Abstract[*]

It has been shown in many studies that life style and high-fat diets can contribute to the risk of development of cancer. Arachidonic acid (AA) and eicosapentaenoic acid (EPA) are fatty acids that are abundantly present in foods, mainly in meat and fish, and can also be produced by the body itself from essential fatty acids linoleic acid and α-linolenic acid. Biological active fatty acid-derivatives formed from AA and EPA, like prostaglandins, leukotrienes and thromboxanes, play important roles in the regulation of the immune response, platelet aggregation. However these fatty acids are also implicated in cancer progression. This review will discuss how fatty acids are produced, how they are excreted in the circulation and how they can be transported in the circulation. Furthermore, we will focus on possible routes that can be used to enter target cells, their functions and interactions, possible intermediate factors that determine their activity and their role in cancer.

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[*] Due to confidentiality reasons this is not the original version of my thesis. For additional information please contact my supervisor prof. E. E. Voest (e.e.voest@umcutrecht.nl)

1. *What are fatty acids?*

Fatty acids (FA) consist of a long, hydrophobic hydrocarbon chain and a terminal hydrophilic carboxylate group and they have four major physiological roles. First, fatty acids are the building blocks for phospholipids, which are important components of cellular membranes. Second, fatty acids are as source of energy, they are stored in the body as triglycerides in adipocytes. The high caloric value of fatty acids makes them ideal as an alternative source of energy in addition to carbohydrates. Third, many proteins are posttranslationally modified by covalent binding to fatty acids, these modifications will target the proteins towards membrane locations. Fourth, fatty acids and derivatives of fatty acids can function as hormones and intracellular signalling molecules [1].

Some fatty acids are considered essential fatty acids (EFAs) since the body can not produce these fatty acids itself, and therefore need to be ingested via the diet. There are 2 EFAs, α-linolenic acid (ALA), an omega-3 fatty acid and linoleic acid (LA), an omega-6 fatty acid (see figure 1). ALA is abundantly present in fish and fish oil, whereas LA is more abundant in meat. The denotation of omega-3 and omega-6 is based on the position of the first double carbon-carbon bond, counting from the terminal methyl carbon. So in the case of an omega-3 fatty acid, like ALA, the first double bond is on the 3th carbon-carbon bond counting from the methyl carbon end of the molecule [1].
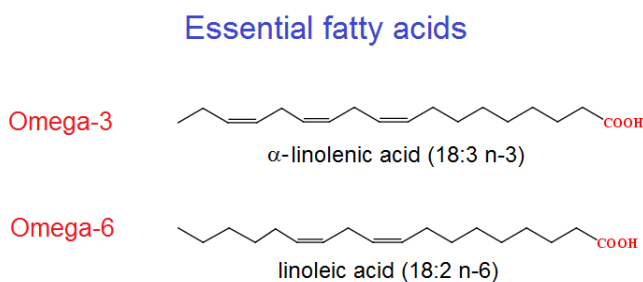

Figure 1: Structural formulas of the two essential fatty acids, α-linolenic acid and linoleic acid. α-linolenic acid is an omega-3 fatty acid and linoleic acid is an omega-6 fatty acid. This denotation is based on the position of the first double bound carbon-carbon bond counting from the methyl carbon end of the molecule. The human body is incapable of producing these fatty acids and therefore need to be ingested via food.

2. *General functions of omega-3 and omega-6 fatty acids and its metabolites*

In general the fatty acids derived from AA have pro-inflammatory functions like, among others, increasing vascular permeability, neutrophil chemotaxis, platelet aggregation and bronchial smooth muscle contraction [2]. The EPA-derived fatty acids have anti-inflammatory functions, like inhibition of platelet aggregation and blood clotting [3]. Next to immunity-related functions some AA-derived fatty acids are also implicated in cancers of the lung, breast, colon, prostate and head and neck. These PUFAs can promote tumor progression via cell proliferation, survival and anti-apoptosis. In addition, the overexpression of several AA-derived PUFAs is associated with poor prognosis in various cancers (see also page 20) [4]. In

contrast to this, the EPA-derived fatty acids have been implicated to have protective effects against several diseases, like atherosclerosis, thrombosis, hypertriglyceridaemia, high blood pressure, asthma and psoriasis. Furthermore, EPA is thought to have protective properties against breast, prostate and colon cancer although some contradictory results are present within the literature [5,8].

3. Omega-3 and omega-6 fatty acid metabolism

The two essential fatty acids, ALA and LA, are taken up in the body via the intestine. The fatty acids are present as triacylglycerols when they are ingested via the diet. These triacylglycerols need to be digested to fatty acids before transport across the intestinal epithelium can occur. Bile salts solubilize the triacylglycerols within the intestinal tract, leading to the formation of micelles. Pancreatic lipases present in the small intestine digest the lipids into monoacylglycerol and free fatty acids, which can then be transported across the epithelium. Within the intestinal epithelial cells the monoacylgyerols and free fatty acids are resynthesized into triacylglycerols and together with apolipoprotein B-48 packaged into chylomicrons. These chylomicrons are then released into the lymph system and via there also in the blood. Once in the blood, the chylomicrons can bind to membrane-bound lipoprotein lipases present on cells, here the triacylglyerols are broken down again into free fatty acids and monoacylglycerol, which can then be transported into peripheral tissues. Once inside the cell, the essential free fatty acids can be further metabolized [1].

