The relationship between inflammatory mediators, n-6 and n-3 long-chain polyunsaturated fatty acids in red blood cell membranes and postoperative atrial fibrillation following open heart surgery

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Thesis for the degree of Master of Science
University of Iceland
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HÁSKÓLI ÍSLANDS

Tengsl bólguþátta, ómega-6 og ómega-3 fjölómettaðra fitusýra í himnum rauðra blóðkorna og gáttatifs eftir opna hjartaskurðaðgerð

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Ágrip

Opin hjartaskurðaðgerð veldur vefjaskemmdum og kemur af stað bólgumyndunarferli, en bólga er talin eiga þátt í meinmyndun gáttatifs eftir hjartaskurðaðgerð. Fyrri rannóknir hafa bent til gagnsemi ómega-3 fjölómettaðra fitusýra (FÓFS), sem koma úr sjávarfangi, við að draga úr bólgu og fyrirbyggja gáttatif eftir hjartaskurðaðgerð. Niðurstöður rannsókna hafa verið misvísandi hvað varðar áhrif þessara fitusýra á gáttatif hjá sjúklingum sem gangast undir kransæðahjáveituaðgerð. Markmið rannsóknarinnar var að kanna tengsl milli styrks ýmissa bólguhvetjandi og bólguletjandi frumu- og flakkboða, og bráðfasa próteins í blóði, hlutfalls ómega-3 FÓFS í himnum rauðra blóðkorna (RBK) og tilkomu gáttatifs hjá sjúklingum sem gangast undir opna hjartaskurðaðgerð.

Rannsóknin var framsýn, slembiröðuð, tvíblind, lyfleysustýrð, klínísk samanburðarrannsókn á meðferð ómega-3 FÓFS til að fyrirbyggja gáttatif eftir opna hjartaskurðaðgerð. Blóðsýni voru tekin úr hverjum sjúklingi við upphaf meðferðar (grunngildi), rétt fyrir aðgerð (á aðgerðardegi) og á þriðja degi eftir aðgerð. Heildarfituefni voru einangruð úr RBK og fitusýrusamsetning ákvörðuð í gasgreini. Styrkur bólguþátta var mældur í upphafi meðferðar, rétt fyrir aðgerð og á þriðja degi eftir aðgerð. Endapunktur rannsóknarinnar var tilfelli gáttatifs sem stóð lengur en fimm mínútur samkvæmt hjartasírita.

Styrkir IFN-γ og TNF-β í plasma voru lægri og styrkir IL-6, IL-8, IL-10, IL-18 og hs-CRP voru hærri á þriðja degi eftir aðgerð en á aðgerðadegi. Könnuð voru tengsl milli styrks bólguþátta og hlutfalla fitusýra í plasma og himnum RBK. Á aðgerðardegi voru tengsl milli hærra hlutfalls eikósapentaensýru (EPA) í plasma posfólípíðum (PL) og lægri styrks hs-CRP og á milli hærra hlutfalls dókósahexaensýru (DHA) og lægri styrkja IL-12 og IL-18. Í RBK voru tengsl milli hærra hlutfalls arakidónsýru (AA) og hærri styrks TNF-β, og hærra hlutfalls DHA og lægri styrks IL-18. Á aðgerðardegi voru tengsl milli hærra hlutfalls AA Í plasma PL og meiri hækkunar á styrk IL-10 og minni hækkunar á styrk TGF-β, en hærra hlutfall EPA tengdist minni hækkun á styrk IL-10. Aftur á móti voru tengsl milli hærra hlutfalls AA í RBK og meiri lækkunar í TNF-β og minni hækkunar á styrk TGF-β, en hærra hlutfall EPA tengdist meiri hækkunar í styrkjum IL-1 β og TGF-β.

Engin aldurstengd áhrif voru milli aldurs og styrkja bólguhvetjandi né bólguletjandi þátta við upphaf meðferðar, en á aðgerðardegi voru neikvæð tengsl milli styrks TGF-β og aldurs ($r = -0.165$, $P = 0.036$). Á þriðja degi eftir aðgerð voru jákvæð tengsl milli styrks IL-6 og IL-8 ($r = 0.179$, $P = 0.036$ og $r = 0.180$, $P = 0.022$) og neikvæð tengsl milli styrks TGF-β ($r = -0.168$, $P = 0.033$) og aldurs.

Sextíu og tveir sjúklingar sem fóru í kransæðahjáveituaðgerð (CABG) (49.6%) fengu gáttatif eftir aðgerðina. Þeir sem fengu gáttatif voru eldri (69 (45-82) ár á móti 65 (43-79) ár, $P=0.001$) og með lægri líkamsþyngdarstuðul (BMI) (26.7 (17.2-38.1) kg/m^2 á móti 28.3 (20.9-41.3) kg/m^2, $P<0.05$) miðað við þá sem fengu ekki gáttatif. Engin munur var á styrk bólguþátta á aðgerðardegi hjá þessum hópum. Í báðum hópunum eftir aðgerð voru styrkir IL-6, IL-8, IL-18, IL-10 og hs-CRP hærri ($P<0.05$) og styrkur TNF-β lægri ($P<0.01$) en fyrir aðgerð. Á þriðja degi eftir aðgerð var aðeins styrkur IL-6 hærri hjá þeim sem fengu gáttatif borið saman við þá sem ekki fengu gáttatif. Á aðgerðardegi var munur á hlutfalli ómega-6 og ómega-3 FÓFS í himnum RBK milli þessara hópa. Þeir sem fengu

gáttatif höfðu lægra hlutfall AA og hærri hlutföll heildar ómega-3 FÓFS og DHA borið saman við þá sem ekki fengu gáttatif ($P < 0.05$). Enginn munur var á hlutfalli EPA milli hópanna ($P > 0.05$). Fjölþátta aðhvarfsgreining sýndi bólguþættina ekki sem sjálfstæða áhættuþætti fyrir gáttatif. Hins vegar jókst áhættan á gáttatifi þegar hlutfall DHA í RBK var tiltölulega hátt fyrir aðgerð (OR (95% CI) = 1.506 (1.010-2.246)) og þegar hlutföll EPA (OR (95% CI) = 1.952 (1.043-3.654)), DHA (OR (95% CI) = 1.983 (1.260-3.121)) og heildar ómega-3 FÓFS (OR (95% CI) = 1.440 (1.119-1.853)) voru tiltölulega há í himnum RBK eftir aðgerð ($P < 0.05$). Meiri líkur voru á gáttatifi við hærri fjórðunga DHA og heildar ómega-3 FÓFS í RBK á aðgerðardegi ($P = 0.006$ og $P = 0.010$). Á þriðja degi eftir aðgerð var marktækur munur á áhættu gáttatifs milli fjórðunga DHA og heildar ómega-3 FÓFS ($P = 0.007$ og $P = 0.034$) með marktækt meiri líkur á gáttatifi með hærri fjórðungum ($P = 0.001$, $P = 0.002$ og $P= 0.001$).

Niðurstöður rannsóknarinnar benda til þess að ómega-6 og ómega-3 FÓFS í himnum RBK gegni mikilvægu hlutverki í bólgusvörun eftir skurðaðgerð. Ennfremur gefa niðurstöðurnar til kynna að styrkur bólguþátta tengist ekki tilkomu gáttatifs eftir aðgerð og að hátt hlutfall ómega-3 FÓFS í himnum frumna geti verið einn af áhættuþáttum gáttatifs eftir opna hjartaskurðaðgerð.

Abstract

During open heart surgery, mechanical manipulation of the heart and pericardium leads to a local tissue trauma and local inflammatory response which both increases the risk of postoperative atrial fibrillation (POAF). In clinical studies focus has been on the anti-inflammatory and anti-arrhythmic effect of n-3 long-chain polyunsaturated fatty acids (LC-PUFA), but the effect on the incidence of POAF in patients undergoing coronary artery bypass grafting (CABG) has been contradictory. The aims of this study were to examine the association between circulating inflammatory mediators, n-6 and n-3 LC-PUFA in red blood cell (RBC) membrane lipids and the incidence of POAF in patients undergoing open heart surgery.

The study was a part of a prospective, randomized, double-blinded, placebo-controlled clinical trial on the use of n-3 LC-PUFA for a week prior to open heart surgery to prevent postoperative atrial fibrillation. Blood samples were obtained from each patient a week before surgery (baseline), immediately before surgery (perioperatively) and on the third postoperative day. Total lipids were extracted from RBC membrane and the fatty acid composition determined by using a gas chromatograph. Inflammatory mediators were measured at baseline, perioperatively and postoperatively. The study endpoint was defined as an episode of POAF lasting more than five minutes by continuous electrocardiographic monitoring.

Plasma concentrations of IFN-γ and TNF-β were lower than those of IL-6, IL-8, IL-10, IL-18 and hs-CRP were higher on the third postoperative day than perioperatively. The relationship between concentrations of inflammatory mediators and fatty acid levels in plasma phospholipids (PL) and RBC membrane lipids was investigated. In perioperative plasma PL, higher level of eicosapentaenoic acid (EPA) was associated with lower concentration of hs-CRP, and higher docosahexaenoic acid (DHA) level was associated with lower IL-12 and IL-18 concentrations. In RBC, higher arachidonic acid (AA) level was highly associated with higher concentration of TNF-β, whereas higher DHA level was associated with lower IL-18 concentration. In perioperative plasma PL, higher level of AA was associated with more increase in IL-10 and lesser increase in TGF-β, whereas higher level of EPA was associated with lesser increase in IL-10. On the other hand, in RBC higher AA level was associated with more pronounced decrease in TNF-β, and lesser increase in TGF-β, whereas higher level of EPA was associated with more increase in IL-1 β and TGF-β.

No associations were observed between age and plasma concentrations of either pro-inflammatory or anti-inflammatory mediators at baseline, but perioperatively the concentration of TGF-β was negatively associated with age ($r = -0.165$, $P = 0.036$). On the third postoperative day the concentrations of IL-6 and IL-8 were positively associated ($r = 0.179$, $P = 0.036$, and $r = 0.180$, $P = 0.022$, respectively), and TGF-β negatively associated ($r = -0.168$, $P = 0.033$) with age.

