Cell Membranes, Endothelia, and Atherosclerosis – The Importance of Dietary Fatty Acid Balance

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Abstract

Atherosclerosis begins at the fragile blood vessel endothelia, which may be the Achilles' Heel of the circulation. The endothelia are thin cell sheets vulnerable to injury, yet from their cell membranes come fatty acid metabolites (prostanoids, other eicosanoids) that coordinate vessel wall homeostasis and help protect against atherosclerosis. Oxidation products of fatty foods threaten endothelial integrity, and dietary fatty acids protected by dietary antioxidants can slow or perhaps even reverse atherosclerosis.

Eicosanoid messenger molecules, generated from 20-carbon fatty acids associated with membrane phospholipids, are among the major factors ensuring vessel wall homeostasis. Among their functions is to help control blood vessel tone, retard platelet aggregation, and discourage white blood cells and platelets from adhering to the vessel wall. Eicosanoid balance is delicately poised between anti-inflammation and pro-inflammation, with the dietary intake of fatty acids and antioxidants influencing this balance. The 2-series metabolites from arachidonic acid are quantitatively the most abundant, while the 1-series (from DGLA) and the 3-series (from EPA) counterbalance the 2-series and enhance atherosclerosis protection and heart disease prevention.

Dietary long-chain, unsaturated fatty acids also benefit the blood vessel wall by enhancing cell membrane fluidity and regulating membrane receptors. Inconsistent clinical results with the dietary fatty acids may be related to dose. High doses of any one of the n-3, n-6, or n-9 FAs may create deficiencies of others. For consistent, effective nutritional support against atherosclerosis, a diversified intake of fatty acids should only be undertaken in conjunction with a generous intake of antioxidants.
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Atherosclerosis continues to be the major killer disease of Western society. After intense research efforts that have spanned decades, a consensus is emerging that the major causative factors in atherosclerosis are oxidized breakdown products of dietary fats.[1-3] But fats cannot simply be excluded from the diet, most especially because of the essential fatty acids. These function much as vitamins function, fitting the classic deficiency yardstick for vitamins.[5] This review is an effort to sort out the potential contributions of the essential fatty acids to the prevention and management of atherosclerosis through a critical assessment of their respective roles in blood vessel homeostasis. Because the literature on atherosclerosis is so massive, only initiation and the earlier stages of progression will be considered in detail.

Atherosclerosis initiation and early progression is almost invariably a result of abnormal interactions between circulating oxidized lipids and the blood vessel walls.[1-6] White blood cells (monocytes, macrophages) and platelets tend to become involved subsequent to the initial damaging event. The pivotal target zone for atherosclerosis initiation is the endothelial system that lines all blood vessels.[3,4] Situated at the vital interface between the circulating blood and the deeper layers of the vessel wall, the endothelia are thin sheets of flattened cells. Being only one cell thick, the endothelial sheet is delicate and is extremely vulnerable to physical or chemical attack. The fragility of the vessel wall endothelia may be the Achilles' Heel of the circulation as a whole.

Selected Abbreviations and Acronyms	
FA	= Fatty acid
EFA	= Essential fatty acid
PUFA	= Polyunsaturated fatty acid
Eicosanoids	= Metabolites of cell membrane fatty acids; includes prostanoids (PGs, Txs) + peroxide metabolites + leukotrienes
Prostanoids	= Prostaglandins (PGs) and thromboxanes (Txs), metabolites of cell membrane 20-carbon fatty acids via cyclo-oxygenase
OxLDL	= oxidized low-density lipoproteins
OxChol	= oxidized cholesterol derivatives
n-6	= omega-6 fatty acid, first double bond at the 6th carbon after the methyl group
n-3	= omega-3 fatty acid, first double bond at the 3rd carbon after the methyl group
n-9	= omega-9 fatty acid, first double bond at the 9th carbon after the methyl group
AA	= arachidonic acid, 20 carbons, 4 double bonds, omega-6. (C20:4 n-6) Precursor to the 2-series prostanoids
DGLA	= dihomo-gamma-linolenic acid, 20 carbons, 3 double bonds, (C20:3 n-6) omega-6. Precursor to the 1-series prostanoids
GLA	= gamma-linolenic acid, 18 carbons, 3 double bonds, omega-6 (C18:3 n-6)
LA	= linoleic acid, 18 carbons, 2 double bonds, omega-6. Precursor to (C18:2 n-6) the eicosanoid 13-HODE via 15-lipoxygenase
EPA	= eicosapentaenoic acid, 20 carbons, 5 double bonds, omega-3. (C20:5 n-3) Precursor to the 3-series prostanoids
DHA	= docosahexaenoic acid, 22 carbons, 6 double bonds, omega-3 (C22:6 n-3)
OA	= oleic acid, 18 carbons, 1 double bond, omega-9 (C18:1 n-9)
PG, Tx	= prostaglandins, thromboxanes (prostanoids)

Endothelial Dysfunction is Linked to Atherosclerosis

Endothelial dysfunction or disruption has been linked to diverse vascular abnormalities, including vasospasm, intimal hyperplasia, coagulopathies, and microcirculatory dysfunctions (see Figure 1), in addition to the "Big 3"—diabetes, hypertension, and atherosclerosis.[1-4]

The first detectable stage in atherosclerosis is a slightly raised, fatty "streak" in the arterial wall.[1] Among Western populations, these can be found in most individuals beginning as early as the teenage years. The fatty streak is much like a localized inflammatory response to the "wounding" of a vessel wall.[2] But for a fatty streak to develop, the vessel endothelium must first have been breached by some injurious agent.
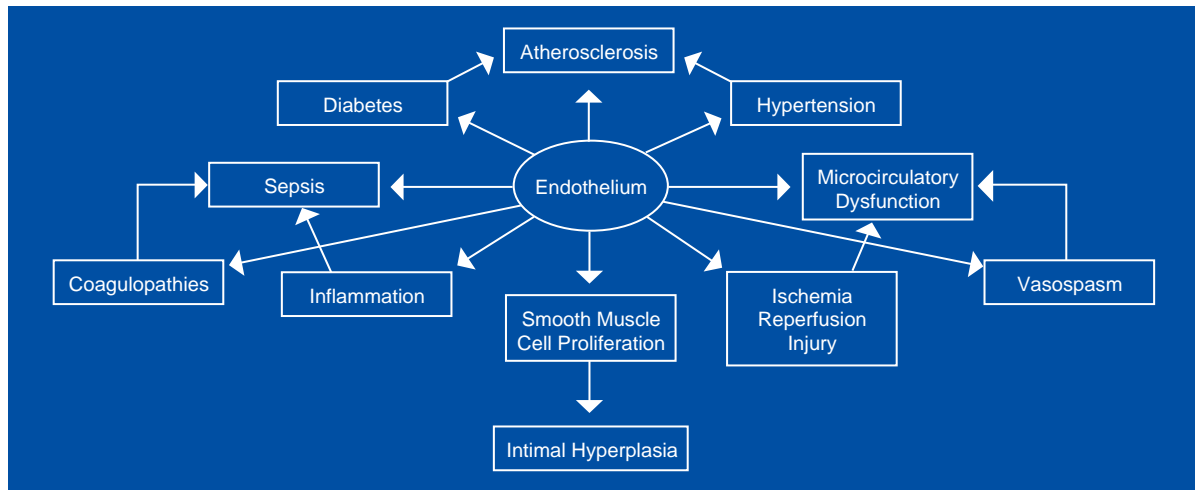
A small gap between adjoining endothelial cells, or the stripping away of a stretch of endothelium ("denudation") can allow oxidized LDL or other oxidants into the vessel wall.[1] The exposed subendothelial wall is "sticky," and as the sheet is interrupted white cells begin to approach the area and attach by way of adherence receptors.[6] An inflammatory focus is initiated, and after inflammatory cytokine messengers are produced, smooth muscle cells begin to proliferate out of control. The inflammatory cascade worsens, and eventually a chronic inflammatory, atherosclerotic lesion is established in the vessel wall.[1,2]

Vulnerable as they may be, the endothelia do possess biochemical defense mechanisms, which fall into two major

FIGURE 1.

classes: antioxidant defenses, and "prostaglandin" (really prostanoid) cascades that originate from membrane fatty acids. These two classes of endothelial defense have extensive biochemical-metabolic overlap, and are tightly intertwined in their support of blood vessel homeostasis. Since antioxidant defense mechanisms are more generally understood at this point, herein a more detailed emphasis will be placed on the fatty acid defenses.

