

# The role of the tissue omega-6/omega-3 fatty acid ratio in regulating tumor angiogenesis

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**Abstract** Angiogenesis is a necessary step in tumor growth and metastasis. It is well established that the metabolites of omega-6 and omega-3 fatty acids, which must be obtained through the diet and cannot be synthesized *de novo* in mammals, have differential effects on cellular processes. Omega-6 fatty acid (*n*-6 FA)-derived metabolites promote angiogenesis by increasing growth factor expression whereas omega-3 fatty acids (*n*-3 FA) have anti-angiogenic and antitumor properties. However, most studies thus far have failed to account for the role of the *n*-6 FA/*n*-3 FA ratio in angiogenesis and instead examined the absolute levels of *n*-6 and *n*-3 FA. This review highlights the biochemical interactions between *n*-6 and *n*-3 FA and focuses on how the *n*-6/*n*-3 FA ratio in tissues modulates tumor angiogenesis. We suggest that future work should consider the *n*-6/*n*-3 FA ratio to be a key element in experimental design and analysis. Furthermore, we recommend that clinical interventions should aim to both reduce *n*-6 metabolites and simultaneously increase *n*-3 FA intake.

**Keywords** Omega-3 fatty acids · Omega-6 fatty acids · Fatty acid ratio · Angiogenesis · Cancer · Inflammation

## 1 Introduction

Angiogenesis, defined as the formation of new blood vessels, is controlled by a balance of pro-angiogenic growth

factors and anti-angiogenic growth inhibitors. Although angiogenesis is a natural process that occurs during wound healing, placental growth, and exercise, abnormal angiogenesis underlies many chronic diseases [1, 2]. Excessive angiogenesis is associated with cancer, obesity, arthritis, and diabetic retinopathy whereas insufficient angiogenesis is associated with heart attack, stroke, osteoporosis, and pre-eclampsia [3].

Understanding the factors that regulate angiogenesis is important for interventions across a multitude of diseases that share malfunctions in angiogenesis. Research has demonstrated pro-angiogenic effects of the metabolites of omega-6 fatty acids (*n*-6 FA) and anti-angiogenic effects of the metabolites of omega-3 fatty acids (*n*-3 FA). This review will examine how manipulating the tissue ratio of *n*-6 FA/*n*-3 FA may be a promising approach to modulating tumor angiogenesis as a part of cancer therapy.

### 1.1 Angiogenesis and cancer

Angiogenesis is maintained by endothelial cells, which line the inner membrane of the vasculature and act as receptors for a variety of chemical stimuli. The general mechanism of angiogenesis can be explained as follows: tissue secretes an angiogenic signal that binds to receptors on pre-existing vessels, causing (a) existing vessels to dilate, vascular permeability to increase, and the extracellular matrix to degrade; (b) endothelial cells to proliferate and migrate towards the angiogenic signal; (c) endothelial cells to aggregate and begin tube formation, connecting the tissue-secreting growth factor to the pre-existing vasculature; and (d) capillaries to stabilize through pericyte recruitment and remodeling of the vasculature [4].

One of the hallmarks of cancer is the body's ability to promote sustained angiogenesis because the rapid rate of tumor expansion necessitates a constant and expanding blood

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supply [5, 6]. Microscopic breast cancer cells can be found in approximately 50 % of middle-aged women, but these cells are typically dormant and asymptomatic; however, in some of these women, the microscopic cancer cells become active and proliferate uncontrollably until they begin to metastasize [7]. It has been hypothesized that the ability to induce angiogenesis underlies the switch between dormant, microscopic cancer cells, and an active tumor [8, 9].

Various molecules are responsible for promoting and sustaining angiogenesis. Common growth factors and their functions include vascular endothelial growth factor (VEGF), which causes dilation of blood vessels and endothelial cell proliferation/migration, platelet-derived growth factor (PDGF), which recruits smooth muscle cells to stabilize new vessels, fibroblast growth factors (FGF), which promote endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures, matrix metalloproteinase (MMP), which causes breakdown of the basement membrane, and angiopoietin, which mediates vascular remodeling and maintains vascular integrity [10].

