic Pathways (Figure 2)
   A. Cyclooxygenase pathway (PGs, PGIs, TXs)
   B. Lipoxygenase pathway (HPETEs, LTs)

III. Cyclooxygenase Pathway (Figure 3)
   A. Nature of substrates - eicosanoic acids
   B. Cyclooxygenase reaction (prostaglandin endoperoxide synthase)

IV. Lipoxygenase Pathway (Figure 4). In leukotriene nomenclature, LT stands for leukotriene, additional capitol letters symbolize specific structural features (different from those of PGs), and numerical subscripts indicate the number of carbon-carbon double bonds. As shown in the figure, since arachidonic acid is the predominant eicosanoic acid in humans, the majority of LTs are of the “four” series, such as LTA₄, LTC₄, LTD₄, and LTE₄.

V. Regulation of Eicosanoid (PG, PGI and TX) Synthesis and Concentration
   A. Substrate production (Figure 5)
   B. Cyclooxygenase activity (Figure 6)
   C. Degradation (Figure 7)

VI. Overview of Eicosanoid Metabolism and Role of Eicosanoids in Platelet Aggregation (Figures 8 and 9)

Figure 1. Structures of relevant eicosanoids
The six principal naturally occurring prostaglandins

8,11,14-Eicosatrienoic acid
5,8,11,14-Eicosatetraenoic acid
5,8,11,14,17-Eicosapentaenoic acid

(arachidonic acid)

(Figure 1, continued)
General biosynthetic pathways

Figure 2. Overview of eicosanoid biosynthesis

Arachidonic acid (AA), the precursor of PGs and LTs, is ingested in the diet or synthesized from the essential fatty acid linoleic acid. The two main branches of AA metabolism are named after their initial enzymes: the cyclooxygenase branch produces PGs (including PGIs and TXAs) while the lipoxygenase branch produces LTs. The concentration of free AA in cells is very low and limits activity of the pathway. Both the cyclooxygenase and lipoxygenase branches compete for the same AA precursor pool, so their activities are mutually interdependent. Drug-induced blockade of the cyclooxygenase branch, for example, increases AA availability for metabolism via lipoxygenase ("shunting").

Cyclooxygenase pathway

Cyclooxygenase, a membrane-bound microsomal enzyme, catalyzes a two-step reaction to produce the unstable endoperoxide PGH$_2$ via the transient enzyme-bound intermediate PGG$_2$. Both oxygen atoms introduced in these steps arise from heme-bound molecular oxygen. Cyclooxygenase is irreversibly inhibited by aspirin, and reversibly inhibited by other Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) such as ibuprofen and indomethacin. PGH$_2$ is converted to other PG subtypes by individual synthases. The type of PG produced by a given cell is probably controlled at the level of synthase transcription.

Two major cyclooxygenase isoforms are currently known: COX I, an enzyme constitutively expressed in all tissues but enriched in platelets, renal collecting tubules, and gastric mucosa, and COX II, with a more limited tissue distribution but enriched in endothelial cells and in cells mediating fever, pain, and inflammation. COX II activity is transcriptionally regulated by mitogens such as cytokines and Platelet Derived Growth Factor, and transcriptionally repressed by steroids such as dexamethasone and hydrocortisone. Reduction of COX II transcription may thus be one of the mechanisms by which steroids can reduce inflammation. Transcription factor binding sites in the COX II gene enhancer region include those for Jun, CREBP, other factors activated by MAPK pathways, SP1, and the glucocorticoid receptor. In contrast, the only sites identified in the COX I gene enhancer region are those for SP1.
Figure 3. Outline of cyclooxygenase-mediated biosynthetic pathways
Formation of leukotrienes.
5-lipoxygenase catalyzes two reaction steps: conversion of AA to 5-hydroperoxyeicosatetraenoic acid (5-HPETE), and dehydration of 5-HPETE to form LTA₄. LTA₄ can be hydrated to form LTB₄, or conjugated with glutathione to form LTC₄. LTD₄ is formed from LTC₄ by γ-glutamyl transpeptidase. Subsequent loss of glycine creates LTE₄. The cysteine-containing LTs, especially LTD₄ and LTE₄, are potent bronchoconstrictors of great importance in asthma and allergic responses. 5-lipoxygenase inhibitors such as zileuton and leukotriene receptor antagonists such as montelukast (“Singulair”) are available, but there is much debate concerning their clinical efficacy in some conditions. For example, many asthma patients do not respond to these drugs.