Inside the cell the essential fatty acids can be metabolized to several signaling molecules. Both LA and ALA can be metabolized to prostaglandins, prostacyclins, leukotrienes and thromboxanes. These signalling molecules play important roles in immunity and blood clotting. Overall the signalling molecules formed from linoleic acid counteract the signalling molecules formed from α-linolenic acid and vice versa. When the LA and ALA enter the cell they are modified via a series of enzymes to arachidonic acid (AA) and eicosapentaenoic acid (EPA), respectively. The enzymes responsible for these modifications are, in order, Δ6 desaturase, elongase and Δ5 desaturase. However, AA and EPA are also present in foods, so the modification from LA and ALA to AA and EPA are not always a necessity [6-8].

3.1. AA metabolism

In the cell AA and EPA can both be processed by two enzymes, cyclooxygenase 1 and 2 (COX1/2) and lipoxygenase-5 (5-LOX). The COX1/2 pathway of AA and EPA leads to the formation of prostaglandins, prostacyclins and thromboxanes. The 5-LOX pathway of AA and EPA leads to the formation of leukotrienes. The best defined pathway is that of AA (see figure 2) [4]. Extracellular stimuli like membrane damage and increases in intracellular calcium concentrations can lead to the release of phospholipids from the cell membrane and conversion of these phospholipids into AA by phospholipase A2 [2,9,10].

Figure 2: Schematic flow chart of the omega-6 pathway. LA, which is ingested via the diet, is converted to EPA. EPA can be further metabolized into prostaglandins, prostacyclins, thromboxanes and leukotrienes. The functions of these fatty acids are generally pro-inflammatory, as depicted in the green boxes. Enzymes responsible for the conversion of one fatty acid to another are depicted in red.

To date 15 different phospholipase A2's have been identified, however it has been shown that the cytosolic form of phospholipase A2 group IV (cPLA2) is responsible for the hydrolysis of phospholipids leading to the production of AA [7]. When AA is released from the membrane, 5-LOX can convert AA into 5-hydroperoxyeicosatetraenoic acid (5-HPETE). This is a precursor molecule for the formation of leukotrienes A4, B4, C4, D4 and E4. The COX1/2 pathway of AA will lead to the production of prostaglandin H2. This is a precursor for the formation of other prostaglandins, prostacyclins and thromboxanes. Which fatty acid derivative gets formed from prostaglandin H2 depends on the enzyme, prostacyclins are made by prostacyclin synthase, thromboxanes are made by thromboxanes synthase and prostaglandins are made by prostaglandin synthases [7].

Omega-3
Anti-inflammatory

Essential Dietary FFA

α-Linolenic Acid (ALA)

Plasma membrane

α-Linolenic Acid (ALA)

Eicosapentaenoic Acid (EPA)

5-LOX — 5-HPEPE

COX1/2 — Prostaglandin G3

Leukotriene B5

Leukotriene A5

Thromboxane A3   Prostacyclin 3   Prostaglandin E3

Leukotriene C5

anti-thrombotic effects   inhibition of platelet aggregation
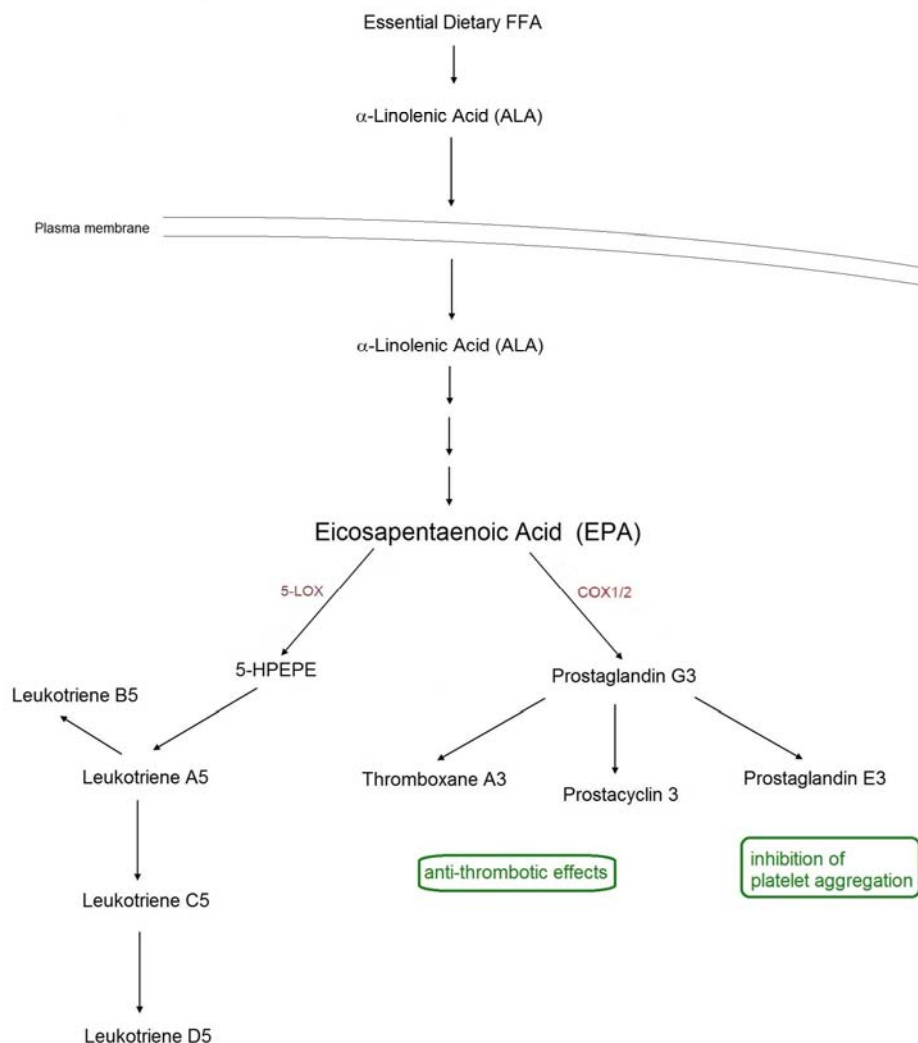
Leukotriene D5

Figure 3: Schematic flow chart of the omega-3 pathway. Uptake of essential dietary fatty acids like ALA leads to the production of EPA. Which can be further metabolized into prostaglandins, prostacyclins, thromboxanes and leukotrienes. The omega-3 fatty acid derivatives have general anti-inflammatory functions as depicted in the green boxes.