Most growth factors, including VEGF, PDGF, and angiopoietins, are receptor tyrosine kinases. Binding of the growth factor to its endothelial receptor activates signal transduction, ultimately causing transcription of the growth factor in the nucleus [11]. Some pro-angiogenic molecules regulate the production of angiogenic growth factors. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), an inflammatory cytokine, increases expression of VEGF and MMP production [12]. Another macrophage-derived chemokine, interleukin (IL)-8, upregulates MMP-2 and MMP-9 [13]. Therefore, molecules that regulate growth factor expression, production, or downstream effects will likewise impact angiogenesis.

## 1.2 The biochemistry of omega-6 and omega-3 fatty acids

Both  $n-6$  and  $n-3$  FA are long-chain, polyunsaturated fats.  $n-6$  FA contain two or more double bonds, with the first double bond on the sixth carbon from the methyl end of the molecule;  $n-3$  FA contain three or more double bonds, with the first double bond on the third carbon atom from the methyl end.  $n-3$  FA and  $n-6$  FA play crucial biological roles that include altering the properties of cell membranes, providing substrates for the production of signaling molecules or functioning mediators, and modulating gene expression [14]. These fatty acids are considered “essential” because they cannot be produced *de novo* by the body and must be obtained from the diet.

The primary  $n-6$  FA is linoleic acid (LA), which can be converted to arachidonic acid (AA). The three main  $n-3$  FAs are  $\alpha$ -linolenic acid (ALA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Through the same desaturase and elongase enzymes, the  $n-3$  FA ALA can be converted into EPA and DHA (Fig. 1). However, because

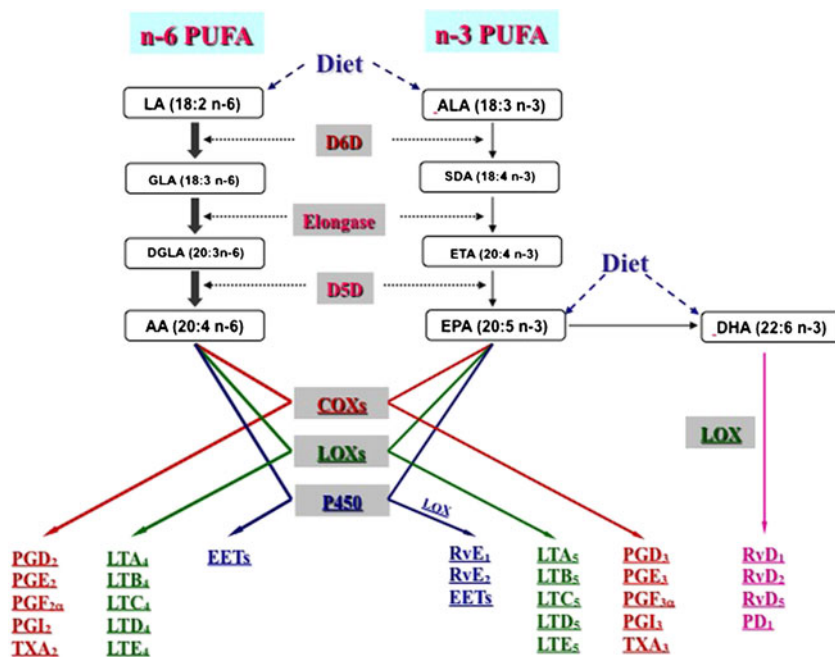
these conversions are inefficient, consuming preformed EPA and DHA is necessary to meet dietary requirements [15].

The enzymes responsible for the metabolism of both  $n-6$  FA and  $n-3$  FA are the cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 (CYP 450; Fig. 1). Several  $n-6$  FA-derived eicosanoids promote tumor angiogenesis, such as the prostaglandins (PGH<sub>2</sub>, PGE<sub>2</sub>, PGI<sub>2</sub>), leukotrienes (4-series LTs), thromboxanes (TXA<sub>2</sub>), and hydroxyeicosatetraenoic acids (12-HETE, 15-HETE) [16–24]. These eicosanoids make the tumor microenvironment more favorable for neoplasms and metastasis by encouraging the transcription of angiogenic growth factors, increasing the rate of endothelial cell migration and proliferation, and increasing the rate of vascularization. In contrast,  $n-3$  FA metabolism produces leukotrienes and prostaglandins that attenuate excess vascularization.  $n-3$  FA and  $n-6$  FA compete for incorporation into the cell membrane in addition to enzymes for eicosanoid production, including COX-2 and 5-LOX (Fig. 1). Thus, high levels of tissue  $n-3$  FA can reduce angiogenesis through decreased production of pro-angiogenic AA-derived eicosanoids and through  $n-3$  FA's intrinsic antitumor properties [16–27]. Furthermore,  $n-3$  FA have been found to down-regulate expression of angiogenic growth factors VEGF, PDGF, IL-6, and MMP-2 [28, 29].