Figure 4. Lipoxygenase pathway.
Figure 5. Release of arachidonic acid from membrane lipids. The binding of a stimulus to its receptor activates pathway 1 or 2.

AA is stored predominantly in esterified form, at the 2 position of membrane phospholipids, usually choline-, inositol-, or ethanolamine-phosphoglycerides.

The lipase that releases AA from membrane phospholipids of most cells is regulated. For example, Phospholipase A₂, which removes AA in a single step from the 2 position of choline phosphoglycerides, can be activated by phosphorylation. (Both cAMP-dependent and cAMP-independent phosphorylation mechanisms are known.) Phospholipase A₂ is inhibited by lipocortins, proteins which are synthesized in response to certain steroids such as cortisone (usually administered as the more stable hydrocortisone or dexamethasone). Thus, steroids limit PG- and LT-mediated inflammation in part through inhibition of AA mobilization. AA is released from platelet membranes by a different pathway: phospholipase C cleaves the inositol moiety from inositol phosphoglycerides, leaving diacylglycerol, which is attacked by diacylglycerol- and monoacylglycerol-lipases to yield free AA.
Aspirin can diffuse across cell membranes, probably in a non-dissociated form. Its plasma levels peak 30-40 minutes after ingestion and the drug has a half life of about 20 minutes. In humans aspirin acetylates COX I at serine 529 and COX II at serine 516.

Aspirin has been prescribed traditionally to alleviate fever, pain, and inflammation. However, it is now known that COX II, the cyclooxygenase enriched in endothelial cells and cells mediating these processes, is much less sensitive to aspirin inhibition than is COX I. As a result, effective aspirin inhibition of COX II often requires drug levels that result in serious side effects due to the concomitant more severe inhibition of COX I. For example, as discussed below, aspirin stimulates bleeding. In order to reduce or eliminate such side effects, use has been made of the known differences in structure between COX I and II to design COX II-specific inhibitors known as COXIBs. Two COXIBs that have been widely used are Celebrex and Vioxx.

**Figure 7. Eicosanoid degradation**
**Figure 8. Overview of eicosanoid metabolism.** Eicosanoids are produced from fatty acids released from membrane phospholipids. In humans, arachidonic acid is the major precursor of the eicosanoids, which include the prostaglandins, leukotrienes, and thromboxanes. Note the inhibitory effects of glucocorticoids on the generation of arachidonic acid, and of NSAIDs on cyclooxygenase activity.
Figure 9. Role of eicosanoids in platelet aggregation. Mature platelets express only COX I while endothelial cells express both COX I and COX II. The major agonist for endothelial cells appears to be blood flow (hemodynamics) which activates cell surface mechanoreceptors. Such receptors not only activate phospholipases, but also upregulate COX II expression. COX II is the major ‘source’ of PGI$_2$ in endothelial cells. Two of a variety of agonists for platelets are thrombin (which binds to a G protein-coupled receptor) and collagen (which binds to a member of the integrin family of cell surface integral membrane proteins). Platelets become exposed to collagen in the extracellular matrix of ruptured blood vessel walls.