3.2 EPA metabolism

The metabolism of EPA is less well defined. In the cell EPA can be metabolized by COX1/2 and 5-LOX (see figure 3). As for the metabolism of AA, the LOX pathway of EPA leads to the formation of leukotrienes. In contrast to the leukotrienes formed from AA, the function of the EPA-derived leukotrienes are not well known. The COX pathway of EPA leads to the formation of prostaglandin G3, which can be further metabolized by three different enzymes; thromboxane synthase can convert prostaglandin G3 to thromboxane A3 by, prostaglandin E synthase can metabolize prostaglandin G3 to prostaglandin E3 and finally prostacyclin synthase can convert the prostaglandin G3 precursor to prostacyclin 3 [8].

4. Free fatty acid transcellular biosynthesis

As complex as the pathways of AA and EPA may already seem, it has been postulated that several cell types can be involved in the production of several end products from the AA pathway, this process is referred to as transcellular biosynthesis [13]. Evidence for this hypothesis comes from studies in which human umbilical cord endothelial cells that were treated with indomethacin, a non-selective COX1 and 2 inhibitor, and cultured in the presence of platelet rich plasma were still able to produce prostacyclin 2. Suggested was that the human umbilical cord endothelial cells could receive the COX product, prostaglandin H2, from the platelets, thereby still being able to produce prostacyclin 2 and finish the pathway. Evidence supporting this hypothesis was delivered by Marcus *et al*, who showed that radioactive labelled prostaglandin H2 could be taken up by endothelial cells and converted to prostacyclin, despite treatment of the endothelial cells with the COX1/2 inhibitor aspirin [14]. These results indicate that transport of the lipophilic prostaglandin H2 can occur from platelets to endothelial cells, in addition to this Karim *et al*. showed that the reverse is also possible. Aspirin treated platelets were able to take up prostaglandin H2 from endothelial cells and restore thromboxane A2 production [15].

Important *in vivo* evidence for transcellular biosynthesis of AA derived signalling molecules came from Fabre *et al*. in 2002. They used 5-LOX knockout mice. The 5-LOX knockout mice are unable to produce 5-HPETE, the precursor of all AA-derived leukotrienes. These mice were lethally irradiated and injected with bone marrow from LTA4-H knockout mice. These mice can still form all AA-derived leukotrienes except leukotriene B4, since it lacks the enzyme responsible for the conversion of leukotriene A4 to leukotriene B4. Remarkably, injecting 5-LOX -/- mice with LTA4-H -/- bone marrow still led to production of leukotriene

B4 which was due to transcellular biosynthesis of leukotriene A4 from the LTA4-H -/- bone marrow derived cells to the 5-LOX -/- cells and subsequent conversion to leukotriene B4 by these cells [16].

A lot of research has been done *in vitro* and *in vivo* on the transcellular biosynthesis of AA-derived fatty acids, However, no evidence yet exists that similar mechanisms of transcellular biosynthesis occurs within the EPA pathway. However, it has been shown that administration of exogenous EPA can increase production of EPA-derived leukotrienes in rabbit lung tissue, so exogenous EPA can be transported into the cells where conversion towards end products can occur, although no research has been done on the involvement of different cell types in the exchange of intermediate products leading to production of end products of the EPA pathway [17].

These studies done on transcellular biosynthesis have shown that several molecules in the AA pathway, like prostaglandin H2 and leukotriene A4, can travel from one cell type to another to complete the formation of end products of this pathway. The potential role for intermediate cell types points to a multilayered regulation of the production of AA-derived PUFAs. Not only the presence of the enzymes that are involved in the AA-pathway can regulate the production but also the presence of different cell types can regulate the production of AA derivatives.

5. *Regulation in the production of AA and EPA-derived PUFAs*

The biologically active PUFAs from AA and EPA counteract each others functions; therefore it is likely that AA and EPA-derived PUFAs somehow regulate each other. To start with, the relative amount of AA or EPA present in the cell membrane is factor that determines with pathway is activated. Also, there is competition between LA and ALA for the enzymes that convert them into AA and EPA, respectively. These enzymes are required for both pathways, thereby regulating the formation of AA and EPA-derived metabolites. Further downstream in the pathway AA and EPA also compete for COX and LOX. In general AA is more abundant in the cell, however EPA has higher affinity for these enzymes [5]. In addition various studies report the presence of inhibitory functions of AA metabolites on the EPA pathway and vice versa. It has been shown in fibroblasts, addition of AA to the culture medium can block formation of EPA-derived PUFAs. However, EPA was not able to reduce the production of AA-derived PUFAs [18]. Similar feedback mechanisms are present in other cell types. Whatley

et al. reported that endothelial cells cultured under EPA-enriched conditions produce relatively less of the AA product prostacyclin 2 than controls, indicating the presence of feedback mechanisms between the AA pathway and EPA pathway [19] and research by Tsunomori *et al*. has shown that 15-HPEPE, a metabolite of the EPA pathway, is able to inhibits AA metabolism in platelets [20]. Furthermore, results from Ishihara et al. indicated that ((16:4) n-3) is able to suppress AA-derived leukotriene B4 and C4 production in mouse mast cells. However, the level of inhibition was less than the level of inhibition seen upon EPA treatment [21]. The various levels of AA-EPA regulation make that these pathways are in balance with each other.