Unlike other fatty acids that are widely available in foodstuffs,  $n-3$  FA are primarily found in fatty fish and certain vegetables and nuts. Single-celled algae and phytoplankton produce  $n-3$  FA and because these organisms enter our food web through various routes (*e.g.*, fish that have eaten phytoplankton), they are considered to be the primary origin of most of the  $n-3$  FAs in the human diet [30]. Currently, fish populations are diminishing and the price of seafood is increasing, rendering dietary  $n-3$  FA increasingly scarce. However, whereas  $n-3$  FA are present in few foods,  $n-6$  FA are abundant in the Western diet. Corn and soybean oils, processed foods containing these oils, and grain-fed meat are all high in  $n-6$  FA.

Throughout our evolution as hunters and gatherers, humans thrived on a meat and fish diet that was high in  $n-3$  FA and low in  $n-6$  FA; the ratio of  $n-6$  FA/ $n-3$  FA was likely close to 1:1 [28]. In more recent human history, this ratio has drastically changed. During the last century, the emergence of agribusiness alongside processed foods, grain-fattened livestock, and hydrogenation of vegetable fats have reduced the content of  $n-3$  fatty acids and increased  $n-6$  fatty acids. Now, the ratio of  $n-6$  FA/ $n-3$  FA is approximately 15:1 or higher [29]; our bodies may not be accustomed to utilizing such high levels of  $n-6$  FA [31]. This is considered to be one of many factors responsible for the relatively recent rise in chronic diseases, predominantly those associated with inflammation including cancer, heart disease, arthritis, and diabetes [32].

**Fig. 1** The metabolic pathways of *n*-6 FA and *n*-3 FA. Cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 (CYP 450) convert *n*-6 and *n*-3 FA into bioactive lipid mediators. The *n*-6-derived eicosanoids, which include the prostaglandins (PGH<sub>2</sub>, PGE<sub>2</sub>, PGI<sub>2</sub>), leukotrienes (four-series LTs), thromboxanes (TXA<sub>2</sub>), and hydroxyeicosatetraenoic acids (12-HETE, 15-HETE), promote tumor angiogenesis. In contrast, *n*-3 FA metabolism produces eicosanoids that attenuate excess vascularization, including leukotrienes and prostaglandins



## 2 The effects of omega-6 fatty acid metabolites on angiogenesis

### 2.1 COX-2 metabolites

COX-2 is the enzyme that catalyzes the formation of PGE<sub>2</sub>, PGI<sub>2</sub>, PGH<sub>2</sub>, and TXA<sub>2</sub> from AA, which have been shown to promote angiogenesis by encouraging the expression of growth factors and creating favorable conditions for growth in the tumor microenvironment [33]. Downstream products of COX-2 induce tumor vascularization by regulating multiple steps of angiogenesis including the production of VEGF and matrix metalloproteinases, promoting vascular sprouting, migration, and tube formation, and enhancing endothelial cell survival [34].

COX-2 is upregulated in tumor tissue and leads to an increased production of pro-angiogenic eicosanoids. Moderate to strong levels of COX-2 have been found in pulmonary, colonic, and mammary tumors as compared to negligible levels of COX-2 in non-neoplastic epithelium [35]. Similarly, COX-2 mRNA and protein expression are elevated in the tumor cells of head and neck cancer patients [36]. Furthermore, patients with metastases have higher COX-2 expression, PGE<sub>2</sub> levels, and microvessel densities than cancer patients without metastases [36]. COX-2 levels are also higher in the tumor front zone, which is characterized by active proliferation, than in the tumor core. These results indicate increased levels of COX-2 are linked to the degree of tumor progression and angiogenesis.