A heart attack can result when a coronary artery is blocked. Prostaglandins affect the circulation through their effects on vascular smooth muscle and platelets. Platelets are anucleate cell fragments in the blood that act as molecular first aid kits for damaged blood vessel walls. Platelets coat injured vascular endothelial cell surfaces, releasing wound-healing and immune-activating molecules. The platelet aggregate can become a focus for serious blockage. Prostacyclin (PGI$_2$) is produced through COX II by healthy, intact vascular endothelial cells and inhibits platelet aggregation. Platelets produce their own aggregation-promoting signal, thromboxane (TXA$_2$) through COX I. The local ratio of prostacyclin to thromboxane is one of the factors that control
the degree of platelet aggregation and, secondarily, the number of immune cells recruited by fac-
tors released from platelet aggregates. Thus, keeping blood vessels clear requires a high ratio of
prostacyclin to thromboxane. Aspirin is a drug used to achieve this goal. Aspirin is an irreversi-
ble inhibitor of cyclooxygenase. This enzyme is required for both prostacyclin and thromboxane
synthesis. Low doses of aspirin on a daily or alternating day basis can effectively inactivate
platelet thromboxane synthesis while leaving vascular endothelial cell prostacyclin synthesis
relatively intact. Endothelial cells can synthesize new cyclooxygenase protein while platelets,
having no nuclei, cannot. (The platelet population is renewed about every 10 days from bone
marrow precursors.) Thus, a low dose of aspirin permanently reduces thromboxane synthesis in
exposed platelets but only transiently blocks prostacyclin synthesis in exposed vascular endothe-
lial cells.

The fact that low dose aspirin selectively inhibits COX I (and thus platelet TXA₂ production),
enhances its effect such that for all practical purposes TXA₂ synthesis is nearly completely sup-
pressed after one week. As noted above, the PGI₂/TXA₂ ratio is just one of the factors regulating
blood clotting (hemostasis). TXA₂ is not absolutely required for hemostasis, but low TXA₂ levels
increase bleeding time and significantly reduce atherothrombosis.

Aspirin stimulates bleeding by inhibiting blood clotting. The initial investigation of the effec-
tiveness of low dose aspirin in preventing heart attacks, the so-called “Physicians’ Health Study”
(1982-1988) used 325 mg aspirin (a single tablet) every other day and resulted in about a 30%
reduction in heart attacks in men (albeit a low-risk group, who had had less than a 1% annual
risk for such an event before treatment). Women were not studied. Further investigations showed
that 100 mg aspirin every other day was just as effective, but reduced the risks of gastro- and
non-gastrointestinal bleeding, hemorrhagic strokes and peptic ulcers. A recent investigation of
the effectiveness of low dose aspirin in preventing heart attacks, the so-called “Women’s Health
Study” (1993-2004) used 100 mg aspirin every other day, also in a low risk population, and re-
sulted in no reduction in heart attacks in women under the age of 65. Men were not studied. Sur-
prisingly, in the Physicians’ Health Study, low dose aspirin had no effect on the risk of stroke,
while in the Women’s Health Study the stroke risk was reduced by 17%.

A possible reason for the different responses of men and women in these studies may be that
women are less sensitive than men to heart attack prevention by aspirin, and therefore require
doses higher than 100 mg every other day. This possibility has been supported by a different in-
vestigation in which daily 100 mg dosages reduced rates of heart attacks in both men and
women.
An eicosanoid related chronology.
From general aspirin usage in 1899, to the withdrawal of Vioxx in 2004, the eicosanoids have been of major interest to both physicians and patients. Some relevant dates are:

- **Prehistory to the 19th Century C.E.**: Extracts of various herbs or plants, such as willow bark, used to treat fever, pain, and inflammation. Most extracts contain salicylates.
- **1897**: The acetylated form of synthetic salicylic acid prepared by the Bayer company in Germany.
- **1899**: Common aspirin usage
- **1930s**: Prostaglandin discovery
- **1950s**: Prostaglandin structural elucidations
- **1960s**: Prostaglandin biosynthetic pathways
- **1971**: Report of mode of action of aspirin as a cyclooxygenase inhibitor
- **1970s**: Thromboxane and leukotriene discovery
- **1991**: COXII identification
- **1994**: COXI structural elucidation
- **1996**: COXII structural elucidation
- **1999**: Celebrex release
- **1999**: Vioxx release
- **1998**: Singulair release
- **2004**: Vioxx withdrawal