6. Various cell types produce AA and EPA-derived PUFAs

Many of the end products of the AA and EPA pathway are involved in regulation of immune responses. Therefore, it makes sense that many cells of the immune system are able to produce these biologically active PUFAs. Macrophages, monocytes, neutrophils and mast cells are the main producers of AA and EPA derived PUFAs. However, there are some quantitative differences in the production between cells. Monocytes and macrophages produce large amounts of prostaglandin E2 and prostaglandin F2, neutrophils produce moderate amounts of prostaglandin E2, and mast cells produce only prostaglandin D2. Leukotrienes are produced by monocytes, macrophages and mast cells [2]. Furthermore, blood platelets are also important in the formation of AA and EPA-derived PUFAs. Stimulation of platelets, by for instance thrombin, leads to increased calcium concentration and massive release of AA from phospholipids and production of active metabolites [22]. Endothelial cells are also able to produce AA and EPA-derived metabolites. The production of either AA or EPA-derived fatty acids is determined by the presence of these PUFAs in the plasma membrane [19]. Another cell type that is able to convert AA and EPA into its metabolites are fibroblasts. Williard *et al*. showed by radioactive labelling of EPA that EPA-derived PUFAs like tetradecatrienoic acid ((14:3) n–3) were formed by β-oxidation in peroxisomes in these fibroblasts. Peroxisomal β-oxidation is a important cellular mechanism to metabolize very long chain fatty acids (C≤20) and it is not coupled to ATP production like the β-oxidation that takes place in mitochondria [23].

7. Omega-3 and omega-6 fatty acid metabolism in micro-organisms

Multiple species are able to produce and metabolize omega-3 and omega-6 fatty acids. The production of AA and EPA has been described in many micro-organisms. The research of AA

and EPA production in micro-organisms dates back several decades. In 1988, Yasawa *et al.* isolated several bacteria strains that were able to produce EPA. In the bacteria from the genus Altermonas the EPA production was 40% of the total fatty acid content [25]. Also, lower fungi of the class of Phycomyecetes (especially the order of Mucorales) are able to produce AA and EPA. Interestingly, the temperature in which the microbes were grown determined whether AA or EPA was made [8]. This form of regulation between AA and EPA production in micro-organisms shows similarities between the regulation of the AA and EPA pathways in mammals.

Furthermore, a wide variety of marine algae are able to produce EPA. These algae are also responsible for the high content of EPA in fish since many fish eat these algae leading to accumulation in the food-chain [26].

8. *Release of AA and EPA-derived PUFAs into the circulation*

After the formation of the fatty acids derived from the AA and EPA pathway, these metabolites are secreted into the circulation. A lot of research has been done on how prostaglandins, thromboxanes and leukotrienes are released from cells. For some time researchers thought that the release of the lipophilic AA and EPA metabolites occurred via passive diffusion through the membrane. However, their high lipophilicity was able explain the migration of the molecules into the membrane, but not their migration through the membrane. So specific transporters are needed to transport the AA and EPA metabolites through the membrane. The multidrug resistance protein 4 (MRP4) has been shown to facilitate the active transport of prostaglandins and leukotrienes out of the cell. This efflux pump is a member of the ATP binding cassette transporter family of proteins. *In vitro* studies have shown that cells with MRP4 overexpression facilitate the export of anticancer drugs from cells [29]. Thereby this transport pump can contribute to protection of the tumor to medication.

Another mechanism by which AA and EPA-derived PUFAs can be excreted in the circulation is via exosomes. Exosomes are small vesicles released from the cell from multivesicular bodies or late endosomes. A wide variety of stimuli can activate the production of exosomes, like cytokines, cell differentiation, senescence, ATP exposure, activation of oncogenes, loss of the p53 tumor suppressor gene, and stress. Exosomes can contain various proteins, mRNAs and miRNAs and they have been implicated in cancer [30]. It has been shown by Skog *et al.* that

glioblastomas can secrete exosomes containing angiogenic proteins, cell proliferation, migration, immune response and angiogenesis-related mRNAs and miRNA. These exosomes were able to influence the tumor environment. Uptake of the exosomes in nearby cells promoted tumor growth and angiogenesis [31]. Similar results were shown for colorectal cancer. Exosomes secreted from these tumors were also able to promote tumor progression, mainly via enhancing endothelial proliferation and angiogenesis [32]. Interestingly, both the reports of Skog *et al.* and Hong *et al.* showed that several mRNAs in the exosomes were enriched above levels present in the donor cells, indicating that a specific mechanism is responsible for the selection of mRNAs for exosomal secretion. Research by Subra *et al*, has shown that exosomes secreted by mast cells contain AA, phospholipase A2 and several AA-derived PUFAs. In addition, it was shown that these exosomes could be efficiently internalised by other mast cells, reaching sufficient intracellular concentrations to be biologically active [33].