Moreover, high COX-2 activity in tumors leads to increased expression of VEGF. Under hypoxic conditions, COX-2/PGE<sub>2</sub> enhances HIF-1 transcriptional activity and

increases VEGF levels, facilitating the angiogenesis that enables tumor cell survival in this hostile environment [37, 38]. COX-2 activity has also been positively associated with lymphoangiogenesis, which is a key step in metastatic progression. Lymphoangiogenesis is stimulated by COX-2 upregulation of VEGF-C through the Her/Neu pathway, which activates NFκβ [39, 40]. In leukemic cells, COX-2 induction of VEGF can be mediated through the JNK pathway in which C-jun binds to CRE [41]. The wide range of signal transduction pathways affected by COX-2 illustrates the diverse mechanisms through which COX-2 metabolites can facilitate angiogenesis.

A convincing line of research shows that inhibition of COX-2 reduces the synthesis of pro-angiogenic prostaglandins and thromboxane A<sub>2</sub>, effectively mitigating tumor growth and excess vascularization. Indomethacin, a nonsteroidal anti-inflammatory drug that inhibits COX-1 and COX-2, has been shown to induce cell death in tumors and reduce tumor size and vascularization in mice with mammary tumors [42]. Furthermore, NS-398, a COX-2 inhibitor, reduces the expression of VEGF mRNA in mouse carcinoma cells [43]. Gene knockdown of COX-2 also influences growth factor production; siRNA of COX-2 in MDA-MB-231 (breast cancer) cells reduces VEGF-C production [44]. These findings suggest a link between COX-2 and angiogenesis.

### 2.2 LOX and cytochrome P-450 metabolites

Relative to COX, less research has focused on the roles of 12-LOX, 5-LOX, and CYP in angiogenesis. 12-LOX is an enzyme that catalyzes the formation of 12-HETE from AA

and 5-LOX catalyzes the formation of HPETE and subsequently several inflammatory leukotrienes. 12-HETE is produced by tumor cells and induces tumor metastasis by activating growth factors such as bFGF and PDGF [45]. When prostate cancer cells (PC3) with or without 12-LOX were injected into mice, 12-LOX PC3 mice had larger tumors, increased vascularization, and decreased tumor necrosis compared to PC3 control mice [46]. Similarly, the inhibition of 12-LOX blocks the stages necessary for angiogenesis. BHPP, a 12-LOX inhibitor, reduces VEGF-induced cell migration whereas overexpression of 12-LOX stimulates endothelial cell motility [22]. In rodent tumor models, inflammation, upregulation of 5-LOX, and increases in MMP-2 and VEGF have been found to occur concomitantly [47, 48]. Overall, an anti-angiogenic cancer treatment that inhibits both COX and LOX classes of enzymes may be more effective than blocking either COX or LOX separately because the production of AA derived pro-angiogenic metabolites would be minimized [49].

Cytochrome P450 transforms AA into epoxyeicosatrienoic acids (EETs) [50]. Cells that over-express CYP show increased angiogenesis *in vitro* and enhanced matrix metalloproteinase activity [51]. Furthermore, EET incubation causes endothelial cells to proliferate in a dose-dependent manner in addition to increasing endothelial cell migration, with EET signaling through the MAPK and PI3/kinase pathways [52]. Moreover, EETs may function as crucial second messengers in response to VEGF; EET antagonists abrogate VEGF-induced endothelial tube cell formation [50].

To summarize, two lines of evidence support the pro-angiogenic effects of AA metabolites; first, high eicosanoid concentration due to high activity of COX-2, 12-LOX, 5-LOX, and CYP 450, upregulates growth factors through a variety of mechanisms. *In vivo*, tumor tissue shows increased vascularization in response to high levels of AA metabolites. Second, inhibition of COX-2, 12-LOX and 5-LOX attenuates angiogenesis through downregulating growth factor expression.

### 3 The effects of omega-3 fatty acid metabolites on angiogenesis

#### 3.1 Decreased production of pro-angiogenic *n*-6 eicosanoids

*n*-3 FA modulate angiogenesis by suppressing downstream metabolites of AA. Two critical elements of AA metabolism are the availabilities of substrate and enzyme. *n*-3 FA target both factors related to AA metabolism, displacing *n*-6 FA from the cell membrane and competing for COX, LOX, desaturases, and elongases [53]. Supplementation with fish oil lowers production of the pro-angiogenic, AA-derived metabolites PGE2 and LTB4 [54]. *n*-3 FA work as natural

inhibitors of *n*-6 eicosanoids and have the added benefit of antitumor properties, which include increased cancer cell apoptosis [55], altered estrogen metabolism, and decreased production of free radicals or reactive oxygen species [56].