Horrobin recently published[5] a well-reasoned proposal that "the causes of vascular disease are abnormal membrane phospholipid concentrations of the 20-carbon and 22-carbon essential fatty acids (EFAs) of the n-6 and n-3 series." He makes a persuasive case that abnormal levels of these fatty acids are predictive of death from coronary heart disease (CHD), by demonstrating that in controlled trials provision of the EFAs or their longer-chain derivatives reduced mortality. The likely focus for the protective actions of the EFAs is the vessel endothelia.

Endothelial Homeostasis and Defense Mechanisms

The 5,000 square meters of endothelia that line the blood vessels of the adult human are 1% of the body's mass and function virtually as an organ.[4] They make up a vessel endothelial system that manages blood vessel homeostasis, provides a thin line of defense for the deeper segments of the vessel wall, and after damage has occurred, coordinates healing of the wall. When functioning optimally, the endothelial system is intensely active. It maintains a moment-to-moment, delicately-poised, homeostatic balance in the blood vessel networks. The system functions to control vessel tone and to manage inflammatory cascades initiated in response to injury.

By producing and releasing multiple messenger factors, the endothelial sheet maintains (a) a state of relative vasodilation of the vessel wall, mediated by the smooth muscle; (b) a state of "anti-adherence" to white blood cells, delicately balanced and able to quickly shift to pro-adherence in case of injury; (c) relative growth inhibition of the smooth muscle cells deeper in the wall, able to shift to growth promotion in response to injury; and (d), an anti-coagulation state of the platelets, also poised to quickly shift to procoagulation once the vessel wall becomes damaged.[3, 4]

The endothelial system must continually be in a state of readiness to cope with threats to its integrity—once its thin sheet has been breached, the deeper layers of the vessel wall lie naked and vulnerable. Unfortunately, modern living features an array of stressors that can injure, damage, or poison the endothelial system.

Life Stressors and Endothelial Protective Mechanisms

Damage to the blood vessel endothelium is more or less an obligatory step in the development of atherosclerotic vascular disease. Sources of damage to the arterial endothelium include:

Oxidized serum lipids. The circulating lipoproteins in undisturbed native form do not appear to be significantly irritant or toxic to the endothelium; rather, oxidized low-density lipoproteins (OxLDL) and oxidized derivatives of cholesterol (OxChol) are the main offenders.[1-4] Oxidative damage to the endothelial sheet lowers endothelial barrier function and increases access of toxic agents to the underlying smooth muscle cells. As the subendothelial layers become involved, control over the growth of the smooth muscle cells becomes lost, and their proliferation drives the development of an atherosclerotic lesion.

Turbulent blood flow.[3,4] The endothelial cells are mechanosensors, and can transduce the physical forces produced by the blood flow into biochemical signals to which the vessel wall can respond. Both rapid and longer-term responses are engineered by the endothelia. A disrupted zone of endothelium can create blood flow turbulence, which then excerbates damage at this vulnerable site or elsewhere.

Angioplasty and vessel grafting.[3,4] These procedures both result in endothelial damage, the former as a direct result of the mechanical "artero-rooter" invasion of the vessel and the latter due to deterioration of the endothelium during the course of its removal and re-placement. In the second case, atherosclerosis in the grafted vessel commonly occurs; sometimes the grafted vessel heals itself, and sometimes an "accelerated atherosclerosis" occurs that quickly results in re-stenosis.

Inflammatory mediators.[1-4] A variety of these are carried in the circulating white cells and in platelets, to be released in response to appropriate stimulation. This is part and parcel of the normal inflammatory cascade, but even under favorable conditions the cascade is finely poised. In a situation of oxidant excess or prostanoid imbalance, pro-inflammatory mediators can escape homeostatic control and create havoc in the vessel wall.

Circulating "free radical" and other oxidant toxins.[7-10] Foremost among these are the myriad oxidant substances from cigarette smoke, but also included are pesticides and herbicides, solvents, and many pharmaceuticals. Oxygen radicals produced endogenously from respiration—superoxide, hydrogen peroxide, hydroxyl—also can cause damage when they escape intrinsic antioxidant defenses and accumulate to excess. Auto-oxidation products of circulating catecholamines, produced from ongoing emotional stress, may add to the free radical/oxidant burden of the circulating blood.

Hyperglycemia.[8] Glucose circulating in the blood can undergo an oxidation-type reaction with amino groups of proteins. This results in highly crosslinked AGE (advanced glycosylation endproducts) which can gum up metabolism. AGE have strong oxidant character, and have been linked to the production of vasoconstrictive prostanoids and oxygen free radicals. Diabetics characteristically carry high blood AGE, and endothelial dysfunction is an intrinsic feature of diabetic blood vessel disease.

Nutrient imbalances.[3, 9,10] Probably the most severe nutrient imbalance for the circulation is homocysteine buildup. Homocysteine is a sulfur amino acid, an oxidized metabolite of methionine. As an essential amino acid, methionine is a precursor molecule for many metabolic pathways, and is continually in a metabolic balance with homocysteine. The latter can be recycled back to methionine, but only if vitamins B6, B12, and folate are available. In the absence of all or any of these required metabolic cofactors, homocysteine

builds up in the circulating blood and becomes toxic to the vessel wall.

Other toxins or circulating irritants. These include alcohol, heavy metals, infectious agents that reach the circulation, toxins produced by infectious agents located elsewhere (as in the gut), and a myriad of natural or manufactured allergenic substances.

The health of the blood vessel wall is a net outcome of complex interactions between the various toxic and protective factors that impinge upon it. Endothelial protective factors fall into the following classes:

Intrinsic antioxidant factors.[3,7,10-17] Healthy endothelial cells carry a substantial complement of antioxidant factors, including ascorbate (C), tocopherol (E), and glutathione, as well as the enzymes superoxide dismutase, catalase, and those that synthesize, utilize, and recycle glutathione. Selenium, an essential mineral cofactor for the enzyme glutathione peroxidase, in conjunction with glutathione and vitamin E helps keep down the levels of peroxides that threaten the endothelium. Zinc is both an antioxidant enzyme cofactor and a stabilizer of cell membranes. It is vital to endothelial integrity, and may also protect against the potent, pro-inflammatory PAF (platelet activating factor). Copper, an essential cofactor for superoxide dismutase (SOD), is protective at low levels but can become pro-inflammatory at higher levels. Extracellular SOD occurs at very high levels in the healthy blood vessel walls.

Magnesium is not known as an antioxidant cofactor, yet its deficiency can exacerbate atherosclerosis progression.[10] Endothelial cells when depleted of magnesium produce peroxides that can result in the cells' self-destruction. Iron buildup in the blood, to a point where it exceeds the buffering capacity of transferrin and other proteins, can facilitate a classic Fenton Reaction.[10] This reaction can transform the relatively benign hydrogen peroxide into hydroxyl radical, the most reactive of the known oxygen radicals. Superoxide radicals can cause the release of stored iron and exacerbate the Fenton reaction.

Membrane phospholipids and associated essential fatty acids.[5,56] Every living cell has to maintain homeostasis internally while being responsive to stresses or messages from the outside. The outermost membrane (called the cell membrane) is a sorting-switching-signalling complex, a master switch for the cell. The cell membrane controls (a) the entry of nutrients into the cell and the exit of waste endproducts; (b) ion movements, acidity-alkalinity, and oxidation-reduction (redox) state inside the cell; (c) the passage of molecular messages into and out of the cell; (d) shape changes, expansion, and mobility of the cell; and (e) the cell's capacities to identify, communicate with, and associate with other cells.