9. *Transport of PUFAs in the circulation*

After the release of PUFAs from the cell, long distance transport occurs via the circulation. The high hydrophobicity of PUFAs makes that they do not dissolve well in aqueous solutions like blood. However, sufficient transport of PUFAs is necessary, not only for the biologically active metabolites of AA and EPA but also for the dietary fatty acids that require transport to peripheral tissues in need of energy supplies. This requires transporters in the blood that are able to meet up to the demand. Triacylglyerols are transported through the circulation in large particles consisting of lipids and lipoproteins, called low density lipoproteins (LDL) and high density lipoproteins (HDL). LDL transports excess dietary lipids in the blood from the liver to the periphery. HDL performs the reverse reaction, it transports lipids from the periphery to the liver and therefore is also important in the excretion of lipids from the body [1]. Lipids are transported through the circulation in different ways than free fatty acids. The major free fatty acid transporter in the blood is albumin, which is able to bind many different fatty acids. Each albumin protein contains 7 binding sites for fatty acids. Some disagreement exists about the affinity of the individual binding sites, but generally it is excepted that there are 2-3 sites with high affinity and 4-5 sites with intermediate affinity [35]. Little research has been done on how the fatty acids bound by albumin in the circulation can be transferred to target cells, but 4 hypotheses have been proposed (see figure 4). Route 1 represents diffusion between endothelial cells into the interstitium, where fatty acids are dissociated from albumin and ready for uptake in or binding to target cells. Route 2 represents direct transport of the albumin-fatty acid complex through the endothelial cells followed by dissociation in the

interstitium and accessibility of fatty acids to target cells. Route 3 reflects dissociation of the fatty acids from albumin in the blood stream and transport of fatty acids via diffusion between the endothelial cells.
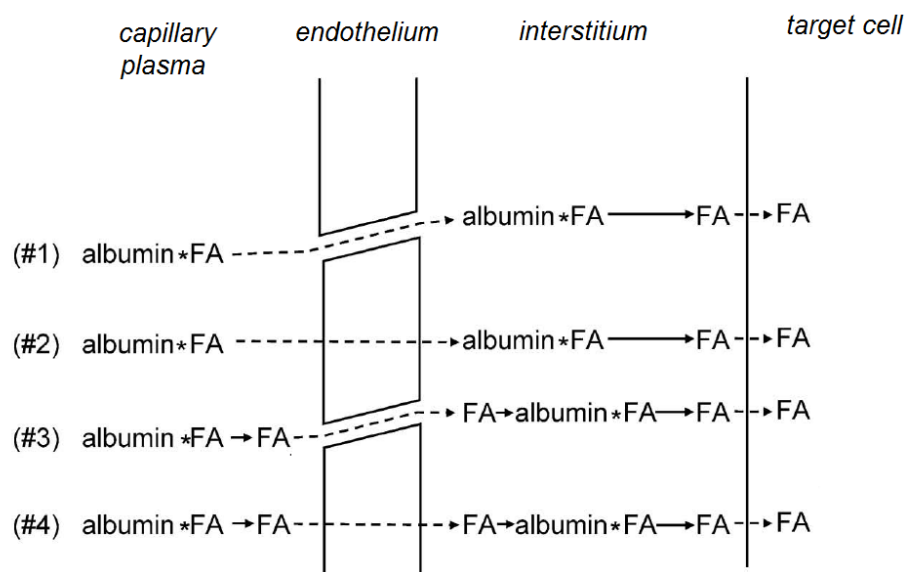

Figure 4: schematic picture of the 4 hypothetic routes of fatty acid delivery to target cells. FA: fatty acid [35].

Thereafter, the fatty acids will bind albumin again in the interstitium for further transport. Route 4 represents dissociation of the fatty acids from albumin followed by transport through the endothelial cells and binding to albumin again in the interstitium. Research has revealed that route 1 and 3 are unlikely since the endothelium presents a major barrier that does not allow passive diffusion of large molecules like fatty acids or albumin [36,37]. There is some evidence from electron microscopy studies that route 2 is possible at least in cardiac endothelium via vesicular transport [38]. However, the estimated transport time was in the order of 5 minutes, which is, based on the quick acting functions of most AA and EPA derived PUFAs and the high demand of the heart for fatty acids as an energy supply, not the main route of action. The discovery that albondin, a protein present on endothelial cells, enhances dissociation of fatty acids from albumin and facilitates uptake in the endothelium renders route 4 as the most likely transport route [39].

10. Transporters and receptors of PUFAs in target cells

In order to exert its functions, the AA and EPA derived PUFAs need to interact with target cells, either by uptake in the cell or via ligand-receptor interactions. In general, long chain fatty acids can be transported into the cell via CD36, plasma membrane associated fatty acid binding protein (pmFABP) and the six members of the family of fatty acid transporter

proteins (FATP1-6). In muscle CD36 is also involved in the regulation of fatty acid uptake. CD36 is also present on endosomes, which upon contraction of the muscle cell can translocate to the membrane, thereby increasing the cell's capability to take up long chain fatty acids [40]. In respect to AA and EPA, researchers have shown that CD36 can bind AA and that increased CD36 expression on platelets enhances AA uptake [41]. Furthermore, EPA can increase CD36 expression in macrophages and muscle cells, thereby increasing fatty acid uptake in these cells [42,43]. In addition to CD36 various other transporters and receptors have been identified for AA-derived PUFAs (see figure 5 and 6). In contrast, very little is known about possible receptors or influx pump of the PUFAs derived from EPA [5].
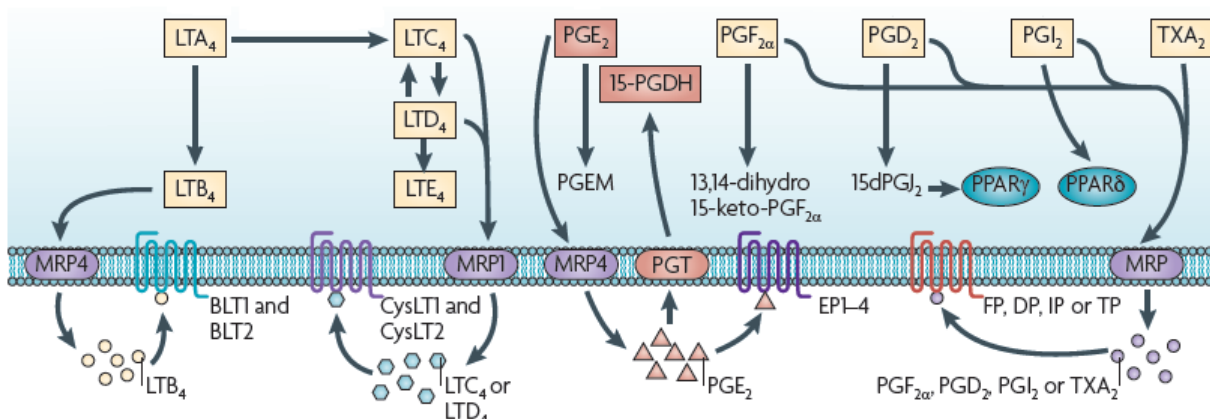