Sixty-two CABG patients (49.6%) developed POAF. The patients who developed POAF (POAF group) were older (69 (45-82) years versus 65 (43-79) years, P=0.001) and their body mass index (BMI) was lower (26.7 (17.2-38.1) kg/m^2 versus 28.3 (20.9-41.3) kg/m^2, P<0.05) compared with those who did not develop POAF (sinus rhythm (SR) group). No difference was in perioperative

inflammatory mediators between the POAF and SR groups. In both groups postoperative IL-6, IL-8, IL-18, IL-10 and hs-CRP concentrations were higher ($P<0.05$), and that of TNF-β was lower ($P<0.01$) than the perioperative concentrations. Only postoperative concentration of IL-6 was higher in POAF group compared with the SR group. Perioperatively, the SR and POAF groups differed in the n-6 and n-3 LC-PUFA levels of RBC membrane lipids. The POAF group had lower level of AA and higher levels of total n-3 LC-PUFA and DHA compared with the SR group ($P < 0.05$). No difference in the EPA levels was found between the two groups ($P > 0.05$). Multivariable logistic regression analysis did not reveal inflammatory mediators as independent predictors of POAF. However, the risk of POAF increased with higher perioperative concentration of DHA (OR (95% CI) = 1.506 (1.010-2.246)), and higher postoperative concentrations of EPA (OR (95% CI) = 1.952 (1.043-3.654)), DHA (OR (95% CI) = 1.983 (1.260-3.121)), and total n-3 LC-PUFA (OR (95% CI) = 1.440 (1.119-1.853)) in RBC membrane lipids ($P < 0.05$). Perioperatively, there was a trend for an increasing incidence of POAF with higher quartiles of DHA and total n-3 LC-PUFA ($P = 0.006$ and $P = 0.010$, respectively). Postoperatively, there was a significant difference in POAF incidence between quartiles of DHA and total n-3 LC-PUFA ($P = 0.007$ and $P = 0.034$, respectively) with a significant trend for an increasing incidence of POAF with higher quartiles of these fatty acids ($P = 0.001$ and $P = 0.002$, respectively).

The study suggests that n-6 and n-3 LC-PUFA in RBC membrane lipids play an important role in modulating the inflammatory response following surgical injury. Further, it indicates that inflammatory mediators are not strongly associated with the development of POAF, and that higher levels of n-3 LC-PUFA in cell membranes may be a risk factor for POAF.

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List of abbreviations

AA	Arachidonic acid
AF	Atrial fibrillation
ALA	Alpha-linolenic acid
BHT	Butylated hydroxytoluene
BMI	Body mass index
BSA	Bovine serum albumin
CABG	Coronary artery bypass grafting
COX	Cyclooxygenase
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FA	Fatty acids
FAME	Fatty acid methyl ester
GC	Gas chromatography
hs-CRP	High-sensitive C-reactive protein
IFN	Interferon
IL	Interleukin
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acids
LOX	Lipoxygenase
LT	Leukotrienes
MIP	Macrophage inflammatory protein
MUFA	Monounsaturated fatty acids
NF	Nuclear factor
NK cell	Natural killer cell
OA	Oleic acid
PBS	Phosphate buffered saline
PG	Prostaglandin
PGI	Prostacyclin
PL	Phospholipids

POAF	Postoperative atrial fibrillation
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell
SEM	Standard error of the mean
SFA	Saturated fatty acids
SR	Sinus rhythm
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TX	Thromboxanes

Inflammation in itself is not to be considered as a disease . . . and in disease, where it can alter the diseased mode of action, it likewise leads to a cure; but where it cannot accomplish that salutary purpose, . . . it does mischief.

John Hunter: Treatise on the Blood, Inflammation, and Gunshot Wounds, London, 1794

1 Introduction

Open heart surgery includes mechanical manipulation of the heart and pericardium that leads to a local tissue trauma and local inflammatory response which may both increase the risk of postoperative atrial fibrillation (POAF) (1). POAF is a frequent complication after open heart surgeries and does often require specific therapy, adds to the length of hospital stay, increases health care costs, and may increase mortality (2, 3). Recently there has been increased interest in the role of specific dietary fatty acids and their effect on health and disease. In light of the beneficial effect of n-3 long-chain polyunsaturated fatty acids (LC-PUFA) on inflammation (4, 5), researchers have in clinical studies also focused on the anti-arrhythmic effect of n-3 LC-PUFA. However, the effect of n-3 LC-PUFA on the incidence of POAF in patients undergoing a coronary artery bypass graft (CABG) surgery or a more complex surgical intervention has been contradictory (6-10).

1.1 Acute inflammation triggered by surgery

Inflammation is a normal part of the body‘s immediate response to infection or injury. It is defined by heat, pain, redness and swelling. All of these responses reflect the effects of cytokines and other inflammatory mediators on local blood vessels as a result of increased blood flow and leakage of fluid into the tissues. During open heart surgery, mechanical manipulation of the heart and pericardium leads to a local tissue injury which triggers an acute inflammatory response (Figure 1). The localized inflammatory response is advantageous as it is designed to help fight and clear infection, repair damaged tissue and organ systems (1, 11). However, these responses must be ordered and controlled because the failure to resolve the inflammation and return the target tissue to homeostasis can result in disease (12, 13).

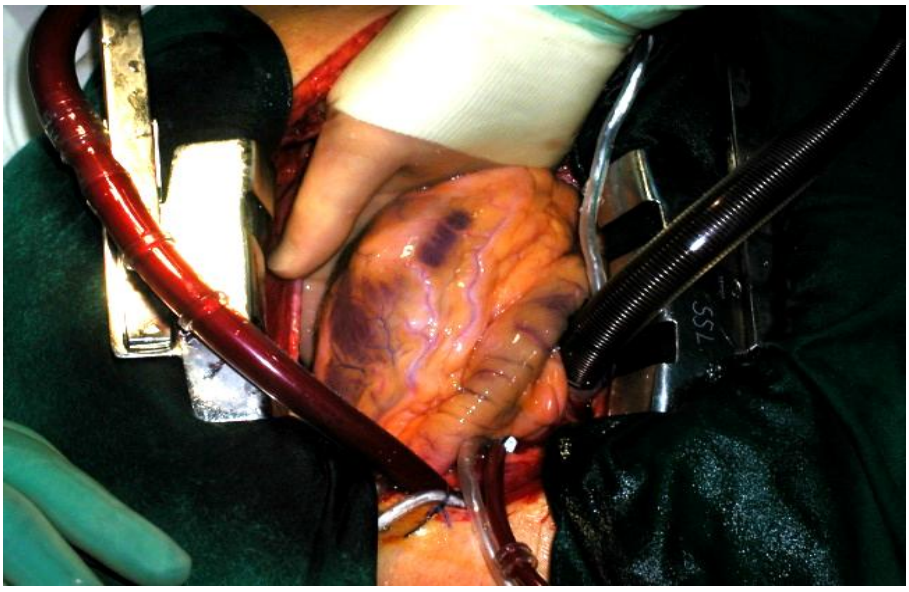

Figure 1. Heart surgery at Landspitali – The National University Hospital of Iceland (14).

1.1.1 Inflammatory mediators

The severity of the inflammatory response following cardiac surgery is influenced by nonspecific (surgical trauma, blood loss, transfusion and hypothermia), and specific (cardiopulmonary bypass (CPB)) activators (1, 15). In addition, the inflammatory response may be influenced by the ability of patient to maintain the balance between pro-inflammatory and anti-inflammatory mediators (16, 17).

Multiple cells may be involved in the inflammatory response (Figure 2). Endogenous mediators referred to as cytokines are produced by diverse cell types at the site of injury and by systemic immune cells (17). The relatively rapid appearance of the cytokines after injury reflects active gene transcription and translation by the injured or stimulated cell.

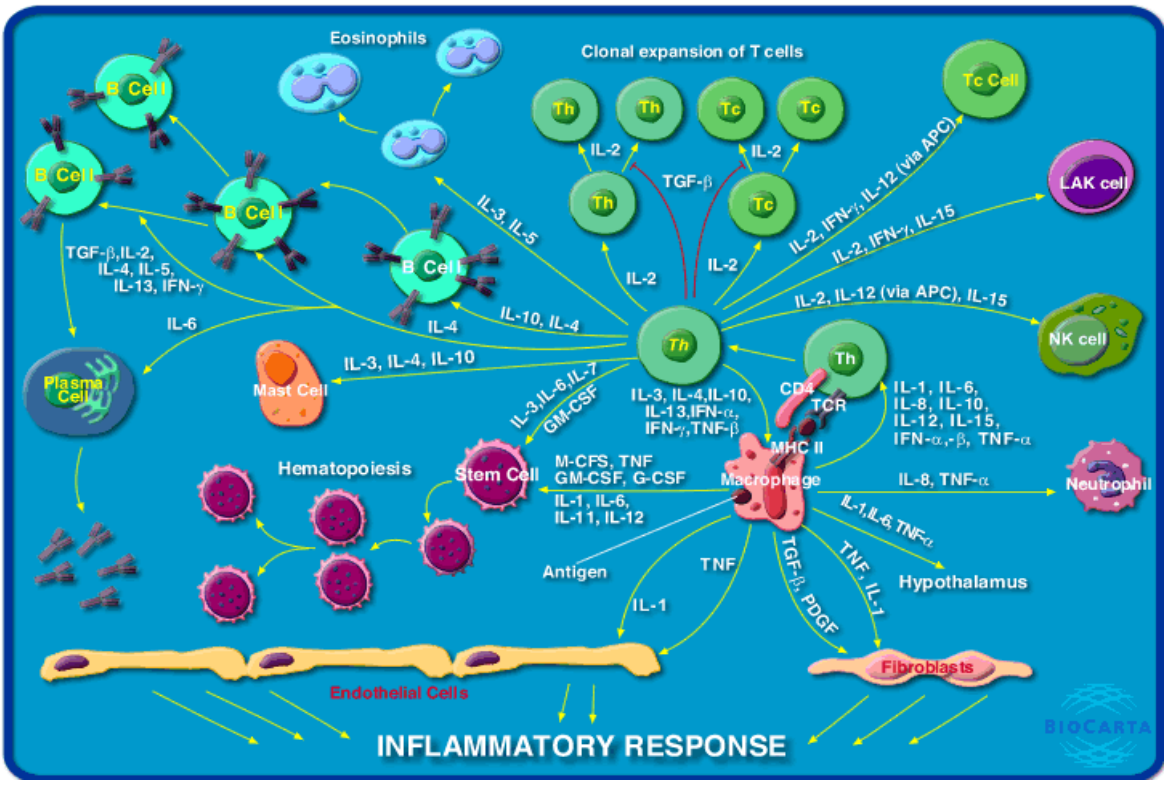

Figure 2. Inflammatory pathway of leukocytes and cytokines. Inflammation is a protective response to infection by the immune system that requires communication between different classes of immune cells to coordinate their actions. Acute inflammation is an important part of the immune response, but chronic inappropriate inflammation can lead to destruction of tissues in autoimmune disorders and perhaps neurodegenerative or cardiovascular disease. Secreted cytokine proteins provide signals between immune cells to coordinate the inflammatory response. Some cytokines such as IL-1, IL-6 and TNF act to broadly provoke the inflammatory response while others act on specific types of immune cells. Macrophages and other phagocytotic cells provide a front-line defense against bacterial infection. Macrophages stimulate the inflammatory responses of neutrophils, fibroblasts, and endothelial cells in response of infection by secreting IL-1 and TNF. Fibroblasts and endothelial cells respond to IL-1 and TNF by recruiting more immune cells to the site of inflammation. Secreted IL-8 is a chemokine that attracts neutrophils to sites of infection. Macrophages also present antigen to T helper cells that play a central role in coordinating immune responses. TGF-beta is a negative regulator of proliferation in many cells, have anti-inflammatory actions in some settings (18).