The membrane components which manage these master switch activities are the ion pumps, transport molecules, enzymes, and receptors, and all are mostly protein in nature. For the membrane to function at its best, each of these molecules must be provided with just the right mix of fluidity, charge, and redox state (antioxidation/oxidation balance).[56] Phospholipids and a small amount of cholesterol make up the membrane matrix, and fatty acids associated with the phospholipids help integrate these proteins into the membrane. As a result, signalling both from the outside of the cell inward and from the inside of the cell outward is subject to control by the phospholipids and their associated fatty acids.

Prostanoids (prostaglandins and thromboxanes).[5,19-20] These are a class of physiological effector molecules chemically related to the 20-carbon prostanoic acid, which technically is 7-[2-(1-Octanyl)cyclopentyl] heptanoic acid. The term "prostaglandin" was coined for these substances because their activity was first noted in the prostate gland and first characterized in semen.[20] "Prostanoids" is a term that includes both the prostaglandins

(PG) and the thromboxanes (Tx), which tend to have opposing biological actions. The broader term "eicosanoids" encompasses peroxides and leukotrienes as well as PGs and Txs, all derived from cell membrane fatty acids.

Endothelium-derived relaxing factor (EDRF), and nitric oxide (NO).[3,4] These are small molecules that share many of the vasoactive properties of the PG_{I2}/prostacyclin, since they relax smooth muscle and inhibit platelet aggregation. The best-understood relaxing factor is nitric oxide (NO), which is biosynthesized by the enzymatic conversion of l-arginine. A minimum of 5 cofactors are required for this pathway, including nicotinamide adenine dinucleotide phosphate (NADP), tetrahydrobiopterin, flavin adenine nucleotide (FAD), flavin mononucleotide (FMP), and heme. NO acts by stimulating cell membrane guanylate cyclase, which then raises cyclic GMP inside the cell.

NO does not carry all EDRF activity. Other metabolites of NO such as thiols (sulfhydryls, cysteine-derived) appear to have EDRF activity, and EDHFs (endothelium-derived hyperpolarization factors) such as acetylcholine and other chemical transmitters account for some of the rest. Also, recent findings suggest that NO is not unconditionally protective for the endothelia—toxic influences can convert NO into a potentially toxic molecule. EDRF/NO activity can be protected by the antioxidant enzyme superoxide dismutase, but is subject to destruction by oxygen free radicals.

Longer-term regulators of endothelial activity.[3,4] These include (a) the endothelins, (b) locally-produced renins and angiotensins, and (c) bradykinin. All three of these classes of peptide regulatory substances act mainly through the prostanoid cascades of the endothelia.

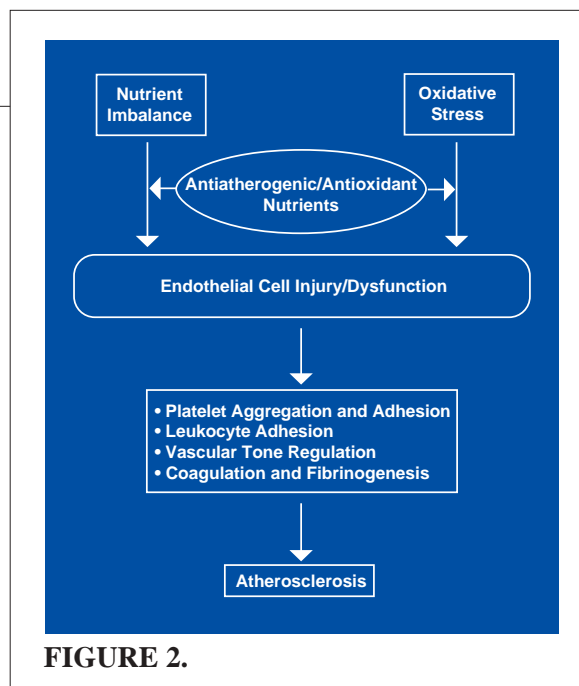Under optimal conditions, along every endothelial segment a delicate balance is main-

FIGURE 2.

tained between the fluxes of prostacyclins versus thromboxanes, and between NO and oxygen free radicals, and with other factors so as to maintain a healthy vascular tone. Oxidative attack or other events that disrupt this complex "yin-yang" equilibrium can lead to blood cell (platelet-monocyte-neutrophil) adherence, release of a variety of vasoconstrictive factors,[4] and a shift away from homeostasis towards potentially pathophysiologic consequences.[18-23]

Oxidized Lipids Trigger Atherosclerosis

While any of the aforementioned sources of endothelial damage can exacerbate pre-existing damage and thereby speed atherosclerosis progression, a growing body of evidence indicts oxidant/free radical agents as the major initiators of vessel wall damage.[1-4] The most common sources of such oxidants/free radicals are foods—frying oils, precooked meat products, dried dairy and egg products, margarine and butter, and any food with substantial content of rancid fats. As they reach the bloodstream, oxidized food derivatives pose a direct threat to the vessel endothelia (see Figure 2).

However minor and localized the injury, any interruption of the endothelial cell sheet is likely to reduce its effectiveness in protecting the deeper vessel wall.[3,4,21-24] Among the major agents threatening the endothelia are the polyunsaturated fatty acids (PUFA), because they are so easily oxidized.[25-27] When the longer-chain PUFA are ingested at high doses, it is unlikely any amount of added antioxidants will totally guarantee against rancidity. Substantial dietary intakes of these highly reactive fatty acids, without the requisite protection against oxidation, sets the stage for a paradoxical toxicity from these nutrients.[28,29]

Much the same is true for the ingestion of oxidized cholesterol derivatives versus pure cholesterol.[3] The pure material does not appear to be atherogenic, whereas its oxidized derivatives are highly toxic to the endothelium and correspondingly atherogenic. Cholesterol is relatively resistant to oxidation, but food processing often accelerates its oxidative breakdown. OxChol derivatives are ubiquitous in foods coming from animal sources.

The essential fatty acids (EFAs) are really vitamins, as judged from the occurrence of deficiency states.[5,31,32] Two fatty acids that belong to 2 different chemical classes have been proven essential in humans. One is linoleic acid (LA, C18:2 n-6), of the omega-6 class; the other is alpha-linolenic acid (ALA, C18:3 n-3), of the omega-3 class. Exposure of cultured endothelial cell sheets to impure preparations of these EFAs resulted in decreased barrier function, i.e., albumin or LDL passed across the endothelium more freely.[25-27] The extent of endothelial damage was roughly proportional to the number of double bonds in each fatty acid (FA), and the experimental addition of small amounts of the peroxides of these FAs markedly increased damage to the endothelial cell sheets. Oxidized cholesterol derivatives also were devastating to the endothelia, even when present in small amounts. Interestingly, saturated FAs (C16:0, C18:0) were not toxic to the endothelia in these experiments.