Figure 5: schematic overview of transporters and receptors that have been identified to play a role in AA-derived PUFA release, uptake and signalling. For the efflux from cells, most AA-derived PUFAs rely on members of the multidrug resistance family of proteins (MRP). Influx of prostaglandin E2 (PGE2) into the cell is mediated by a specific prostaglandin transporter (PGT). Multiple receptors have been found to bind the AA-derived leukotrienes and prostaglandins. Leukotriene B4 (LB4) can bind to BLT1 (high affinity receptor) and 2 (low affinity receptor). leukotriene C4 and D4 (LTC4 and LTD4) can bind to CysLT1 and 2 receptor. Multiple receptors, like EP1-4, DP and IP, are known to bind the different prostaglandins. For thromboxane A2 (TXA2) one receptor, TP, has been identified [4].

Ligand	Receptor
Prostaglandins	
E2	PGT, EP1, EP2, EP3, EP4
F2	PGT, FP
D2	PGT, DP, GPR44
Prostacyclins	
2	IP
Thromboxanes	
A2	TP
Leukotrienes	
B4	BLT1, BLT2
C4	CysLT1, CysLT2
D4	CysLT1, CysLT2

Figure 6: scheme with AA-derived PUFAs and their receptors.

Several receptors and transporter have been identified for the different prostaglandins. The prostaglandin transporter (PGT) is an influx pump that facilitates the uptake of prostaglandin E2, F2, D2 and in lesser extent also thromboxane B2 [44,45]. In addition to PGT, that physically imports the prostaglandins into the cell, several receptors activate intracellular cascades in

target cells upon binding to prostaglandins (see figure 6). All of these receptors belong to the family of G-protein coupled receptors. Prostaglandin D2 can bind to DP and GPR44. Prostaglandin E2 can bind to EP1, 2, 3 and 4 and prostaglandin F2 can interact with FP. For prostacyclin 2 only one receptor has been identified and that is IP. Also thromboxane A2 has one receptor, TP. Leukotriene B4 can bind to BLT1 and BLT2, however binding to the first occurs with higher affinity that the latter. To complete the list, leukotriene C4 and D4 can both bind CysLT1 and CysLT2, again the first has higher affinity for both leukotrienes than the latter [4]. In addition to the binding of AA-derived PUFAs to membrane bound receptors, some PUFAs can bind nuclear receptors of the PPAR family. Prostaglandin D2 and its hydrolysed form 15dPGJ2 can bind and activate PPARγ and prostacyclin 2 can also activate PPARγ [46]. In addition, EPA is also a PPARγ ligand [5].

11. Functions of AA and EPA-derived PUFAs

Many functions have been described for the AA-derived PUFAs. This in contrast to the EPA-derived PUFAs. Reports have been made that EPA has anti-proliferative effects via activation of PPARγ [5]. In respect to the AA-derived PUFAs, it has been shown that prostaglandin E2 can function as a potent neutrophil chemoattractant, it can cause vascular dilation and induce fever [2]. Furthermore, it has been shown that it can increase cell proliferation via Ras-ERK pathway and the GSK3β–β-catenin pathway. Also a role in anti-apoptosis has been proposed for prostaglandin E2, since it can upregulate the expression of Bcl-2, an anti-apoptosis protein, thereby inhibiting apoptosis [5]. Prostglandin E2 is also involved in angiogenesis. It can upregulate the expression of CXCL1, which is a pro-angiogenic protein [4,48]. Prostaglandin D2 derived from mast cells can increase the production of hyaluronic acid in fibroblasts by upregulating the expression of hyaluronic acid synthase 2 [49]. Hyaluronic acid is an important component in the extracellular matrix and is implicated in cell proliferation and migration via Rho, PI3K and MMP [50]. The hydrolysed form of prostaglandin D2, 15d-PGJ2 can bind to and activate PPARγ. PPARγ can activate transcription of genes involved in inhibition of cell proliferation and angiogenesis and stimulation of apoptosis and cell differentiation [51,52]. A similar function has been described for prostacyclin 2 as it can also bind PPARγ [53]. Prostaglandin F2-FP signalling can enhance the expression of fibroblast growth factor 2 (FGF2), interestingly FGF2 signalling can also enhance COX-2 expression, forming a positive feedback loop between FGF2 signalling and AA metabolism [54].