The earliest cells to appear at inflamed sites are platelets or thrombocytes. They are important for wound healing, both as initiators of coagulation and through the release of growth factors such as transforming growth factor β (TGF-β), that plays a significant role in the repair and regeneration of connective tissues. Neutrophils are the most abundant type of white blood cells (or leukocytes) in mammals and form an essential part of the innate immune system. They arrive very early after tissue damage with monocytes (which transform into macrophages in tissue) and lymphocytes (i.e. T cells, B cells and natural killer (NK) cells) appearing later (11). Neutrophils, monocytes and macrophages are the main effector cells during acute inflammation where they are involved in pathogen killing, clearing up cellular and tissue debris and in tissue repair. Mast cells, derived from circulating basophils, are recruited somewhat later at sites of injury and act mainly as secretory cells. Each of these inflammatory cell types releases various mediators (cytokines and chemokines) and growth factors that are presumed to play a role in tissue repair signals and wound closing as well as magnifying the inflammatory signal, drawing in more neutrophils, macrophages and mast cells to the wound site (11).

Cytokines are small soluble proteins that act as signaling molecules of the immune system important in mediating and regulating inflammation (13). The cytokines refereed to in this thesis include tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-6, IL-10, IL-12, IL-18, interferon-γ (IFN-γ), TGF-β (Table 1). A single cytokine can have several functions and similar functions can be stimulated by different cytokines. One cytokine can influence the synthesis of other cytokines, enhancing or suppressing their production, and they can act together or have antagonistic effect on each other (19).

Chemokines are a group of small proteins that posess the ability to induce cell migration or chemotaxis in numerous cell types (13). The chemokines refereed to in this thesis include CCL3, which common name is macrophage inflammatory protein (MIP) 1α, and CXCL8, which common name is IL-8 (Table 1).

1.1.2 Pro-inflammatory mediators TNF-α, TNF-β, IL-1β, IL-6, and CRP

The inflammatory response following open heart surgery is an extremely complex process, initiated by the production of the pro-inflammatory cytokines TNF-α and IL-1β. The primary sources of TNF-α synthesis are monocytes / macrophages and T cells (20). TNF-α and TNF-β induce the expression of IL1-β (21). TNF-α and IL-1β, bind to specific receptors on the endothelium (Figure 2), initiating diverse signal transduction pathways, which activate a specific set of genes (termed activation genes) within the nucleus of the endothelium cell (22).

IL-6 is the most important regulator of the hepatic acute phase response (17), and its sources are virtually all cells and tissues, however, primary monocytes / macrophages cells (23). TNF-α and IL1-β act synergistically to stimulate the production of IL-6 from virtually all cells and tissues, which leads to increased plasma concentration of CRP in the circulation (24, 25). An anti-inflammatory role has been demonstrated for IL-6 (26, 27), such as during injury by attenuating TNF-α and IL-1 activity (26).

The acute phase protein CRP is the inflammatory mediator that has evolved as the strongest and most reproducible mediator of inflammation, and CRP measurements can provide information about the current inflammatory status of the patient (28). Primarily the liver synthesizes CRP in response to the inflammatory mediator IL-6. The biological function of CRP is not fully understood. Evidence

suggests that elevated high sensitivity CRP (hs-CRP) concentrations are among the most reliable and reproducible inflammatory mediators (29, 30), and represent one of the strongest and most independent predictive risk factor for cardiovascular diseases (31-33).

1.1.3 Pro-inflammatory mediators IL-12, IL-18 and IFN-γ

The pro-inflammatory cytokine IL-12 is most importantly secrated from dendritic cells, but also from other cells such as macrophages and B cells (13). IL-12 is involved in growth and function of T cells, and along with IL-18 promotes cell-mediated immunity and stimulates the production of IFN-γ from T and NK cells (34). IL-18 is produced by macrophages and other cells, and in contrast to most cytokines, it is constitutively expressed. IL-18 has an important role in cellular adhesion (13). Much of IL-12 and IL-18 biology is mediated via IFN-γ (17). When released into the circulation, IFN-γ is detectable in vivo by 6 hours and may be persistently elevated for as long as 8 days. Injured tissues, such as operative wounds, also demonstrate the presence of IFN-γ production 5 to 6 days after injury. IFN-γ has important roles in activating circulating and tissue macrophages. Alveolar macrophage activation mediated by IFN-γ may induce acute lung inflammation after major surgery or trauma.

1.1.4 Pro-inflammatory mediators IL-8 and MIP-1α

The pro-inflammatory mediators IL-8, synthesized primarily in macrophages, and MIP-1α are essential regulators of cell migration defined as chemokines (13). TNF-α and IL-1β induce expression of IL-8 by endothelial cells, which may play an important role in reperfusion injury (35). Oxidantive stress increases IL-8 secretion and causes recruitment of inflammatory cells, whereby it induces a further increase in oxidative stress mediators (36). The fact that IL-8 secretion is increased by oxidant stress and conversely, makes it a key parameter in localized inflammation.

MIP-1α, also known as CCL3, is along with MIP-1β (CCL4) crucial for immune responses towards infection and inflammation (37). It both plays an important role in the recruitment of monocytes, macrophages and neutrophils to the inflamed tissue, and in homeostasis (13, 38).

1.1.5 Anti-inflammatory mediators IL-10 and TGF-β

Key anti-inflammatory mediators include the cytokines IL-10 and TGF-β. Monocytes and B cells are the major sources of IL-10 in human subjects (39). IL-10 is a potent inhibitor of the production of TNF-α, IL-1β and IL-8 (40), and IL-10 has been shown to be released together with pro-inflammatory mediators during and after CPB (41). IL-10 can inhibit the production of pro-inflammatory mediators at several different levels, it can reduce cytokine gene transcription, promote cytokine mRNA degradation and deactivate accessory cell function (42-44).

TGF-β is produced by numerous cell types and is one of the most potent chemoattractant for monocytes and other cell types within wounds (45). TGF-β down-regulates pro-inflammatory cytokine production (1), and, in general, inhibits proliferation and induces apoptosis (13).

Table 1. Cytokines and their activities.

Cytokine	Principal producing cells	Main target cells	Function
TNF-α	Macrophages; mast cells; NK cells	Macrophages	Adhesion molecule and cytokine expression
		Tumour cells	Death
TNF-β	Th1 cells; Tc cells	Phagocytes	Phagocytosis, NO production
		Tumour cells	Death
IL-1β	Monocytes; macrophages; B cells; dendritic cells	Th cells	Co-stimulation
		B cells	Maturation and proliferation
		NK cells	Activation
		Various	Inflammation, acute phase response, fever
IL-6	Monocytes; macrophages; Th2 cells; stromal cells	Activated B cells	Differentiation into plasma cells
		Plasma cells	Antibody secretion
		Stem cells	Differentiation
		Various	Acute phase response
IL-12	Dentritic cells; macrophages; B cells	Activated Tc cells	Differentiation into Tc cells (with IL-2)
		NK cells	Activation
IL-18	Macrophages	NK cells; T cells	IFN-γ synthesis
IFN-γ	Th1 cells; Tc cells; NK cells	Various	Inhibition of viral replication
		Macrophages	MHC expression
		Activated B cells	Ig class switch to IgG_{2a}
		Th2 cells	Inhibition of proliferation
IL-8	Macrophages; endothelial cells	Neutrophils	Chemotaxis
MIP-1α	Macrophages	Monocytes; T cells	Chemotaxis
IL-10	Monocytes; macrophages; Th2 cells	Macrophages; B cells	Anti-inflammatory (e.g. decreases TNF-α synthesis)
TGF-β	T cells; monocytes	Monocytes; macrophages	Chemotaxis
		Activated macrophages	IL-1 synthesis
		Activated B cells	IgA synthesis
		Various	Inhibition of proliferation

Tc = Cytotoxic T lymphocytes; TNF = tumour necrosis factor; IL = interleukin; IFN = interferon; MHC = major histocompatability complex; MIP = macrophage inflammatory protein; TGF = transforming growth factor.

1.2 Postoperative atrial fibrillation

Atrial fibrillation (AF) is an arrhythmia frequently seen in the postoperative period of cardiac surgery (46). AF is characterized by an irregular and often rapid heart rate, which originates in the atria of the

heart and results from abnormal electrical impulses in the heart. In AF, multiple impulses travel through the atria at the same time. The atria may contract at a rate of 400 - 600 times per minute. The ventricles pick up a small number of these electrical impulses, since the ventricular rate can approach 180-200 times per minute. Whether at high or low heart rates, the irregular rhythm adversely affects the contractility of the ventricles. This leads to increased stasis of the blood within the atria, increasing the risk of blood clot formation, and insufficient amounts of blood being supplied to the body. This can result in a variety of symptoms and problems such as palpitations, shortness of breath, fatigue, dizziness and chest pain, although it can also be asymptomatic in some patients (47). If AF is not treated it can be followed by serious consequences including stroke, decreased quality of life and increased morbidity and mortality. These consequences may be in large part because AF increases the risk of heart failure and stroke (48-50).

POAF is one of the most common complications after CABG surgery, and even higher incidence has been reported after more complex cardiac surgery like valvular surgery and combined valvular/CABG surgeries. The incidence of POAF is increasing but it has been reported in wide range from 5% to 70% after CABG or/and combined surgeries (46, 51, 52). Most episodes of POAF occur within the first postoperative week, with a peak incidence between the second and third postoperative day (53), but it can occur at any point during the recovery period (3).

The pathophysiology of POAF is not fully understood but is likely due to multiple and complex factors. The multiple reentrant wavelets circulating throughout the atria are in part thought to be due to an atrial myopathy which leads to abnormalities in atrial conduction, in refractoriness and in the triggering of ectopic atrial beats (54). Other factors such as preexisting structural changes of the atria and ventricles, volume overload, intraoperative atrial ischemia, electrolyte imbalance, use of vasoactive drugs and pericardial lesions from the surgery may all play a role in the pathogenesis of POAF (3). Mechanisms involved in the genesis of POAF in the postoperative period may therefore be somewhat different from those causing AF in the general population, involving a number of factors related to the open heart surgery. Prior studies have demonstrated that advanced age, male gender, obesity, diabetes, hypertension, previous history of AF, and the complexity of the surgical procedure increase the risk of developing POAF. POAF prolongs the length of hospital stay and therefore increases healthcare costs (2, 3, 55).