This pessimistic picture of the potential of peroxidized EFAs to cause destruction of the blood vessel endothelia is transformed into its opposite when vitamin E is included in the experimental design.[11-15] Vitamin E was found to protect PUFA from their near-spontaneous degradation to peroxides and other free radical endproducts. These findings are consistent with the proven clinical protection against vascular morbidity and mortality conferred by supra-RDA intakes of vitamin E, as established from epidemiologic studies.[11]

Oxidized PUFA and other oxidant agents also may contribute to endothelial disruption by way of pro-inflammatory factors that they yield in vivo. One such is TNF, or tumor necrosis factor, which has been detected in human atheromatous plaques and is produced at higher rates in lesioned areas of the vessel wall.[18] TNF promotes coagulation and inflammation, and can directly injure endothelial cells by way of generating oxygen radicals. Exposure to TNF can deplete intracellular antioxidant levels, and also may "fool" the damaged endothelium into producing "adhesion molecules" on their surfaces. Such molecules facilitate the attachment of monocytes from the circulating blood, thereby increasing the probability of inflammatory discharges in close proximity to the vessel wall.[6,22]

While the findings to date overwhelmingly suggest that dietary intakes of oxidized fatty acids and cholesterol ("rancid fats") are largely responsible for the initiation and promotion of atherosclerosis, a similarly impressive body of research indicates that **non-oxidized** forms of essential fatty acids are crucial for endothelial homeostasis and for protection of the vessel wall against atherosclerotic progression.
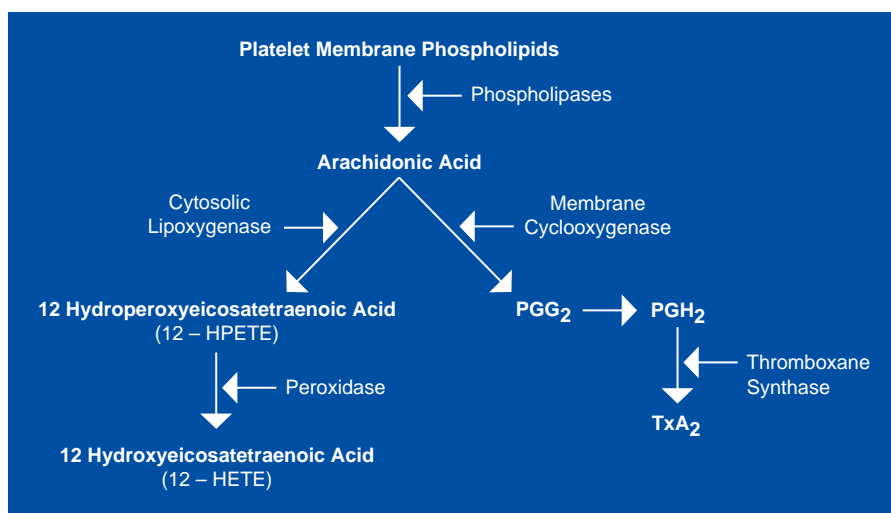
Platelet Membrane Phospholipids

Phospholipases

Arachidonic Acid

Cytosolic Lipoxygenase

Membrane Cyclooxygenase

12 Hydroperoxyeicosatetraenoic Acid
(12 – HPETE)

PGG_2 → PGH_2

Peroxidase

Thromboxane Synthase

12 Hydroxyeicosatetraenoic Acid
(12 – HETE)

TxA_2

FIGURE 3.

Prostanoids and Other Fatty Acid Metabolites

The literature on fatty acid metabolites and their involvements in homeostasis, inflammation, and pathology is so massive that this section can serve only as a modest orientation and introduction.

The 20-carbon fatty acids located in cell membranes can be metabolized to a variety of smaller, messenger-type molecules; this broad group are the eicosanoids. Within the eicosanoid group, metabolites of the enzyme cyclo-oxygenase are termed prostanoids; included in this subgroup are the prostaglandins and the thromboxanes. Metabolites of the lipoxygenase enzyme are called leukotrienes; these are not prostanoids but are taken together with the peroxide metabolites into the broader group of eicosanoids. The prostanoids, rather than the leukotrienes or other eicosanoids, have been most intensely investigated for their effects on the vessel endothelial system.

The first, and rate-limiting, step in prostanoid synthesis is the breaking away of the 2-position fatty acid "tail" from a membrane phospholipid by the enzyme phospholipase A2.[33] While still in the membrane, the resultant "free" fatty acid is attacked by lipoxygenase and other enzymes to eventually generate leukotrienes; or by cyclooxygenase to yield an endoperoxide (see Figure 3). The

endoperoxides are later converted to prostanoids, either by prostaglandin synthetase to prostaglandins, or by thromboxane synthetase to thromboxanes.

Each prostanoid is named for its chemical relationship to prostanoic acid. To the applicable PG or Tx is added a third letter—E, F, A, B, C, or D—denoting functions in the 5-membered ring of prostanoic acid. Each prostanoid is also assigned a number subscript that refers to the number of double bonds in the side chain, and sometimes an alpha or beta is also assigned based on the configuration of substituents on the ring. The primary PGs in vivo are PGE_1, E_2, E_3, F_{1a}, F_{2a}, and F_{3a}. PGE_1 was the first prostaglandin to be chemically defined, and also the first to be employed therapeutically (for a review see Sinzinger et al [20])

Prostanoid tissue concentrations usually are very low (on the order of nanomoles, 10^{-9} molar or less),[20,35] yet the prostanoids are ubiquitous messenger substances. Among their many functions, the prostanoids are involved in the stimulation of smooth muscle and the dilation and constriction of blood vessels; in bronchial dilation and constriction; in platelet aggregation; in gastric secretion; in the induction of labor, abortion or menstruation; and in the regulation of ocular pressure.[34-37] The prostanoids regulate tissue activity on a second-by-second time scale, as evidenced by their activity at very low concentrations, their very short half-lives, and the existence of enzyme systems specifically responsible for degrading them.[20]

The most significant precursor of prostanoids in human tissues is arachidonic acid (AA), C20:4 n-6. AA gives rise to the 2-series prostanoids, which are quantitatively

most abundant in the endothelia as well as all the other tissues. AA can be obtained directly from the diet only by way of animal sources. Plant foods provide precursors of AA, including the shorter chain linoleic acid (LA, C18:2 n-6), gamma-linolenic acid (GLA, C18:3 n-6), or dihomo-gamma linolenic acid (DGLA, C20:3 n-6). In addition to AA, two other 20-carbon fatty acids are sources of prostanoids: DGLA yields the 1-series prostanoids, while EPA (eicosapentaenoic acid, C20:5 n-3) yields the 3-series prostanoids.

Prostanoid Operating Principles

Prostanoid research is still encumbered with unanswered questions and apparent paradoxes. Nonetheless, some "operating principles" about prostanoids are evident. Prostanoid principle number one is that prostanoid biochemistry is interwoven, perhaps to the point of being maddeningly labyrinthine. The many prostanoids, generated from at least 3 starting sources by several different enzyme activities, interfere with each other in ways both constructive and destructive. Prostanoids can inhibit each other by competing for the same receptor sites on target cells, or they can act at different receptor sites to either amplify or dampen each other's effects.[35-39] For this reason alone, any single research finding on a prostanoid is likely to have little predictive value; it must first be assessed in the context of the large body of data about prostanoids that already exists.

Prostanoid principle number two: the 2-series prostanoids (derived from arachidonic acid) are more pro-inflammatory (and potentially more pro-atherosclerotic) than are those of the 1- and the 3-series.[20,39] The differences lie mainly in the thromboxanes, the prostanoids that generally stimulate platelets to aggregate and discharge (these are "pro-thrombotic," whence comes their name). TxA_2 lowers the platelet discharge threshold, thereby increasing the risk of vessel wall damage due to platelet activity: it is highly pro-thrombotic. In contrast, TxA1 (from DGLA) has little pro-thrombotic activity, and TxA3 (from EPA) has even less. Here the concept of prostanoid balance assumes new relevance: atherosclerotic progression can be influenced through dietary manipulation of prostanoid balance.

Principle number three: prostanoids are generated by way of free radical intermediates.[17] Early along the pathway from the 20-carbon FA to its prostanoid end-products, unstable intermediates are generated that have strong free radical character. These include various "lipid peroxides," including endoperoxides and malondialdehyde, which can crosslink biological macromolecules and cause toxic cell damage. Antioxidant factors that down-regulate lipid peroxidation also down-regulate the oxidative prostanoid pathways, steering the outcome towards anti-inflammatory prostanoids.[15-17] High oxidative stress and/or low antioxidant defense favors adverse prostanoid balance (pro-inflammatory, perhaps even pro-atherosclerotic). Prostanoid balance is therefore closely linked to oxidant-antioxidant balance.