Several different functions have been described for the AA-derived leukotrienes. Leukotriene B4 is a potent chemoattractant for neutrophils, mast cells, monocytes and macrophages. It enhances adherence of inflammatory cell to the endothelium by upregulation of adhesion molecules [55]. Furthermore, it can stimulate cell survival via binding BLT1 on target cells and subsequent ERK activation. Leukotriene B4 mediated signaling in target cells can also enhance cell proliferation via Mek-ERK and PI3K-Akt [48]. Leukotriene D4 can increase cell proliferation and survival via the following pathways; GSK3β-β-catenin, PKC-Raf1-ERK and Bcl-2 and COX [4,5]. In addition, thromboxane A2 has been implicated in cellular migration and invasion by activation of Rho [56]. Furthermore, recent research has shown that AA can increase cell motility via epithelial-to-mesenchymal transition (EMT). During this process cells adopt more mesenchymal characteristics, like increased vimentin and N-cadherin expression and decreased E-cadherin expression, leading to increased cellular migration [57]. An interesting link between BLT2 and cancer progression was made by the research of Choi *et al*. They showed that the survival of ER negative breast cancer cell lines is enhanced via activation of BLT2-ROS signaling pathway. Blocking BLT2 increases apoptosis and inhibited proliferation in ER-negative breast cancer cell lines MDA-MB-468 and MDA-MB-453. Since BLT2 enhances ROS formation the authors investigated the levels of ROS in the ER-negative cell lines and showed that these were elevated upon BLT2 activity and could be blocked by BLT2 antagonists. It was shown that the increased ROS production was mediated by NADPH oxidase 1 (Nox-1) and that the ROS production protected the cell lines against apoptosis [58]. An overview of the non-immunological functions and pathways of the AA-derived PUFAs is given in figure 7.

Limited research has been done on EPA and EPA related PUFAs. Research by Tonutti *et al*. has shown that EPA can inhibit endothelial cell migration by disassembly of actin filaments and dysregulated distribution of focal adhesions [61]. Furthermore it was shown by Tsuzuki and collegues that an alkaline treated conjugated form of EPA can inhibit VEGF-induced angiogenesis [62]. Future research should give more insight in the functions of EPA-derived PUFAs, since the data available at this point is little compared to the knowledge about AA-derived PUFAs.
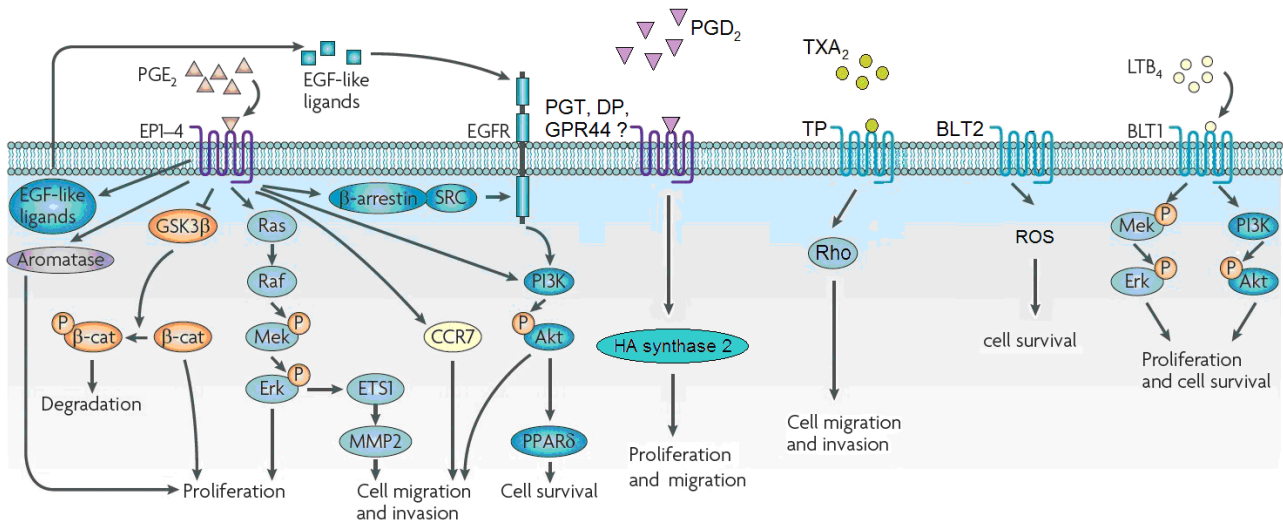
Figure 7: schematic overview of the pathways and functions of AA-derived PUFAs leading to enhanced cell proliferation, survival and migration.

12. Excretion and degradation of AA and EPA-derived PUFAs

In general fatty acids are metabolized and excreted from the body by the liver. High density lipoproteins (HDL) is responsible for the transport of fatty acids and cholesterol from the periphery to the liver. In the liver, lipases are able to metabolize the fatty acids and excrete them via the bile [1]. This is also an important elimination route for the AA and EPA-derived PUFAs [63]. Furthermore, unsaturated fatty acids can also be metabolized via β-oxidation. This process takes place in the mitochondria of cells, where in each oxidation step the fatty acid is shortened by two carbons. Special enzymes are required to break the double carbon-carbon bonds and to breakdown odd number fatty acids [1]. Mitochondrial β-oxidation is mainly described as an important process to yield energy from fat. However, β-oxidation can also take place in peroxisomes, this process is not coupled to ATP formation and it has been shown that fibroblasts can metabolize AA and EPA in peroxisomes [18]. In respect to AA and EPA-derived PUFAs, several specific degradation enzymes have been described. For instance, 15-PGDH is responsible for the rapid intracellular degradation of prostaglandin E2 [4]. Interestingly, the loss of 15-PGDH expression is implicated in bladder cancer progression [64]. In the excretion and elimination of leukotrienes from the body the enzymes γ-glumatmyl transferase and dipeptidase play important roles. These extracellular enzymes are present on the epithelial cells of the kidney and intestine. Studies using radioactively labelled leukotrienes has shown that excretion occurs mainly via feces and urine [65]. In contrast to the already limited literature about the elimination of prostaglandins and leukotrienes, no literature was found on the elimination of thromboxanes. Since the liver and its lipases are the

general elimination route of fatty acids is likely that thromboxanes and other AA and EPA-derived PUFAs follow a similar route of excretion.