1.2.1 Inflammation and postoperative atrial fibrillation

The open heart surgery leads to a local tissue trauma and local inflammatory response which both increase the risk of a POAF (1, 56). After cardiac surgery, the complement system is activated and systemic inflammatory responses are induced (57). Bruins and coworkers (58) were the first to propose the association between inflammation and POAF following CABG surgery. They demonstrated that IL-6 rises initially and peaks at 6 h after surgery and a second phase occurs in which CRP levels peak on postoperative day 2. The incidence of POAF follows a similar pattern and peaks between postoperative day 2 or 3 (3, 58). There is emerging data supporting the association between inflammation and POAF. Baseline plasma hs-CRP concentrations have been associated with higher risk of developing POAF (59) and elevated perioperative concentrations of IL-6 have been

indicated as a strong predictor of POAF (60). Further, patients who have a higher postoperative leukocyte count may be more likely to develop POAF (61).

Whether inflammation is a cause of POAF or only a consequence is currently unknown but a beneficial effect of drugs with anti-inflammatory properties such as statins further implicate inflammation as an important denominator for POAF (62, 63). Many of these drugs however, require close therapeutic monitoring due to their side-effect and drug interaction profiles. In light of that, researchers have focused on n-3 LC-PUFA in finding alternative, safer, preventive and therapeutic strategy due to their beneficial effect on inflammation and cardiac arrhythmias. Further studies are needed to conclusively demonstrate the association between inflammatory mediators and development of POAF.

1.3 n-6 and n-3 Long-chain polyunsaturated fatty acids (LC-PUFA)

Fatty acids (FA) are hydrocarbon chains with a carboxyl group at one end (the alpha end) and methyl group at the other (the omega) end. FA vary in chain lengths and are classified into families depending on the number of double bounds in their chain. Saturated FA (SFA) have only single bonds between the carbon atoms in the chain, monounsaturated FA (MUFA) (e.g. Oleic acid (OA)) have one double bond and FA containing two or more double bonds are called polyunsaturated FA (PUFA).

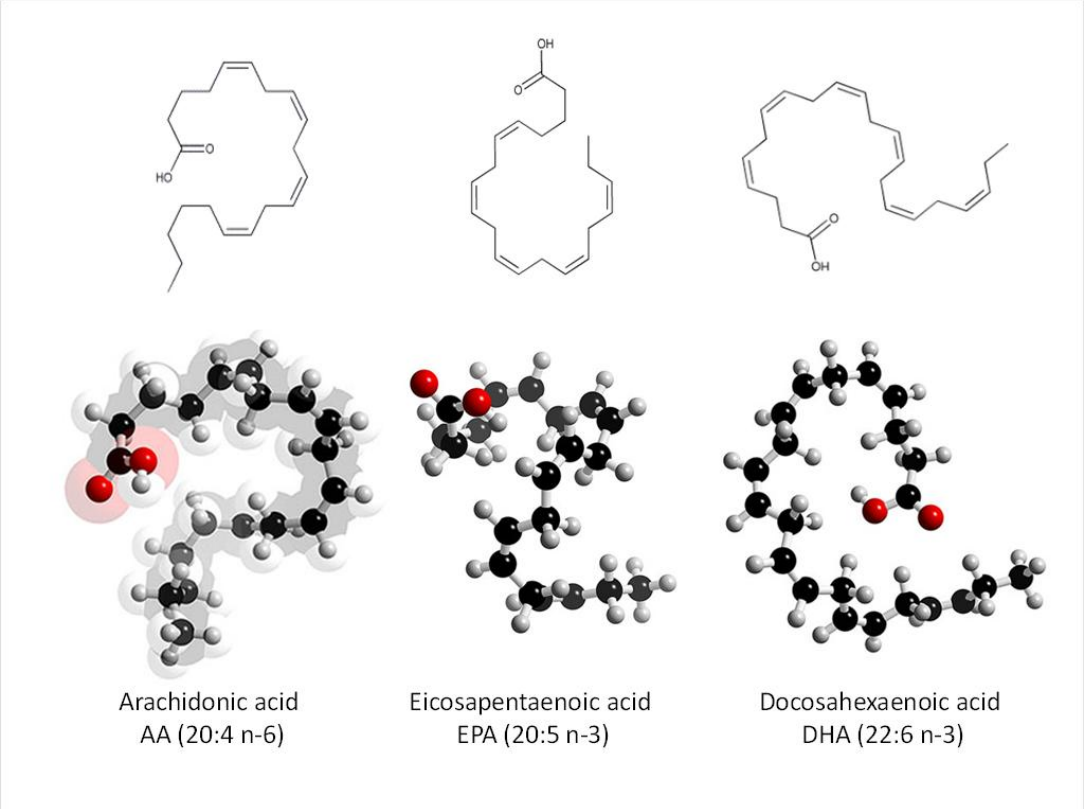

Figure 3. Structures of the n-6 and n-3 long-chain polyunsaturated fatty acids (LC-PUFA).

LC-PUFA contain twenty or more carbons and two or more double bonds in the chain. The position of the double bond counted from the methyl (omega or n) end determines the type of LC-PUFA. The

n-3 LC-PUFA have double bond three carbons away from the methyl end, whereas the n-6 LC-PUFA have a double bond six carbons away from the methyl end (Figure 3). The n-3 LC-PUFA are eicosapentaenoic acid (EPA; 20:5n−3) with 20 carbon atoms and five double bonds, and docosahexaenoic acid (DHA; 22:6n−3) with 22 carbon atoms and six double bonds. The n-6 LC-PUFA is arachidonic acid (AA; 20:4n-6) with 20 carbon atoms and four double bonds.

Mammals lack the ability to introduce double bonds in the FA chain more proximal to the n end than at the seventh carbon atom, which means that they cannot synthesize the precursors of the n-6 and n-3 LC-PUFA linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), respectively. Therefore, LA and ALA are essential in the diet. LA is found in most vegetable oils such as corn oil and safflower oil and ALA is found in some vegetable oils such as rapeseed, flaxseed and soybean oils, as well as green vegetables and nuts. In humans, AA can be synthesized from LA by desaturation and chain elongation and some n-3 LC-PUFA (EPA and DHA) can be synthesized from ALA. However, the conversion is limited in humans and therefore n-3 LC-PUFA may have to be obtained directly from the diet, e.g. from fatty fish and fish oil. The metabolism of n-6 and n-3 PUFA are linked as their metabolic pathways compete for the same enzymes (desaturases and elongases), resulting in a competition between LA and ALA producing AA, and EPA and DHA, respectively (Figure 4).

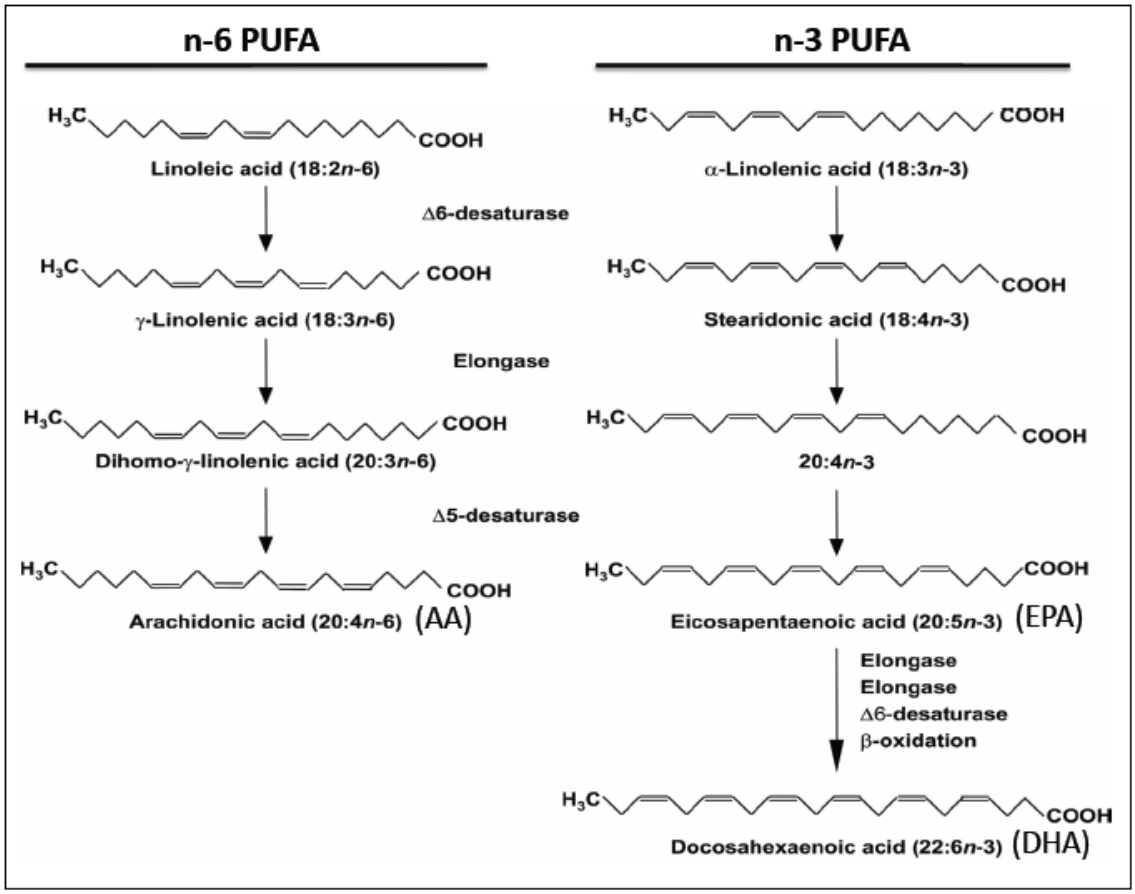

Figure 4. Metabolisms of the n-6 and n-3 long-chain polyunsaturated fatty acids (LC-PUFA) (64).

1.3.1 Fatty acids in the red blood cell membrane lipids

The FA composition of plasma phospholipids (PL) has been most widely used to assess the overall FA status in an individual. Plasma PL are merely transporters of FA in circulation, and the type and amount of n-6 and n-3 PUFA consumed during the previous few days or a week are reflected in the FA composition of plasma PL (65). However, in mature red blood cells (RBC) all of the cell lipids are in the membrane, and their n-6 and n-3 PUFA composition reflects that of plasma PL (65), since FA have to be compensated for by interchange with the FA in plasma PL. In human the RBC membrane n-6 and n-3 PUFA composition is a good indicator of dietary n-6 and n-3 PUFA intake over the preceding three months (65, 66), and the FA composition of the RBC membrane has been shown to be a good indicator of the FA composition of other cell membranes, including the myocardial cells and atrial tissue (67). FA composition of a cell membrane (Figure 5) can influence cell function through a variety of mechanisms, including membrane structure and fluidity, intracellular signaling and gene expression, biosynthesis of lipid mediators like eicosanoids, and lipid raft structure (68).