An adjunct to Prostanoid Principle 3 is that prostanoid synthesis is tightly integrated with cell membrane systems. Crosslinking of the membrane lipids due to toxic/free radical attack; deficiencies in membrane antioxidant protection; oxidative damage to the membrane enzymes of the prostanoid pathways; all can shift the membrane's intrinsic balance towards pro-inflammatory or other abnormal prostanoid patterns. Still, anti-inflammatory prostanoid balance is favored only if dietary fatty acid intake is appropriately balanced in favor of GLA-DGLA or ALA-EPA, the "anti-inflammatory" prostanoid precursors.[39] One concrete example of the importance of antioxidants for prostanoid biology, is that enzymatic synthesis of either of the anti-inflammatory PGE_1 or PGE_2 from AA-endoperoxides requires glutathione as a
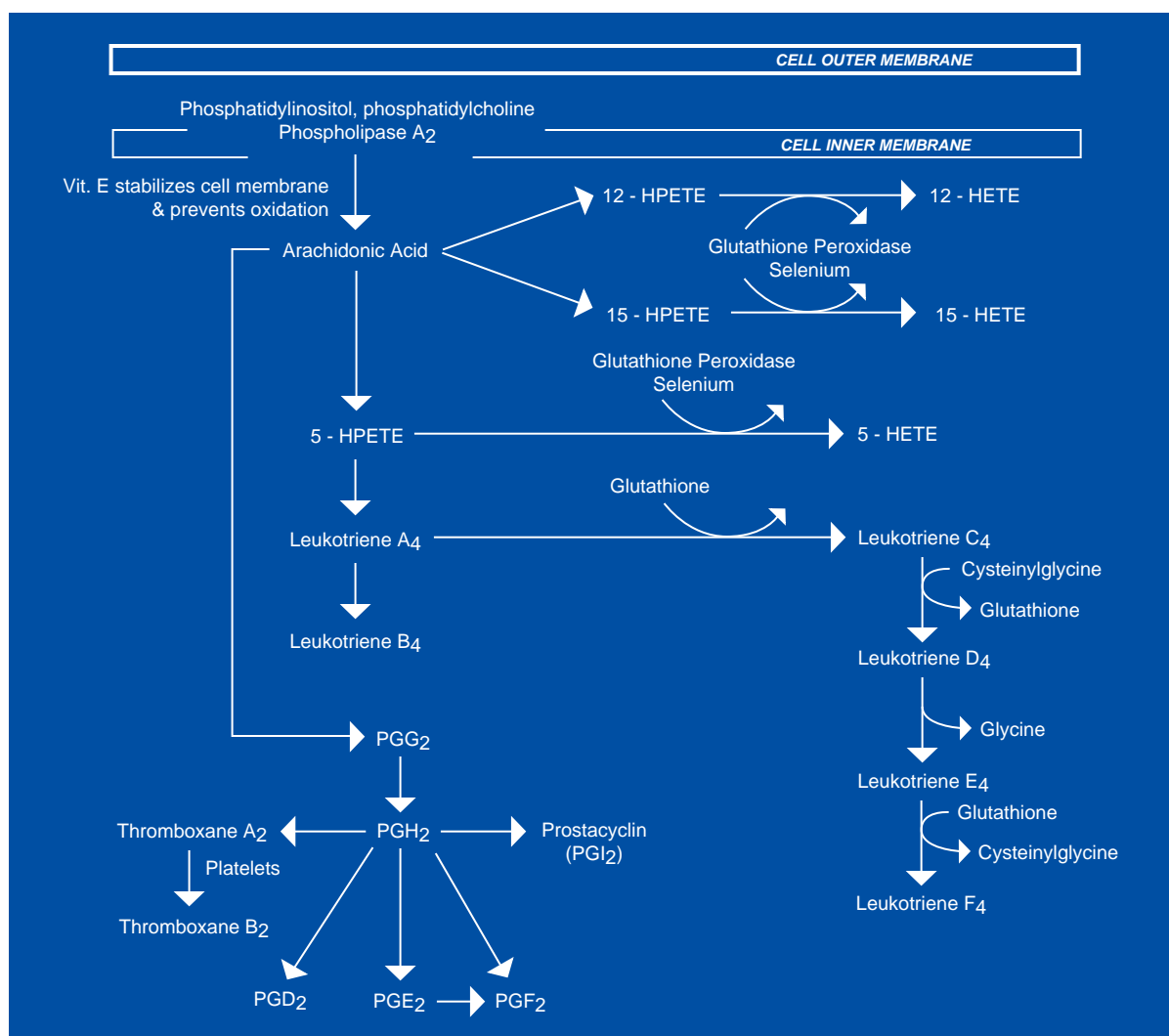
FIGURE 4.

cofactor.[16,17] With depletion of GSH the prostanoid balance is shifted towards thromboxanes. Vitamin E, the antioxidant most tested for endothelial protection, also modulates prostanoid generation towards the prostacyclins.[11-15] Both E and glutathione appear to be major protective antioxidants for the endothelia [17] (see Figure 4).

These Prostanoid Principles are likely to be operative not just in the blood vessel endothelia but in circulating platelets, monocytes, and other white cells. Prostanoid balance tends to respond favorably, i.e., pro-homeostatically, to the appropriate manipulation of dietary fatty acid intake. One puzzling set of observations made with endothelial cell cultures is that LA, ALA, and even the normally innocuous OA when given alone were toxic to the cells.[24-27] This does not seem to follow the same pattern in animal experiments. A plausible explanation for this apparent inconsistency is that a direct exposure to one fatty acid in culture can crowd out other FAs from the cell membrane, reducing the viability of the cell.

GLA, DGLA and 1-Series Prostanoids are Protective

In the endothelial cells, as in other cells of the body, membrane fatty acid distributions are reflective of dietary fatty acid intake.[3,10,19] The phospholipids are the holding sites for FAs in the cell membrane: the position 1 tails most

often carry saturates or mono-unsaturates, while AA occurs often in the tails of position 2.[33,56] Either of the proven-essential fatty acids (LA and ALA) can replace each other (or AA or OA) in the membrane phospholipids, depending on their relative dietary intake levels.[32] Therefore the prostanoid profile of the endothelium (or other particular tissue) at a particular point in time is likely to be an integrated outcome of the membrane fatty acid distributions within that tissue.[5,41]

In the context of dietary fatty acid intakes being the major determinant of endothelial prostanoid balance, the question could be asked, "What is the anti-atherosclerotic potential of gamma-linolenic acid (GLA)?" This FA has been championed as a clinically useful dietary supplement by Dr. David Horrobin.[35,36,39] The first product of dietary GLA (C18:3) after its enzymatic elongation is DGLA (C20:3), which is the immediate precursor of the 1-series prostanoids.

GLA is undoubtedly relevant to prostanoid balance in the human. Horrobin and others have shown that dietary supplementation with GLA from plant oil sources raises the levels of GLA and DGLA in the tissues, and increases the tissue ratio of DGLA/AA.[35] Levels of DGLA can get as high as one-third of the AA levels in human tissues, and in some tissues (adrenal, renal medulla, ovaries, testes) DGLA levels may be equal to or greater than AA: and in almost all human tissues DGLA levels are 2-4 times higher than EPA.[35] Therefore on a quantitative basis, DGLA is a significant prostanoid precursor. In support of a physiologic role for DGLA, Horrobin has pointed out that low DGLA levels appear to be strong markers of risk of coronary artery disease.[5,35]

PGE_1 coming from DGLA is the best studied 1-series prostanoid.[35,37] Horrobin[35] has catalogued an impressive range of clinical benefits from PGE_1, all related to its high potency as a vasodilator, an anti-platelet aggre-gation agent, and a bronchodilator. When administered directly, PGE_1 has anti-inflammatory action, even at very low concentrations (10^{-9} to 10^{-13} molar).[37] PGE_1 has a spectrum of action that differs clearly from the anti-inflammatory actions of PGE_2 or PG_{I2} (prostacyclin), so may have its own, distinctive functional niche.[35]

The drawback to administering PGE_1 as the native orthomolecule is that, like most known prostaglandins, its persistence in the circulation is very short (half-life on the order of a few minutes). Horrobin suggested that oral supplementation with GLA-DGLA plant oil could be the most practical means to shift the tissue prostanoid balance towards PGE_1 and the other 1-series prostanoids.[35,39] Fan, Chapkin and their collaborators at Texas A& M University showed in a series of experiments with mice, that a GLA+omega-3 combination diet (primrose oil plus fish oil) down-regulated mice macrophages and upregulated PGE_1 levels in vivo.[38] As discussed in the next section, this n-6/n-3 combination has clinical potential for moderating atherogenic progression in a dose-dependent fashion.