#### 3.2 Transcriptional downregulation of growth factors

Population studies have demonstrated a strong chemopreventive effect of *n*-3 FA. Populations that ingest high levels of fatty fish have a lower risk of developing cancer. Moreover, the VEGF levels of individuals with lower serum *n*-6 FA/*n*-3 FA ratios show lower circulating blood levels of VEGF [57]. *In vitro*, *in vivo*, and human experiments show that EPA and DHA inhibit angiogenesis growth factors including VEGF, PDGF, COX2 and PGE 2, and MMP 2 [58].

The *n*-3 FA metabolites resolvin D1 (RvD1) and E1 (RvE1) are potent downregulators of VEGF and cytokines. In one study, corneal neovascularization was induced in mice and *n*-3 resolvins were then subconjunctivally injected with aspirin-triggered lipoxin A4 analog (ATLa), RvD1 or RvE1 at 48-h intervals. The mice treated with lipid mediators had reduced mRNA expression of VEGF (A, C and R2), TNF- $\alpha$ , IL-1 alpha, IL-1 beta, and suppressed hemoangiogenesis due to reductions in the production of pro-angiogenic interleukins [20]. Similarly, in a mouse model of pathological retinal angiogenesis, *n*-3 supplementation was found to be effective in reducing pathologic neovascularization; this effect that was largely mediated by the ability of *n*-3 metabolites to block the production of pro-inflammatory cytokine TNF- $\alpha$  [59]. A proposed mechanism by which *n*-3 FA regulate pro-angiogenic and inflammatory cytokines is through the suppression of transcription factor NF $\kappa$ B [60]. Thus, *n*-3 FA have robust and varied nutrigenomic effects and are a natural substance that counteracts the pro-angiogenic actions of a high *n*-6 FA diet [61].

#### 3.3 Suppression of inflammation

Inflammation and angiogenesis have several mechanisms and pathways in common, so it is important to understand that *n*-3 FA inhibit inflammation in several ways; omega-3 FA (1) displace inflammatory omega-6 FA and prevent them from making pro-inflammatory eicosanoids; (2) they downregulate gene transcription of the enzymes (*e.g.*, COX-2) that catalyze the synthesis of lipid mediators; (3) they decrease the generation of inflammatory cytokines (*e.g.*, TNF-2, IL-6, IL-1 $\beta$ ); and (4) they generate novel anti-inflammatory compounds, including the resolvins and protectins [62]. In fact, low *n*-3 FA levels in the Western diet are associated with chronic inflammatory disorders such as heart disease, cancer, Crohn's disease, lupus, psoriasis colitis, pancreatitis, asthma, hepatitis, liver disease, and supplementation with *n*-3 may decrease these diseases [31, 63–72].

The relationship between inflammation and angiogenesis is multidirectional: inflammation can stimulate angiogenesis, angiogenesis can induce inflammation [73], and inflammatory cytokines, macrophages, and leukocytes can release angiogenic growth factors [74]. For instance, IL-1 induces inflammation and also contributes to tumor invasiveness *via* the production of VEGF and TNF $\alpha$  [75]. One disease that has benefitted from adopting both anti-inflammatory and anti-angiogenic approaches to treatment is rheumatoid arthritis. The inflammation of the rheumatoid synovium can be likened to a tumor; blood vessels provide nutrients to the inflamed synovium and are a portal for chemokines, which exacerbate and increase inflammation [76]. An  $\alpha v/\beta 3$  integrin inhibitor was shown to both reduce symptom severity and increase apoptosis of angiogenic blood vessels in a rabbit model of arthritis [77]. Thus, a reduction in inflammation is one of the ways that  $n-3$  FA modulate angiogenesis.