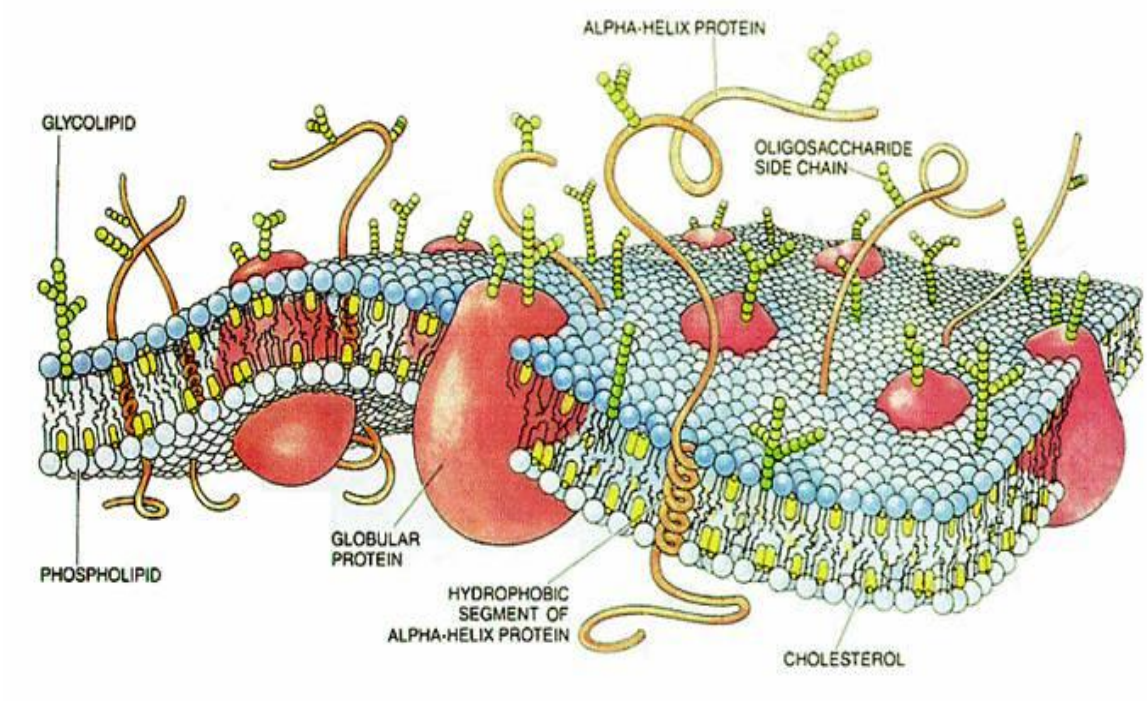

Figure 5. The structure of cell membrane. Cell membrane is a double layer of phospholipid molecules. Proteins of various kinds are inserted through the phospholipid bilayer. Carbohydrates bind to proteins and lipids on the extracellular surface, creating glycoproteins and glycolipids (69).

1.3.2 Anti-inflammatory effect of n-6 and n-3 LC-PUFA

The 20-carbon LC-PUFA AA and EPA are precursors of potent bioactive lipid mediators called eicosanoids that have differential effects on the immune cells. They include prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX) and leukotrienes (LT) that are important mediators of inflammatory responses and in homeostasis (4, 5) (Figure 6 and Figure 7). The same enzymes, cyclooxygenase (COX) and lipoxygenase (LOX) are involved in the metabolism of both types of these

FA, but eicosanoids derived from the AA are more potent mediators of inflammation than those derived from the EPA. It has also been reported that the derivatives of EPA and the 22-carbon DHA, i.e. resolvins, protectins, maresins, and that of AA, lipoxins, are potent anti-inflammatory and pro-resolving lipid mediators (70) (Figure 6 and Figure 7). Therefore, the balance between intake of n-6 and n-3 LC-PUFA may be important for the ratio of n-6 LC-PUFA to n-3 LC-PUFA in the body.

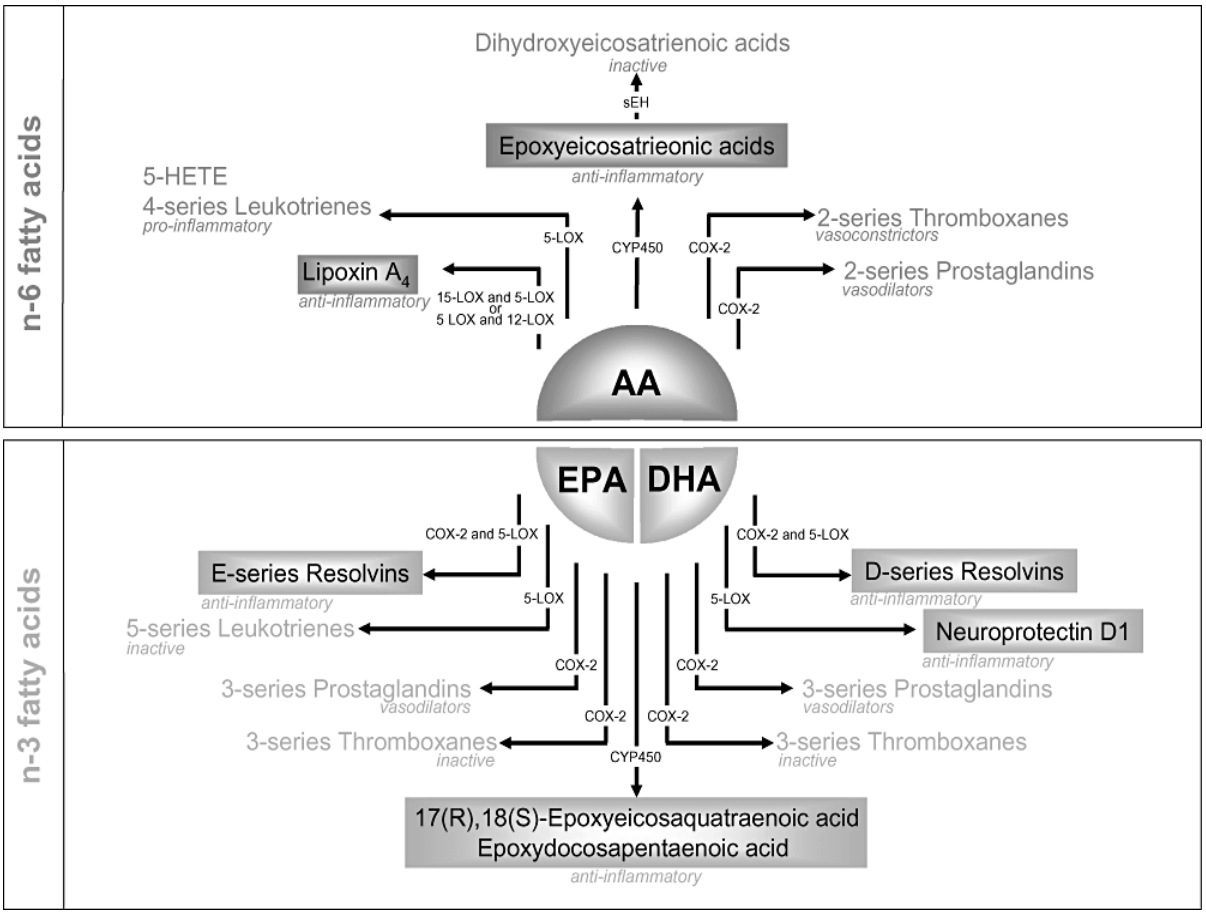

Figure 6. Pathways of eicosanoids and docosanoids from long-chain polyunsaturated fatty acids (LC-PUFA). Pathways in eicosanoid and docosanoid metabolism leading to the generation of pro- and anti-inflammatory, or inactive eicosanoids or docosanoids, from arachidonic acid (AA) and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (71).

Recently, Chapkin and coworkers (72) proposed a molecular model that may explain, in part, the pleiotropic anti-inflammatory and immunosuppressive properties of the n-3 LC-PUFA. The hypothesis is based on the notion that n-3 LC-PUFA in cell membranes and/or in the circulation suppress nuclear receptor activation and thereby NF-κB (Figure 7), that controls the transcription of genes involved in synthesis of many pro-inflammatory mediators, such as TNF-α, IL-1β, IL-6 and IL-8 (22, 73). Therefore, the relative balance between pro-inflammatory and anti-inflammatory mediators may partly depend on baseline levels of AA, EPA and DHA in plasma PL and in cell membrane PL (74).