Mixed Clinical Findings From Fish Oils

Eicosapentaenoic acid or EPA (C20:5 omega-3) is precursor to the 3-series prostanoids. Over the past two decades, a great deal of research has been conducted in efforts to establish whether EPA and the 3-series prostanoids are responsible for the low incidence of atherosclerosis in Eskimos and other fish-eating populations.[22] Fish are generally high in EPA, and as a rule these human populations consume sufficient fish to have marked elevations of EPA in their cell membranes. The fish oil "hypothesis" suggests that, as a consequence of high EPA intake, AA is partly displaced from the cell membranes and the tissue prostanoids shift to a more anti-inflammatory, anti-atherogenic pattern due to the

prostanoids generated from cell membrane EPA.[22,76] Considering the involvement of saturated, monounsaturated, and many polyunsaturated fatty acids in the diet, this assertion may be somewhat simplistic. Yet dietary fish oil supplements unquestionably can extend the lives of subjects with heart disease.[40,41,50-54]

In a randomized, controlled trial Salachas and Saynor reported from Greece that 10 grams of fish oil per day relieved angina and reduced subjects' dependence on glyceryl trinitrate.[40] In a small controlled trial on heart transplant recipients, conducted by Fleischhauer and colleagues at Stanford University, fish oil supplements improved endothelial-dependent coronary artery vasodilation.[50] In a double-blind study by McVeigh and collaborators, dietary fish oil improved vasodilation capacity in both the large arteries and the peripheral vasculature of diabetics.[51]

Concerning the efficacy of the fish oils for directly slowing atherosclerosis, results have not been as clearcut. To date, some eight controlled trials have examined fish oils for the prevention of re-stenosis after angioplasty.[42-49] Of these, 5 reported benefits,[42-46] and 3 did not.[47-49] One variable that was inadequately controlled in these clinical trials was the antioxidant status of the subjects. Also, Horrobin suggested that had the fish oils been administered in conjunction with GLA, anti-inflammatory clinical benefits might have been more completely achieved.[35,39] Recently he reported success in one trial with a mixture of GLA and fish oils against re-stenosis.[5]

Clinical benefits from the fish oils may well depend on dose. When given at high doses the fish oils did not consistently produce clinical benefit; even at very high doses (up to 100 grams per day) they did not consistently lower LDL cholesterol or raise HDL cholesterol. They did consistently lower blood triglyceride levels,[30] though this effect was not proven to lower disease risk and at times was accompanied by rises in LDL cholesterol.[52] Also at high doses (10 grams per day or higher), the fish oils had adverse effects—increased bleeding time and gastrointestinal side effects were common, and immune system competence was threatened. Results from other controlled trials suggest that low doses of fish oils can effectively save lives, and with minimal adverse effects.

Burr's group conducted a large randomized, controlled trial now known as the South Wales Study.[53] They found that a modest intake of fatty fish (2 or 3 portions per week) significantly reduced the risk of death from heart attack in men who had already had one attack. Siscovick's group in Seattle surveyed 334 patients who experienced primary cardiac arrest between 1988 and 1994.[41] By means of interviews, blood testing, and comparisons with a control group, they determined that an intake of 5.5 grams of fish omega-3 FA per month (equivalent to one fatty fish meal per week) was associated with a 70 percent reduction in the risk of primary heart attack. Wallace and colleagues in Northern Ireland gave a similar dose as fish oil (2.4 grams fish oil, or about 0.7 grams EPA+DHA, per day) to healthy women in a double-blind trial.[54] They recorded significant anti-inflammatory effects that were not influenced by the absence of vitamin E from the preparation. Thus the unfavorable benefit-risk characteristics of the fish oils at high doses appears to become far more favorable at low doses.

A Neo-Eicosanoid Pathway to Vessel Wall Protection?

Buchanan and Brister at the McMaster Clinic in Ontario, Canada have demonstrated a pathway for possible vessel wall protection that utilizes, rather than the 20-carbon fatty acids, a linoleic acid (C18:2 n-6) derivative generated by the enzyme lipoxygenase.[19] This derivative could be a new messenger on the

block, different from the archetype 20-carbon-derived prostanoids and other eicosanoids.

Platelets are normally geared to respond to vessel wall injury by secreting procoagulants, proaggregatory agents, and mitogenic factors which homeostatically facilitate hemostasis and vessel wall repair. Under optimal conditions, the platelets circulate freely with the blood and do not interact with the vessel endothelium. Damage to the endothelial sheet, especially if it exposes the underlying subendothelial layer, often causes platelets to become activated. They adhere to the damaged endothelium and initiate fibrin precipitation and clot formation. As with other of the body's mechanisms for response to injury, the platelets are finely poised—if their discharge cascade escapes homeostatic constraints it can worsen damage, exaggerate clot formation, and threaten to occlude the vessel. Antithrombotic strategies are therefore an important facet of atherosclerosis prevention and management, and so far these have mainly involved the use of prostanoid blockers i.e., cyclo-oxygenase enzyme inhibitors such as aspirin.

Buchanan and Brister[19] reviewed the mechanisms involved in platelet stimulation, discharge, and aggregation. They found that, contrary to the prostanoid dogma, lipoxygenase metabolites were produced in far greater amounts by the platelets than was TxA_2, (thromboxane A_2, a major cyclo-oxygenase metabolite). and such metabolites play a crucial role in normal platelet function. Lipoxygenase activity potentially bypasses conventional antithrombotic management and could be pro-thrombotic under conditions of oxidant excess or antioxidant deficiency. For example aspirin blocks the cyclo-oxygenase that generates prostanoids but fails to block the lipoxygenase, and this could allow for a paradoxical enhancement of thrombosis by aspirin.

Buchanan and Brinster claim that under basal, pro-homeostatic conditions, endot-helial cells secrete little of the recognized antithrombotic factors such as prostacyclin, tissue plasminogen activator, or endothelin. These seemingly are produced and released only following trauma of the wall. Instead, unstimulated endothelial cells continually produce 13-HODE (13-hydroxy octadecadienoic acid), from linoleic acid via their 15-lipoxygenase enzyme. 13-HODE confers on the RESTING endothelial cell sheet a marked resistance to platelet or monocyte adherence, apparently by down-regulating adherence receptors at the surfaces of the endothelial cells. Dietary EPA (C20:5, n-3) failed to confer this benefit, though it did decrease platelet adhesivity.

Since it is not generated from a 20-C fatty acid, 13-HODE is not technically a prostanoid, though it could qualify as an eicosanoid, since it is produced by a lipoxygenase enzyme. Human arteries make 6x more 13-HODE than do veins, and its synthesis declines with age.[55] Buchanan and Brinster[19] hypothesized that 13-HODE renders the healthy endothelium (and subendothelium) relatively resistant to fibrin clot or platelet thrombus formation. Their preliminary experimental findings indicate dietary LA can be beneficial, perhaps to help counterbalance thromboxanes derived from AA and so enhance anti-thrombogenic balance in the blood vessel wall.