#### 4 Perspectives

To summarize, thus far this review has emphasized two main points. The first is that  $n-6$  FA metabolites promote tumor angiogenesis through a variety of signaling pathways, encouraging epithelial cell proliferation and migration, and decreasing tumor apoptosis. The second is that  $n-3$  FA and their metabolites can reverse the pro-angiogenic consequences of high  $n-6$  FA metabolite levels. The  $n-3$  FA lower production of  $n-6$  FA-derived prostaglandins and leukotrienes and downregulate growth factor expression (Fig. 2). In this final section, we hope to integrate these two ideas by highlighting a potentially important factor in regulating angiogenesis and tumor growth: the  $n-6$  FA/ $n-3$  FA ratio (Fig. 2).

Given that  $n-6$  FA and  $n-3$  FA have opposing effects and compete for the same enzymes, lowering the ratio of  $n-6/n-3$  FA in tissues shows great potential for controlling dysregulated angiogenesis and reducing cancer risk. However, few epidemiological studies have directed attention towards the  $n-6/n-3$  FA ratio as it relates to their findings, instead favoring absolute levels of  $n-6$  or  $n-3$  FA. This lack of attention may be one factor contributing to the disparity between epidemiological and lab-based studies on the anticancer effects of  $n-3$  FA. Only approximately 1/3 of epidemiological studies have found an association between  $n-3$  FA and cancer risk although *in vivo* and *in vitro* experiments have consistently demonstrated that  $n-3$  FA can modulate angiogenesis, tumor growth, and cancer risk.

Several epidemiological studies have concluded that there is no significant effect of  $n-3$  FA on cancer risk, but these studies have only accounted for absolute as opposed to relative levels of  $n-3$  and  $n-6$  FA. For example, in a meta-analysis that reviewed 38 different prospective cancer cohorts, only 10

out of 65 measures concerning  $n-3$  intake and cancer risk showed positive statistical relationships [78]. While the authors admitted a large discrepancy between their research and lab studies, they concluded that, “Dietary supplementation with omega-3 fatty acids is unlikely to reduce the risk of cancer.” Although most of the studies included in the analysis represented large cohorts, many of the studies that found no beneficial effect of  $n-3$  FA on cancer risk only examined  $n-3$  FA levels but not the  $n-6/n-3$  ratio. For example, a study of Swedish women used the absolute intake of ALA, LA, EPA, and DHA to conclude that no relationship existed between  $n-3$  intake and cancer [79]. Several studies have found that absolute ALA levels are positively correlated with prostate cancer but have not accounted for the  $n-6/n-3$  FA ratio in their analyses [80, 81].