13. Implications of PUFAs in cancer progression

13.1 AA-derived PUFAs and cancer

The implication of AA-derived PUFAs in cell proliferation, survival and migration already indicates that it is possible that there are involved in tumor formation and progression. The most convincing evidence for the role of AA-derived PUFAs in cancer comes from the prostaglandins. Prostaglandin E2 is abundantly investigated in various human cancers and has been shown to be expressed in lung, breast, head and neck and colon cancer, in which it is often correlated with a poor prognosis [66-69]. In addition, prostaglandin E2 can upregulate aromatase in breast cancer cells leading to increased estrogen production and cell proliferation [70,71]. These results from various studies are further strengthened by the observation that 15-PGDH, the enzyme responsible for the breakdown of prostaglandin E2 is often lacking in tumors. The role of prostaglandin D2 in cancer is not clear yet. It has been postulated that it has anti-tumorigenic effects since overexpression of prostaglandin D2 reduces colorectal tumor formation in mice. In addition, deletion of this prostaglandin accelerates tumor formation in mice [4]. Although, deletion of the receptor for prostaglandin D2, DP has no effect, failing to confirm the possible anti-tumorigenic effects of prostaglandin D2. The hydrolysed form of prostaglandin D2, 15d-PGJ2, does have anti-tumorigenic effects. It can bind to and activate PPARγ. PPARγ can activate transcription of genes involved in inhibition of cell proliferation and angiogenesis and stimulation of apoptosis and cell differentiation [51,52]. In a similar fashion prostacyclin 2 is also involved in tumor inhibition. Prostacyclin 2 can activate PPARγ, which leads to decreased tumor progression in lung cancer [53]. No clear evidence exist implicating prostaglandin F2 in cancer progression in human studies, however the positive feedback loop between FGF2 and prostaglandin F2 signalling found in endometrial adenocarcinoma cells *in vitro* can form an important autocrine pathway that could sustains cell growth and therefore tumor progression [54]. In addition, prostaglandin F2 has also been identified as an enhancer of tumor progression in fibroblasts, although prostaglandin F2 itself was not an inducer of tumorigenesis [72].

Less data is present on the role of AA-derived leukotrienes and thromboxanes in cancer. Results from Larré *et al*. and Dreyling *et al*. showed that leukotriene B4 is often expressed in

colon and prostate cancer [73,74]. In addition LTA4-H, the enzyme that converts leukotriene A4 into leukotriene B4 is often overexpressed in esophageal cancer [75]. The expression of leukotriene C4 and D4 receptor CysLT1 in colon and prostate cancer is associated with poor overall survival [76,77]. No reports have been made about a role for leukotriene A4 in cancer. *In vitro* studies have shown that thromboxane A2 can promote cell motility in prostate cancer cell lines via Rho activation [56], however no data is present from human studies that can confirm the role of this PUFA in human cancers. Another interesting finding is that during the production of thromboxane A2 also another side product is formed, named malondialdehyde (MDA). This product is mutagenic because it can covalently bind to adenosine and guanosine in the DNA and therefore could play a role in cancer progression [47].

13.2 EPA-derived PUFAs and cancer

In contrast to AA-derived PUFAs no real evidence exist that EPA-derived PUFAs are enhancing tumor progression. Several studies claim that omega-3 fatty acids like ALA and EPA have protective functions against cancer, albeit that the current data from several clinical trails is not consistent [5]. Many population studies have shown that a relatively higher consumption of fish and fish oil, which is rich in omega-3 fatty acids, is correlated with a lower incidence of colorectal, skin and breast cancer [78-81]. However, some studies show no effect of omega-3 or omega-6 fatty acid intake on cancer [82-84]. One explanation for these differences might be that it is not just the intake of fish oil that protects against cancer, but the ratio between fish oil and animal fat. Second, several studies are based on questionnaires, which could give biased results since the researchers are dependent on the patients capability to objectively recall information about their food intake.

Furthermore, it should be taken in account that little is known about the possible mechanisms of the protective effect. Several hypotheses are proposed, including modulation of the AA pathway, changes in gene expression leading to altered cellular metabolism, altered estrogen production leading to a decrease in estrogen-mediated proliferation, altered ROS production and mechanisms involving insulin sensitivity and membrane fluidity [5]. But further research is still needed to confirm these hypotheses.

Discussion

The omega-3 fatty acids ,ALA and EPA, and the omega-6 fatty acids, LA and AA, can be converted to many different biologically active metabolites that serve many different functions. The majority of research has been on the role of AA and EPA-derived PUFAs in immunity. AA-derived leukotrienes, prostaglandins and thromboxanes are potent chemoattractants for inflammatory cells and have platelet aggregating properties, whereas the EPA-derived PUFAs serve a more anti-inflammatory role. In addition, the AA and EPA-derived PUFAs have also been implicated in cancer progression, Again, here AA-derived PUFAs have an opposing role compared to the EPA-derived PUFAs. Research showed that the AA-derived PUFAs promote tumor progression and their expression in several malignancies is often associated with poor survival. In contrast, the EPA-derived PUFAs appear to protect against cancer formation.

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