Non-Prostanoid Protection by Dietary Fatty Acids

Notwithstanding the central importance of prostanoids in the prevention or promotion of atherosclerosis, dietary long-chain fatty acids (that is, derivatives of n-6 and n-3 EFA ranging from C18 to C22 in chain length) offer benefits to the blood vessel endothelia by additional mechanisms that do not directly involve prostanoids. These include:

1. Moderation of the AA content of the cell membranes. The unsaturated fatty
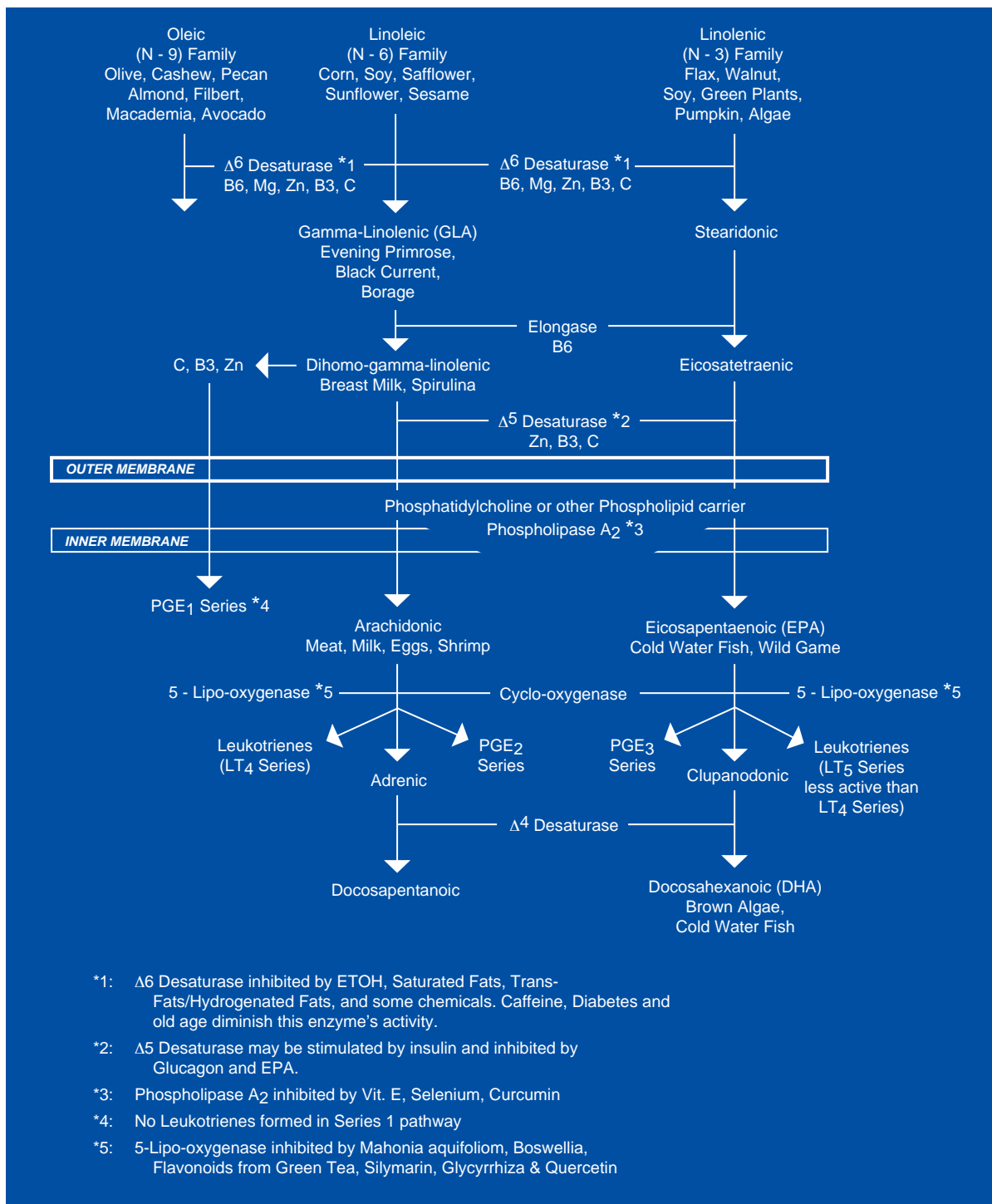
Oleic
(N - 9) Family
Olive, Cashew, Pecan
Almond, Filbert,
Macadamia, Avocado

Linoleic
(N - 6) Family
Corn, Soy, Safflower,
Sunflower, Sesame

Linolenic
(N - 3) Family
Flax, Walnut,
Soy, Green Plants,
Pumpkin, Algae

Δ^6 Desaturase *1
B6, Mg, Zn, B3, C

Δ^6 Desaturase *1
B6, Mg, Zn, B3, C

Gamma-Linolenic (GLA)
Evening Primrose,
Black Current,
Borage

Stearidonic

Elongase
B6

C, B3, Zn ← Dihomo-gamma-linolenic
Breast Milk, Spirulina

Eicosatetraenic

Δ^5 Desaturase *2
Zn, B3, C

OUTER MEMBRANE

Phosphatidylcholine or other Phospholipid carrier

Phospholipase A$_2$ *3

INNER MEMBRANE

PGE$_1$ Series *4

Arachidonic
Meat, Milk, Eggs, Shrimp

Eicosapentaenoic (EPA)
Cold Water Fish, Wild Game

5 - Lipo-oxygenase *5 — Cyclo-oxygenase — 5 - Lipo-oxygenase *5

Leukotrienes
(LT$_4$ Series)

PGE$_2$
Series

PGE$_3$
Series

Leukotrienes
(LT$_5$ Series
less active than
LT$_4$ Series)

Adrenic

Clupanodonic

Δ^4 Desaturase

Docosapentanoic

Docosahexanoic (DHA)
Brown Algae,
Cold Water Fish

*1: $\Delta6$ Desaturase inhibited by ETOH, Saturated Fats, Trans-
Fats/Hydrogenated Fats, and some chemicals. Caffeine, Diabetes and
old age diminish this enzyme's activity.

*2: $\Delta5$ Desaturase may be stimulated by insulin and inhibited by
Glucagon and EPA.

*3: Phospholipase A$_2$ inhibited by Vit. E, Selenium, Curcumin

*4: No Leukotrienes formed in Series 1 pathway

*5: 5-Lipo-oxygenase inhibited by Mahonia aquifoliom, Boswellia,
Flavonoids from Green Tea, Silymarin, Glycyrrhiza & Quercetin

FIGURE 5.

acids compete with each other (and with the saturates and omega-9 monounsaturates) for inclusion in the phospholipids that make up the cell membranes. In particular, the various dietary PUFAs compete with AA for the 2-position tails of the phospholipids; at high enough intakes other FAs literally can crowd AA out of the membrane's phospholipid matrix. However, the fact that AA itself is homeostatically conserved in cell membranes

suggests that a range of optimal AA levels exists.[56] An over-substitution of membrane AA could interfere with signal transduction, the carrying of signals into the cell by way of the membrane (as contrasted with AA prostanoids carrying signals from the membrane to the environment outside the cell). A corollary here, is that overdosing either with the fish oils, with GLA, or with linoleic acid, is possible and has negative implications for vessel wall homeostasis.

2. Inhibition of AA synthesis from its precursors. Here 18-and 20-carbon, omega-3 precursor fatty acids compete with omega-6 precursor fatty acids for the active sites on the elongase and desaturase enzymes. The net outcome should resemble dietary down-regulation of cell membrane AA (see Figure 5).