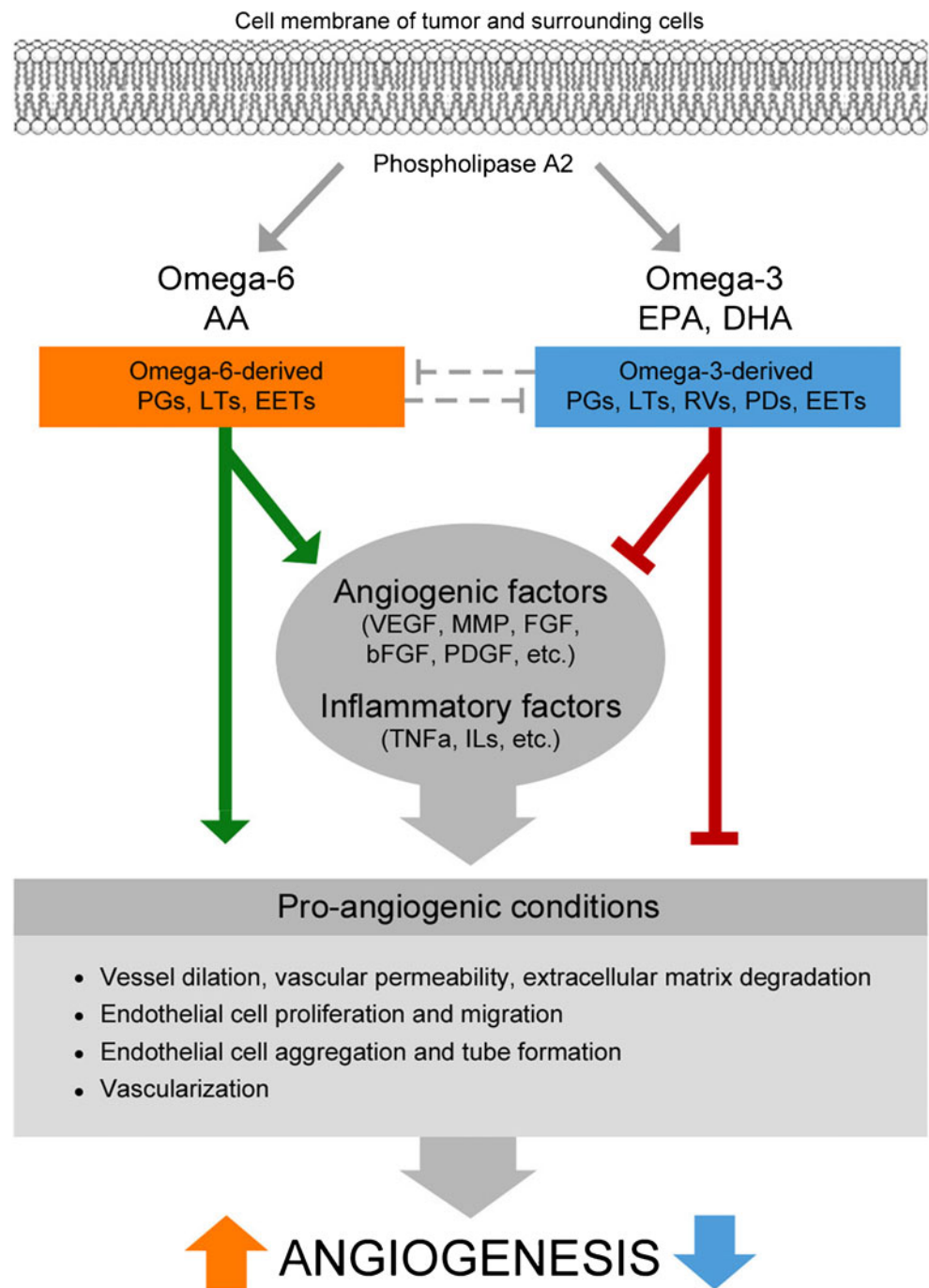
The Health Professionals Follow-Up Study chose the absolute intake of ALA, AA, LA, EPA, and DHA as the basis for their conclusion that ALA intake is positively associated with advanced prostate cancer [82]. However, when looking at the highest and lowest quintiles of relative risk, there was one statistic “LA: EPA+DHA” that served as a rough estimate of the  $n-6/n-3$  FA ratio. For the lowest quintile of relative risk, this value was  $<23.57$  whereas for the highest quintile of relative risk the LA/EPA+DHA ratio was  $>102.34$ . This indicates that the  $n-6$  FA/ $n-3$  FA ratio may be important and that accounting for AA and LA intake along with ALA levels may have shifted the authors’ conclusions. It would be interesting to reanalyze these data and those from other epidemiological studies using the  $n-6$  FA/ $n-3$  FA ratio.

Several studies have found significant correlations between the  $n-6$  FA/ $n-3$  FA ratio and cancer risk. The EURAMIC study showed a decrease in the risk for developing breast cancer when the ratio of  $n-6$  FA/ $n-3$  FA was decreased, but no association when  $n-6$  FA intake and  $n-3$  FA intake were considered separately [83]. After examining adipose tissue in breast cancer patients and in patients with benign breast disease, the ratio of  $n-6$  FA/ $n-3$  FA was significant; women in the highest tertile of the long-chain  $n-3$ /total  $n-6$  ratio had an odds ratio of 0.33 [84]. In addition, a high ratio of  $n-6$  FA/ $n-3$  FA in Caucasian men has been found to be associated with an increased risk of prostate cancer [85]. Standardizing analysis methods using the  $n-6$  FA/ $n-3$  FA ratio may lead to more consistent results regarding the anticancer effects of  $n-3$  FA.