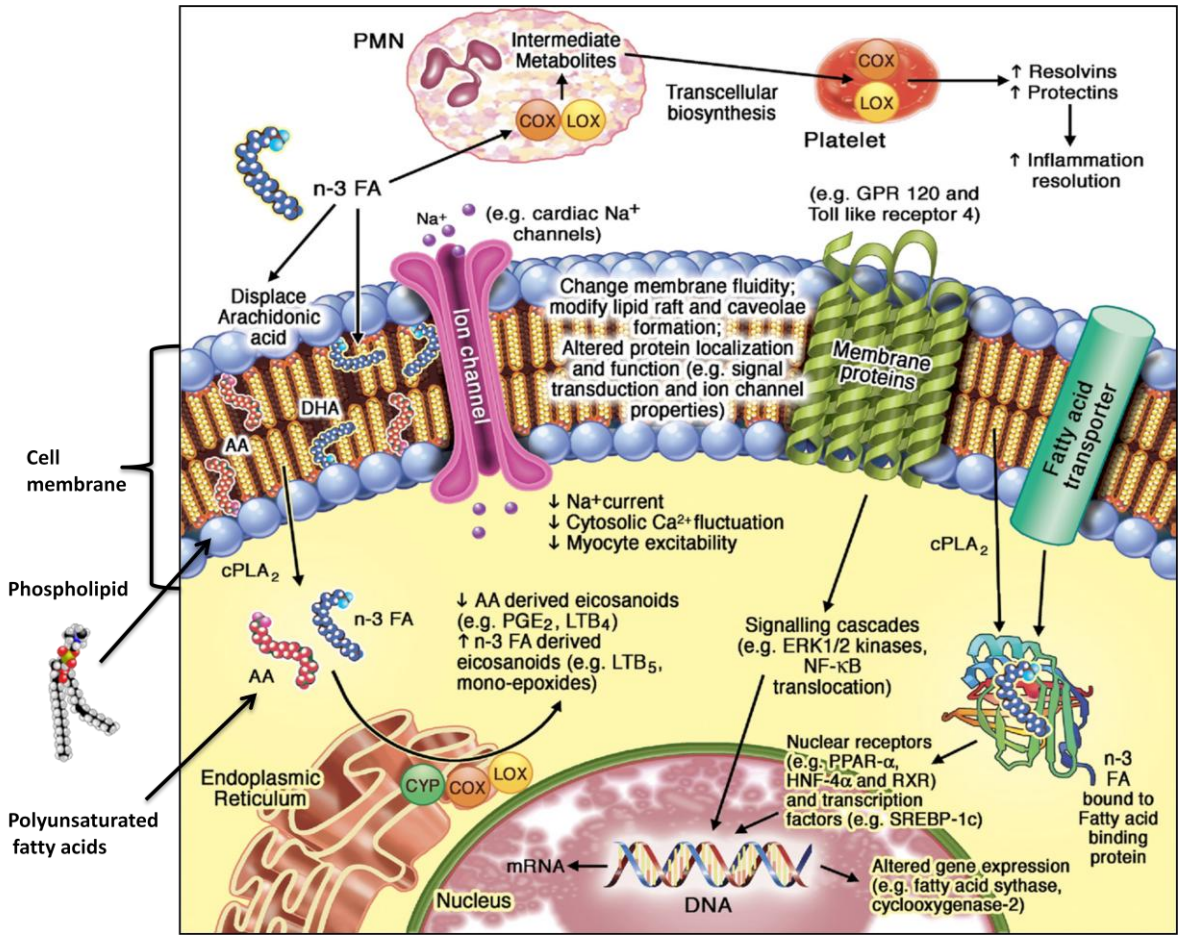

Figure 7. Molecular pathways affected by long-chain polyunsaturated fatty acids (LC-PUFA). n-3 LC-PUFA modulate multiple molecular pathways that together contribute to their physiological effects. The physicochemical properties of cellular and organelle membranes are influenced by their lipid composition. Incorporation of n-3 LC-PUFA into these membranes alters membrane fluidity and biophysics of lipid rafts that modulate protein function and signaling events. Ion channels such as sodium (Na^+), L-type calcium (Ca^{2+}), and Na^+–Ca^{2+} exchangers might be similarly modulated by n-3 LC-PUFA incorporation into lipid membranes. Further, n-3 LC-PUFA seem to directly interact with membrane channels and proteins. n-3 LC-PUFA directly regulate gene expression via nuclear receptors and transcription factors. n-3 LC-PUFA are natural ligands of many key nuclear receptors in multiple tissues. Interactions between n-3 LC-PUFA and nuclear receptors are modulated by cytoplasmic lipid binding proteins (e.g. fatty acid (FA) binding proteins) that transport the FAs into the nucleus. n-3 LC-PUFA also alter function of transcription factors. Such genetic regulation contributes to observed effects of n-3 LC-PUFA on lipid metabolism and inflammatory pathways. After release from phospholipids by cytosolic phospholipase A2 (cPLA2), PUFA including n-3 LC-PUFA are converted to eicosanoids by cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) enzymes. n-3 LC-PUFA displace arachidonic acid (AA) in membrane phospholipids, reducing the production of AA-derived eicosanoids (e.g., prostaglandin E2 (PGE2)) while increasing those generated from n-3 LC-PUFA. This altered eicosanoid profile might influence inflammation, thrombosis, and vascular function. Emerging evidence suggests that n-3 LC-PUFA play an important role in inflammation resolution via specialized pro-resolving mediators, including resolvins or protectins that are n-3 LC-PUFA metabolites derived from actions of COX and LOX. The roles of each of these molecular pathways in the cardiovascular protection of n-3 LC-PUFA represent promising areas for future investigation (75).

1.3.3 Anti-arrhythmic effect of n-6 and n-3 LC-PUFA

In addition to the beneficial effect of EPA and DHA on inflammation (4, 5), researchers have in clinical studies examined the anti-arrhythmic effect of n-3 LC-PUFA, which in part is considered to be due to their effects on human atrial electrophysiology (76-78). This effect is among the most intriguing potential physiological effects of n-3 LC-PUFA, and also the most challenging to document in humans. In vitro and animal experiments suggest that n-3 LC-PUFA directly influence atrial and ventricular myocyte electrophysiology, potentially mediated by effects on membrane ion channels (76, 79). The ionic mechanism underlying the anti-arrhythmic effect of n-3 LC-PUFA is not fully understood in humans. In vitro study on adult human atrial myocytes has shown that EPA and DHA inhibited human atrial I_{to}, I_{Kur}, and I_{Na} in a concentration-dependent manner, which indicates that n-3 LC-PUFA may contribute, at least in part, to the prevention of atrial fibrillation in humans (77, 80, 81). However, the effect of short-term n-3 LC-PUFA supplementation on the incidence of POAF following open heart surgery have yielded conflicting results (6-10). It has been suggested that the anti-arrhythmic effect of n-3 LC-PUFA pretreatment may take longer time to develop than would direct blockade of ion channels (82), since EPA and DHA incorporation into human atrial cell membrane PL may take 2 to 3 months to plateau, but plasma n-3 LC-PUFA levels rise quickly and plateau within days. The anti-fibrillatory effect of n-3 LC-PUFA seems most consistent for coronary heart disease mortality and sudden cardiac death (75), but potential effect of n-3 LC-PUFA on other cardiovascular outcomes are less well established, including AF and POAF.

Anti-arrhythmic drug therapy is a preventive approach for most patients with POAF, but currently available agents are limited by limited efficacy, tolerance and/or safety and cost (83). Although n-3 LC-PUFA have emerged as an interesting therapeutic option for POAF, more studies are needed to demonstrate their efficacy before treatment can be recommend with n-3 LC-PUFA for POAF and other arrhythmias.

2 Aims

Open heart surgery is commonly performed to treat a variety of cardiac diseases, including coronary artery disease and valvular disorders. Cardiac surgery provokes a vigorous inflammatory response that propagates within the injured tissue to initiate the healing process. Recently there has been increased interest in the role of specific dietary FA, especially the n-6 LC-PUFA AA and the n-3 LC-PUFA EPA and DHA, and their effect on health and disease. The inflammatory response following open heart surgery is mediated by a number of complex interacting molecular networks, including eicosanoids and inflammatory mediators. The n-3 PUFA EPA and DHA are potentially useful anti-inflammatory agents as they can affect the production of eicosanoids and inflammatory mediators.

Atrial fibrillation is a common complication after open heart surgery. There is emerging data supporting that inflammation may be important factor in the development of POAF, whereas high CRP and IL-6 concentrations have been associated with higher risk of POAF. In light of the beneficial effect of n-3 LC-PUFA on inflammation, researchers have also focused on the anti-arrhythmic effect of n-3 LC-PUFA. However, the effect of n-3 LC-PUFA on the incidence of POAF in patients undergoing CABG surgery or a more complex surgical intervention has been contradictory.

Since the FA composition of the RBC membrane lipids has been shown to be a good indicator of the FA composition of atrial tissue, the potential benefits of n-3 LC-PUFA in RBC on the incidence of POAF were examined.

The primary aim of this study was to examine the association between circulating inflammatory mediators, n-6 and n-3 LC-PUFA in RBC membrane lipids and the incidence of POAF in patients undergoing open heart surgery.

The specific aims of this study were:

1. To investigate the association of circulating inflammatory mediators with levels of n-6 and n-3 LC-PUFA in RBC membrane lipids.

2. To investigate the association of circulating inflammatory mediators with the risk of POAF.

3. To investigate the association of n-6 and n-3 LC-PUFA levels in RBC membrane lipids with the risk of POAF.

3 Materials and methods

3.1 Subjects and study design

The study is a part of a prospective, randomized, double-blinded, placebo-controlled clinical trial on the use of n-3 LC-PUFA in the prevention of POAF, for which patients were recruited between August 2007 and May 2009 at the Landspitali University Hospital in Reykjavik, Iceland. Details of the study design have been described previously (84). All consecutive patients scheduled for elective or semi-emergent open heart surgery during the study period were evaluated for participation, and a total of 170 patients were enrolled. Exclusion criteria included age younger than 40 years, a history of any form of supraventricular arrhythmias, the use of amiodarone and/or sotalol, and patients undergoing an emergent operation. Once a decision to perform open heart surgery had been made and a scheduled date of the operation was available, the aims and protocol of the study were explained to eligible patients. Those who consented to participation were asked to discontinue intake of cod liver oil and n-3 LC-PUFA capsules if they were taking such supplements but were otherwise advised to remain on their usual diet. The patients were then randomly assigned to one of two study groups. Active treatment (n-3 LC-PUFA group) consisted of a total of 1240 mg of EPA and 1000 mg of DHA as ethyl esters daily and placebo consisted of 2000 mg olive oil in identical capsules. The n-3 LC-PUFA capsules are commercially available in Iceland (Omega Forte, Lysi Inc, Reykjavík, Iceland).

The surgery was cancelled for two patients, leaving 168 patients for further analysis. As there was no difference in the incidence of POAF between the n-3 LC-PUFA and placebo groups (7), the treatment assignment was ignored in the present study. The study was approved by the Bioethics Committee of Landspitali University Hospital and the Icelandic Data Protection Authority. All patients gave written informed consent for their participation in the study (Appendix I).

3.1.1 Study endpoints

Following the operative procedure, the patients were admitted to the intensive care unit and were subsequently transferred to the thoracic surgery ward when their condition was stable. All participants received standard care following the operative procedure and all had continuous electrocardiographic monitoring while hospitalized. The study endpoint, POAF, was defined as an episode lasting more than five minutes. Upon discharge from the hospital or two weeks after the surgery, the patients discontinued the study medication and exited the study.

3.2 Questionnaires and operative characteristics data

Prior to surgery, all participants answered a questionnaire on their fish intake, consumption of liquid cod liver oil and/or n-3 LC-PUFA capsules, smoking habits, alcohol intake, height, body weight and medication use. Since n-3 LC-PUFA may prolong bleeding time, information on possible side effects during the study was meticulously recorded, including operative and postoperative blood loss, major bleeding and number of blood transfusions administered. The need for re-operations in the early postoperative period, death and cerebrovascular accidents were also recorded along with other intra-

and postoperative parameters. At the end of the study, each patient answered a questionnaire regarding their participation, including self-reported side effects of the study medication and whether they thought they were assigned to active treatment group or not (Appendix II).

3.3 Blood sampling and analysis

Venous blood samples were obtained from the patients at three time points during the course of the study: at an average of one week before the surgery (baseline), immediately before the surgery (perioperatively) and at the third postoperative day. The blood samples were collected into disodium ethylenediaminetetraacetic acid (EDTA) tube and the plasma separated from RBC by immediate centrifugation at 1000 *g* for 10 min. The RBC's were washed three times with an isotonic saline solution and the antioxidant butylated hydroxytoluene (BHT), dissolved in methanol, was added to the RBC at a final concentration of 50 mg/L. The plasma and RBC samples were frozen at -76°C and stored until the analysis of the inflammatory mediators and the FA was carried out.