3. Facilitation of membrane proteins through membrane fluidity.[56,57] Transport enzymes, ion gates, receptors, and other integral membrane proteins all function best when associated with phospholipids carrying fatty acids that are highly unsaturated.[56] This comes from a direct, intimate association between the protein and the highly fluid fatty acid "tails;" generally, the more unsaturated its fatty acid tails, the more fluidizing is the phospholipid.[33,56,57] Antigens and membrane-bound antibodies depend on the phospholipid-fatty acid matrix. The ATPase transport enzymes are highly dependent on the lipid matrix for functionality, as are the wide variety of receptors and the membrane enzymes downstream of them—adenylate cyclase and protein kinases, for example. The most fluidizing of the membrane fatty acids is DHA.

Docosahexaenoic acid (DHA, C22:6) is not a prostanoid precursor, but with 6 double bonds it is the most highly polyunsaturated fatty acid of physiologic significance.[5,57] DHA is an omega-3 FA that can be synthesized from the essential ALA, though there is evidence that age and some disease processes

may decrease the activity of this enzyme; it is also a major nutrient constituent of fish and fish oils. DHA is universally present in human cells, and in studies with human endothelia DHA had several anti-atherogenic effects. DHA down-regulates endothelial cell surface adhesion receptors—proteins that promote adhesion of white cells—and so can promote atherosclerosis.[58-60] DHA also down-regulates pro-inflammatory mediators, and turns off the intracellular reading of the genes for these substances.[61,68] In platelets, DHA down-regulates receptor affinity for thromboxanes, inhibiting platelet aggregability.[62-64]

These pro-homeostatic DHA effects were proven distinct from prostanoid effects. Instead, they reflect DHA's capacity to increase membrane fluidity and so improve the performance of proteins built into the membrane. EPA also exhibits these effects, but to a lesser extent than does DHA, which is consistent with EPA's lesser degree of fluidizing capacity.

The strategy of optimizing omega-6 to omega-3 balance in order to benefit the circulation may not be as straightforward as used to be assumed. As an example, EPA and DHA peroxide products were found to facilitate the peroxidation of AA that leads to the generation of series 2 prostanoids.[65] Conversely, peroxidized AA was found to facilitate the generation of 3-series prostanoids from EPA. These complex metabolic entanglements indicate that all the prostanoid precursor fatty acids are homeostatically involved in human health and cardiovascular functioning.[5,66]

Fatty Acid Diversity, Key to Atherosclerosis Prevention

Essential fatty acids and their longer-chain derivatives are located at the core of cell function, the outer membrane system. They are crucial for membrane intregrity through their phospholipid parent macromolecules, and they are the primary sources of messenger molecules that traverse the membrane in all

directions. Metabolic competence to convert cell membrane fatty acids to protective prostanoids is one pillar of endothelial homeostasis. Another pillar is the pro-homeostatic benefit from membrane fatty acids by way of non-prostanoid pathways. Yet another pillar is the net favorable antioxidant status over oxidant challenge. Endothelial functionality is a net outcome of the dietary intake patterns of the 18- and 20-carbon PUFA and their longer-chain derivatives,[5,66,67] translated into metabolites with the intimate participation of membrane antioxidants.

The milieu of the cell membrane is where sophisticated eicosanoid cascades are initiated. It appears that by way of enzymes that interact also with the phospholipid parent molecules and with antioxidant factors, virtually all the unsaturated fatty acids in the membrane can be metabolized to peroxides, hydroperoxides, prostaglandins, thromboxanes, leukotrienes, and other metabolites still being defined. These metabolites interact in complex yin-yang fashion, at times competing and at other times synergizing with each other to support the blood circulation and help protect the vessels against dysfunction or damage.[66]

Horrobin[5,39] has presented convincing evidence that much of the pathophysiology of atherosclerotic progression—elevated arterial lipid deposition, intimal hyperplasia, elevated cholesterol levels, enhanced platelet aggregation, vasoconstriction and increased blood pressure, increased plasma fibrinogen—can be accounted for more plausibly by deficits of EFAs than by any other known risk factor. Yet, from the still-murky perspective of membrane fatty acid metabolism, a great deal remains to be understood before dietary intake recommendations can be accurately developed. Some of the consistent patterns are:

• High intakes of longer chain saturates (C12 through C16) encourage atherosclerosis, since they raise LDL cholesterol levels probably through crowding out unsaturates.[32,69]

The trans-fatty acids are at least as troublesome as the saturates.[5,69] Monounsaturates are beneficial, at least to the extent that they replace saturates. Olive oil or other plant oils high in oleic acid (OA, C18:1) are preferable for frying, since OA is relatively stable to oxidation and does not appear to be toxic to the endothelia.[70] However, Horrobin cautions[5] that extended high intakes of monounsaturates could logically lower membrane EFA and impair homeostasis.

• Arachidonic acid intakes from the SAD (Standard American Diet) are likely to be higher than is consistent with pro-homeostatic, anti-atherosclerotic FA balance. In place of AA from animal sources, LA from plant sources may offer benefit, perhaps as a source of protective 13-HODE and certainly by competing with AA for slots in the cell membrane phospholipids.[19] Here again, too much of a good thing may be possible. AA is central to eicosanoid production and signal transduction,[5,56,57] so an excess of LA intake and/or a compromise of desaturase activity could lead to an excess of membrane LA simultaneous with deficiency of DGLA and AA.

• Omega-3 FAs benefit vessel wall homeostasis, but not in as simplistic a fashion as was earlier hypothesized. Inconsistent clinical results may be related to dose, and lower intakes may be just as clinically effective as high intakes.[40,41] Of the omega-3 FAs, ALA is found in high quantity in flax seed oil, which, though too unsaturated and thus too unstable for cooking, makes (subject to personal taste) an attractive salad dressing. In one clinical trial conducted in France, ALA reduced CHD and total mortality, with blood EPA and DHA found to be significantly elevated.[76] Yet flax seed oil is not an ideal source of omega-3 FA for all individuals: persons with diabetes or with hypertension, alcoholics, and the aged all can have impaired desaturase enzyme capacity, and perhaps also smokers and persons under

stress (see Horrobin[5,72] for references). For these individuals direct supplementation with EPA and DHA may be obligatory.

The recent positive clinical outcomes from fish oil intakes of 2.4 to 5.5 grams per day (EPA+DHA ≥0.7 grams) are consistent with a "crucial threshold" for benefit as suggested by Odeleye and Watson.[71] These lower levels of intake also should lessen the risks of oxidative damage that accompanies high daily intake.

• Real progress is being made towards definition of a desirable ratio for n-6:n-3 intakes. The estimated current intake ratios from the SAD range from 10:1 up to 20:1, in contrast with 2:1 to 4:1 at the end of the 19th Century.[5,66] Most experts agree that current n-3 intakes are far too low, and discussions continue towards formal intake recommendations for some populations.[74] In most cells the ratio is in the 3-5:1 region, and this is likely to have meaning for cell and tissue homeostasis.[5]

All these observed patterns indicate that for consistent nutritional support against atherosclerosis, a balanced intake of a variety of dietary fatty acids is preferable over "megadosing" with any of the omega- 3, 6, or 9 fatty acids. Dietary FAs should be generously augmented with antioxidants, as uncontrolled free radical attack tends to fuel pro-atherosclerotic tendencies, while favorable fatty acid-antioxidant interactions tend to retard atherosclerosis initiation and progression. Periodic assays of red blood cell deformability can help validate antioxidant protocols, but for an overall antioxidant-fatty acid picture, RBC membrane fatty acid assays, which are representative of tissue fatty acid status,[73] should nicely complement the deformability measurements.

Despite the advent of the highly-touted cholesterol-lowering drugs and the implementation of invasive angioplasty and bypass surgery, benefits to the patient have been limited;

atherosclerosis and coronary heart disease are still universal in Western societies. Rational nutritional-orthomolecular approaches to atherosclerosis prevention are fast becoming "mainstream," due to the overwhelming evidence that atherosclerosis progression and endothelial functionality are linked to nutrient status. Particularly if utilized in conjunction with other wholistic protocols, dietary essential fatty acids can be used to heal damaged blood vessel walls, to halt atherosclerotic progression, and over the long term to disarm atherosclerosis as a killer disease.

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