##### 4.1 The fat-1 mouse model

In both animal and human studies, there are technical difficulties in generating accurate dietary ratios of  $n-6$  FA/ $n-3$  FA for research and other dietary nutrients may interact with fatty acid metabolism and its actions. Thus, both epidemiological and *in vivo* animal studies may not give a full

**Fig. 2** The effects of  $n-6$  FA and  $n-3$  FA and their metabolites on angiogenesis. Phospholipase A2 releases  $n-6$  and  $n-3$  from the phospholipid cell membrane. The  $n-6$ -derived eicosanoids, which include the prostaglandins (PGs), leukotrienes (LTs), and epoxyeicosatrienoic acids (EETs) promote tumor angiogenesis and inflammatory factors. In contrast,  $n-3$  FA and their metabolites suppress the activity of angiogenic and inflammatory factors and ultimately inhibit excess vascularization. Overall,  $n-6$  FA promote pro-angiogenic conditions whereas  $n-3$  FA discourage them



picture. The fat-1 mouse is a transgenic animal that has the ability to endogenously convert  $n-6$  FA to  $n-3$  FA because it has a copy of the *Caenorhabditis elegans* fat-1 gene, making it an ideal model for controlling the tissue ratio of  $n-6$  FA/ $n-3$  FA and eliminating the confounding factors associated with dietary supplementation [86]. Two different  $n-6$  FA/ $n-3$  FA ratios can be established in fat-1 mice from the same litter when they are fed an identical, high  $n-6$  FA diet; the wild type mice exhibit a high tissue  $n-6$ / $n-3$  ratio whereas the fat-1 mice will have a lower  $n-6$ / $n-3$  ratio.

Experiments using the fat-1 mouse have shown a reduction in pro-angiogenic molecules, such as down-regulation of the pro-angiogenic factors NF $\kappa$ B, TNF $\alpha$ , and IL-1 $\beta$  [87]. Furthermore, fat-1 mice with melanoma exhibit high levels of  $n-3$  prostaglandins in tumor and surrounding tissues accompanied by decelerated melanoma progression [88]. Studies from our and other laboratories have consistently demonstrated that decreasing the ratio of  $n-6$  FA/ $n-3$  FA conveys antitumor effects [88–96].

#### 4.2 Clinical applications and implications for cancer therapies

Given that  $n-6$  FA increase angiogenesis,  $n-3$  FA decrease angiogenesis, and tumor progression and metastasis depend on angiogenesis, reducing the tissue  $n-6$  FA/ $n-3$  FA would appear to be a new strategy for cancer treatment. The two distinct approaches to addressing angiogenesis in cancer are either (1) using drugs that block the production of AA metabolites or (2) dietary supplementation with  $n-3$  FA. Both treatments only regulate absolute levels of AA-derived eicosanoids or  $n-3$  FA-derived eicosanoids. The ideal therapy would be to combine a reduction in tissue levels of  $n-6$ -derived eicosanoids with an increase in tissue levels of  $n-3$ -derived lipid mediators.

Cancer drugs that target AA eicosanoid production focus on COX-2 and LOX-5 inhibition. For example, in a prospective cohort study of nearly 48,000 men, those who took aspirin more than two times per week had a lower risk for colorectal cancer [97]. However, direct COX-2 inhibition is not a sound approach because this also inhibits production of antitumor  $n-3$ -derived eicosanoids and can be associated with an increased risk of heart attack/stroke and other undesirable side effects. Furthermore, a drug-based approach overlooks other methods of regulating  $n-6$  eicosanoid production, such as increasing dietary intake of  $n-3$  FA [98]. Coupling these inhibitors of AA metabolites with increased  $n-3$  FA intake to slow the growth of metastatic cells not only increases treatment efficacy by further targeting COX-2 and LOX 5 enzymes but is also a natural method with few, if any, harmful side effects. Furthermore,  $n-3$  FA convey benefits to cancer patients beyond modulating  $n-6$  metabolites, including prolonging survival, increasing immune function, decreasing postsurgery complications, and possibly reversing cachexia [99–101].

In conclusion, the upregulation or overproduction of AA-derived metabolites encourages angiogenesis, and this effect is suppressed by the  $n-3$  FA-derived eicosanoids. Overall, epidemiological studies have failed to account for the  $n-6/n-3$  ratio in their analyses but from a biochemical perspective it is clear that a high tissue  $n-6/n-3$  FA ratio promotes inflammation and angiogenesis. One practical clinical intervention would be lowering the  $n-6/n-3$  FA ratio to reduce tissue levels of AA-derived pro-angiogenic eicosanoids by increasing intake of  $n-3$  and decreasing intake of  $n-6$  FA.

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