3.3.1 Assessment of inflammatory mediators

The plasma samples were analyzed for the concentration of the pro-inflammatory mediators IL-1β, IL-6, IL-8, IL-12, IL-18, IFN- γ, TNF-α, and TNF-β, and MIP-1α, and the anti-inflammatory mediators IL-10 and TGF-β as described by Skogstrand et al. (85). The samples were diluted 1:10 in phosphate buffered saline (PBS) containing 0.5% Tween 20 and 1% bovine serum albumin (BSA). A 50 µL aliquot was mixed 1:1 with a suspension of capture-antibody-conjugated beads in multiwell plates. After incubation for 1.5 hours at room temperature with gentle shaking, the beads were washed twice with PBS and subsequently reacted for 1.5 hours with a 50 µL mixture of corresponding biotinylated antibodies, each diluted 1:1000. Streptavidin-phycoerythrin (50 µL) was added to the wells and the incubation continued for additional 30 min. Finally, the beads were washed twice and resuspended in 125 µL of PBS and analyzed on the Luminex 100™ platform (Luminex Corp, TX, USA). Samples were measured in duplicates and standard curves were fitted with a five parameter logistic equation (Logistic-5PL) using BioPlex™ Manager 5.0 (Bio-Rad Laboratories, CA, USA).

Plasma hs-CRP was measured on a Hitachi 911 analyzer using a commercially available latex-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). The lower detection limit of the assay was 0.1 mg/L. The total coefficient of variation for hs-CRP measurements of the internal controls was 1.1% at a concentration of 3.73 mg/L and 1.9% at a concentration of 0.68 mg/L. In addition, plasma CRP was measured by a conventional assay daily following the surgery as a part of the regular care of the patients. We used these measurements to define the maximal (peak) CRP in the postoperative phase.

3.3.2 RBC total lipid extraction and analysis of fatty acids

RBC total lipids were extracted as described by Bligh & Dyer (86) exept isopropanol was used instead of methanol (isopropanol/chloroform 2:1, v/v, Merck, Darmstadt, Germany). The antioxidant BHT (50mg/L) was added to the extraction medium. The FA in RBC total lipids were transmethylated for 45 min at 110°C using 14% boron trifluoride/methanol (Sigma Chemical Co., St. Louis, MO). The fatty acid methyl esters (FAME) were analyzed using gas chromatography (GC) (Agilent 6890 N, Agilent,

Palo Alto, CA) equipped with a flame ionization detector and a Chrompack CP-SIL 8CB column (25 m x 250 µm i.d. x 0.12 µm film thickness). The oven temperature was programmed to have an initial temperature of 150°C for 4 min, then rising at 4°C/min to 230°C and at 20°C/min to 280°C and then held isothermal for 4 min. The injector and detector temperatures were maintained at 280°C and 300°C, respectively. Hydrogen was used as the carrier gas. The FAME peaks were identified and calibrated against those of commercial standards (Sigma Chemical Co.; Nu-Chek-Prep, Elysian, MN). FA values are presented as % (by weight) of total FA with chain length from C_{14} to C_{24}. Instrumental control and data handling was done by HP 3365 Chemstation, Version A.02.12. (Hewlett Packard Co., Palo Alto, CA).

3.4 Statistical analysis

Data were presented as median (range), mean ± standard error of the mean (SEM) or percentages, unless otherwise noted. FA values were presented as percent of total FA.

For the first specific aim of the study we used data from all subjects and examined the association between the FA levels and concentrations of inflammatory mediators at baseline, between the FA levels measured perioperatively and concentrations of inflammatory mediators on the third postoperative day as well as beween the perioperative FA levels and the changes in concentrations of inflammatory mediators from the perioperative time period to the third postoperative day. In this analysis Spearman's correlation coefficient was used to assess the association between the levels of FA in RBC membrane lipids and plasma PL. Wilcoxon signed rank test was used to compare the difference in the concentrations of inflammatory mediators between time points. Parired t-test was used to compare the difference in the FA levels between plasma PL and RBC membrane lipids at different time points. Spearman's correlation coefficient was used to examine the relationship between continuous variables and Wilcoxon-Mann-Whitney test to compare groups. Multivariable linear regression was used to estimate the relationship between the levels of FA and concentrations of inflammatory mediators at baseline, adjusting for age, BMI and smoking. Multivariable linear regression was also used to estimate the relationship between the perioperative levels of FA and postoperative concentrations of the inflammatory mediators and the changes in concentrations of inflammatory mediators from baseline to the third postoperative day, adjusting for age, BMI and smoking.

For specific aims two and three we excluded subjects that underwent valvular surgery to eliminate confounding by the complexity of the procedure. In this analysis differences in baseline and operative characteristics between the patients with POAF (POAF group) and those who remained in sinus rhythm (SR group) were compared with Wilcoxon–Mann–Whitney test for continuous variables and the chi squared test or Fisher's exact test for categorical variables. Wilcoxon-Mann-Whitney test was also used to compare the groups with regard to the inflammatory mediators and Wilcoxon signed ranks test to compare the difference in the concentrations of inflammatory mediators between timepoints. An independent samples t-test was used to compare the levels of FA in RBC membrane lipids between the groups. To examine the association between the levels of individual FA and POAF, we compared the rate of POAF between quartiles of the FA levels using chi squared test and the

Somers'd for ordinal variables. To examine independent association between the level of each FA and/or the concentration of each inflammatory mediator and POAF, a logistic regression analysis was used with POAF as the dependent variable, adjusting for age, BMI and smoking and peak postoperative CRP, although CRP was not used as covariate when examining the association between the concentration of each inflammatory mediators and POAF. Two-sided P value < 0.05 was considered statistically significant. All statistical analysis was carried out using SPSS software (version 17.0, IBM Corporation, Somers, NY, USA).

4 Results

4.1 Patients undergoing open heart surgery

The baseline characteristics of the patients who underwent open heart surgery are shown in Paper I, Table 1. The patients were elderly with a median age of 67 (range, 43-82) years, 79.2% were men, and their median BMI was 27.4 (17.2-41.3) kg/m^2. Seventy two percent of the patients consumed fish once or more a week, 55% took cod liver oil and one-quarter n-3 LC-PUFA capsules as daily supplements.

4.1.1 Plasma concentrations of inflammatory mediators

The median plasma concentrations of pro-inflammatory and anti-inflammatory mediators at baseline, perioperatively and on the third postoperative day are shown in Table 2. On the third postoperative day, the median plasma concentrations of IFN-γ and TNF-β were lower, and those of hs-CRP, IL-6, IL-8, IL-10 and IL-18 were higher compared with perioperative concentrations ($P < 0.05$) (Paper I, Figure 1). No statistical difference was observed in the median concentrations of TNF- α, IL-1β, IL-12, MIP-1α and TGF-β between the perioperative and the third postoperative day.

On the third postoperative day, the median plasma concentrations of hs-CRP, IL-6, IL-8 and IL-10 were higher, and those of TNF-α, TNF-β, IL-12 and IFN-γ lower compared with baseline concentrations (Table 2). No statistical difference was observed in the median concentrations of IL-1β, IL-18, MIP-1α and TGF-β between the baseline and the third postoperative day (Table 2).

Table 2. Median plasma concentrations of pro-inflammatory and anti-inflammatory mediators at baseline, perioperatively and on the third postoperative day.

Inflammatory mediators	**Baseline** (n=159)	**Perioperative** (n=163)	**Third postoperative day** (n=162)
TNF-α (pg/mL)	4 (4-210)	4 (4-181)*	4 (4-224)*
TNF-β (pg/mL)	133.5 (4-403)	120 (4-2083)**	83 (4-1545)**
IL-1β (pg/mL)	4 (4-108)	4 (4-110)	4 (4-90)
IL-6 (pg/mL)	13 (4-277)	10 (4-277)**	104.5 (4-2132)**
hs-CRP (mg/L)	3.56 (0.58-28.70)	2.2 (0.1-144.9)**	166 (7.69-463)**
IL-12 (pg/mL)	10 (4-326)	9 (4-275)*	10 (4-199)*
IL-18 (pg/mL)	364 (65-1239)	302 (52-1033)**	376 (62-1330)
IFN-γ (pg/mL)	4 (4-146)	4 (4-125)**	4 (4-71)**
IL-8 (pg/mL)	4 (4-86)	4 (4-65)	4 (4-356)
MIP-1α (pg/mL)	42 (10-596)	46 (10-603)	38.5 (10-468)
IL-10 (pg/mL)	15 (4-109)	13 (4-131)**	30 (4-221)**
TGF-β (pg/mL)	39 (16-403)	39 (11-402)	55 (17-415)

Data are expressed as median (range). *$P < 0.05$, **$P \leq 0.001$ compared with baseline levels, Wilcoxon signed rank test.

4.1.2 Incorporation of short-term n-3 LC-PUFA supplementation into RBC membrane lipids

Within the n-3 LC-PUFA treated patients the levels of EPA and DHA were increased in RBC membrane lipids from baseline to perioperative levels (1.92 ± 0.1% to 2.32 ± 0.09%, and 7.48 ± 0.14% to 7.58 ± 0.14%, respectively; $P < 0.05$) (Figure 8). On the other hand, within the placebo treated patients the levels of EPA decreased (1.96 ± 0.09% to 1.88 ± 0.08%; $P < 0.05$) in RBC membrane lipids from baseline to perioperative levels, while no change was observed in the levels of DHA (7.31 ± 0.13% to 7.35 ± 0.13%, respectively; $P > 0.05$). No changes were observed in the levels of AA in RBC membrane lipids from baseline to perioperative levels within the n-3 LC-PUFA and placebo treated patients (12.07 ± 0.20% to 12.08 ± 0.20%, and 12.22 ± 0.19% to 12.16 ± 0.18%, respectively; $P > 0.05$).

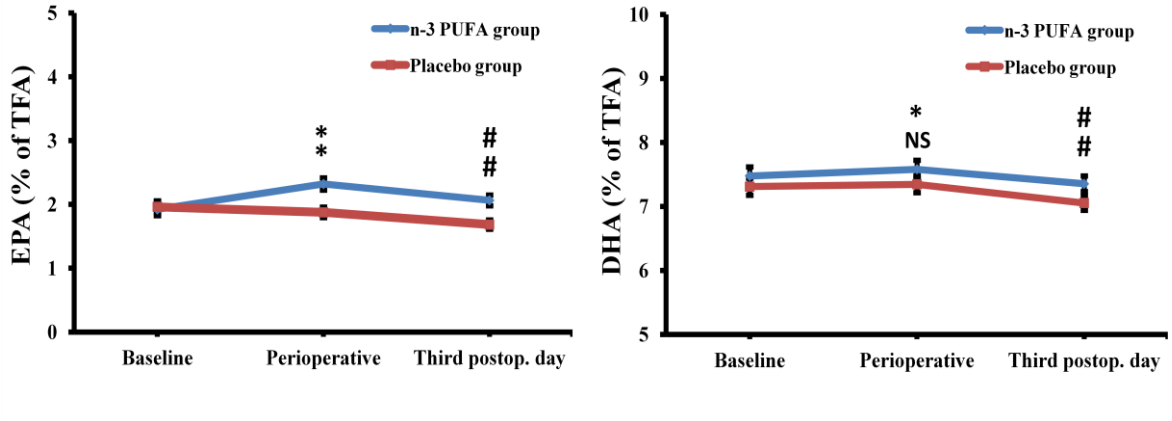

Figure 8. Red blood cell membrane lipid levels (% of total FA, TFA) of a) eicosapentaenoic acid (EPA) and b) docosahexaenoic acid (DHA) in the n-3 LC-PUFA supplement and placebo groups, at baseline, perioperatively and on the third postoperative day. Values are expressed as mean±SEM. *P < 0.05, compared with baseline, #P < 0.05, compared with perioperative value. Wilcoxson signed rank test.

4.1.3 Relationship between FA levels in plasma PL and RBC membrane lipids

The FA levels of plasma PL and RBC membrane lipids at baseline and perioperatively are shown in Paper I, Table 2. RBC contained higher levels of total MUFA, OA, AA, total n-3 LC-PUFA, docosapentaenoic acid (DPA) and DHA, and higher ratio of AA to EPA + DHA than plasma PL ($P < 0.001$). In contrast, the levels of total SFA, total n-6 PUFA, LA and EPA were lower in RBC than in plasma ($P < 0.001$). The levels of all the FA in RBC correlated directly with the corresponding plasma FA at both time points (Spearman's correlation, $P < 0.05$).

The levels of AA, EPA, DHA and EPA + DHA (the Omega-3 Index) in RBC membrane lipids correlated directly with the corresponding plasma levels at baseline (Figure 9), as well as they did perioperatively and postoperatively (data not shown).

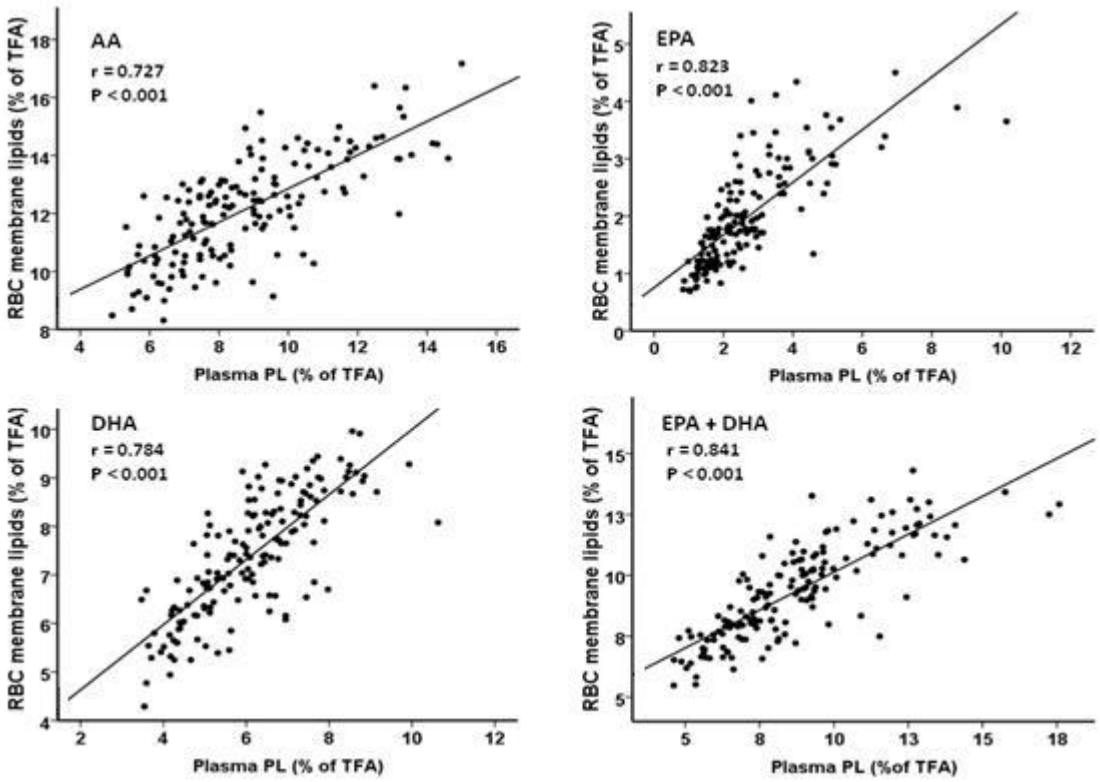

Figure 9. Relationship between levels of arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and EPA + DHA in RBC membrane lipids and levels of the corresponding fatty acids in plasma phospholipids (PL) at baseline. Spearman's correlation.

4.1.4 Relationship between levels of inflammatory mediators and n-6 and n-3 LC-PUFA in RBC membrane lipids

A multivariable linear regression model was used to assess the relationship between the concentrations of circulating inflammatory mediators and levels of AA, EPA and DHA in plasma PL and RBC membrane lipids at baseline (Paper I, Table 3). In plasma PL, higher level of EPA was associated with lower concentration of hs-CRP, and higher DHA level was associated with lower IL-12 and IL-18 concentrations ($P < 0.05$). In RBC, higher AA level was highly associated with higher concentration of TNF-β ($P < 0.01$), whereas higher DHA level was associated with lower IL-18 concentration, and higher levels of EPA + DHA was associated with higher IL-1 β and lower IL-6 and IL-18 concentrations ($P < 0.05$).

The relationship between the concentrations of circulating inflammatory mediators and levels of AA, EPA and DHA in plasma PL and RBC membrane lipids on the day of surgery differed from that at baseline (Paper I, Table 4). In plasma PL, higher level of AA was associated with higher concentration of IL-10, and higher levels of EPA and DHA were associated with lower concentration of IL-18, and higher level of DHA was associated with higher concentration of TGF-β ($P < 0.05$). In RBC, higher AA level was associated with lower concentration of TGF-β and higher concentration of TNF-β, higher level of EPA was associated with higher concentrations of IL-1β and TGF-β, higher DHA level was

associated with lower IL-18 concentration and higher EPA + DHA was associated with higher concentration of IL-1β and lower concentration of IL-18 ($P < 0.05$). In addition there was a borderline significant association between lower postoperative concentration of IL-6 and higher DHA level in plasma PL ($P = 0.05$) and RBC membrane lipids ($P = 0.053$).

A multivariable linear regression model was also used to assess the relationship between the perioperative levels of AA, EPA and DHA in plasma PL and RBC membrane lipids and the changes in the levels of circulating inflammatory mediators from perioperative to the third postoperative day (Paper I, Table 5). In plasma PL, higher level of AA was associated with a more increase in IL-10 and a lesser increase in TGF-β ($P < 0.01$), whereas higher level of EPA was associated with a lesser increase in IL-10 ($P < 0.05$). In RBC, higher AA level was associated with a more pronounced decrease in TNF-β, and a lesser increase in TGF-β, whereas a higher level of EPA was associated with a more increase in IL-1 β and TGF-β. The Omega-3 Index (EPA + DHA) was also associated with a more increase in IL-1β , and TGF-β ($P < 0.05$).

4.1.5 Confounding factors

No associations were observed between age and plasma concentrations of either pro-inflammatory or anti-inflammatory mediators at baseline (data not shown), but the perioperative concentration of TGF-β was negatively associated ($r = -0.165$, $P = 0.036$) with age. Furthermore, the third postoperative day concentrations of IL-6 and IL-8 were positively associated ($r = 0.179$, $P = 0.036$, and $r = 0.180$, $P = 0.022$, respectively), and TGF-β negatively associated ($r = -0.168$, $P = 0.033$) with age. The concentrations of hs-CRP at baseline and IL-10 on the third postoperative day were lower in those who consumed fish once or more per week than in those that consumed less ($P = 0.035$ and $P = 0.050$, respectively). The concentration of MIP-1α was negatively associated ($r = -0.161$, $P = 0.042$) with BMI on the third postoperative day.

4.2 Patients undergoing CABG surgery

A total of 125 patients underwent only CABG surgery, of which 62 patients (49.6%) developed POAF. The baseline and operative characteristics of the patients in the POAF and the SR groups are shown in Paper II, Table 1. The POAF group was older (69 (45-82) years vs. 66 (45-79) years, $P < 0.01$), and their BMI lower (27.0 (19.1-35.0) kg/m^2 vs. 28.4 (20.9-41.3) kg/m^2, $P < 0.05$) compared with the SR group. The patients in the POAF group where more likely to consume fish once or more a week than those in the SR group (82.3% vs. 62.3% more than once per week, respectively, $P < 0.05$). The intake of liquid cod liver oil and/or n-3 LC-PUFA capsules was high and similar in both SR and POAF groups (54.0% vs. 56.4% liquid cod liver oil, and 25.4% vs. 27.4% n-3 PUFA capsules, respectively). No difference was in use of cardiopulmonary bypass (CPB) between the SR and POAF groups ($P > 0.05$).

4.2.1 Plasma concentrations of inflammatory mediators

The median plasma concentrations of pro-inflammatory and anti-inflammatory mediators in the SR and POAF groups at baseline are shown in Table 3, and those perioperatively and on the third

postoperative day are shown in Paper II, Table 2 and Figure 1. The median plasma concentrations of inflammatory mediators at baseline, perioperatively and postoperatively are also shown in Appendix III, Table A1. At baseline the concentration of hs-CRP were higher in the POAF group than in the SR group ($P < 0.05$) (Table 3). No difference was found between the groups with regard to the other inflammatory mediators.

Table 3. Median plasma concentrations of pro-inflammatory and anti-inflammatory mediators at baseline in the sinus rhythm (SR) and postoperative atrial fibrillation (POAF) groups.

Inflammatory mediators	**SR group** (n=63)	**POAF group** (n=62)
TNF-α (pg/mL)	4 (4-210)	4 (4-138)
TNF-β (pg/mL)	104 (4-1520)	139.0 (4-733)
IL-1β (pg/mL)	4 (4-108)	4 (4-85)
IL-6 (pg/mL)	16.5 (4-143)	13.5 (4-50)
hs-CRP (mg/L)	5.87 (0.58-18.80)	3.10 (0.67-19.08)*
IL-12 (pg/mL)	9 (4-252)	9.5 (4-129)
IL-18 (pg/mL)	406.5 (184-1129)	351.5 (65-1000)
IFN-γ (pg/mL)	4 (4-146)	4 (4-117)
IL-8 (pg/mL)	4 (4-86)	4 (4-51)
MIP-1α (pg/mL)	50 (10-577)	35.0 (10-596)
IL-10 (pg/mL)	17.50 (4-109)	14.0 (4-97)
TGF-β (pg/mL)	60 (36-403)	39 (17-328)

Data are expressed as median (range). *P<0.05 compared with SR group, Wilcoxon-Mann-Whitney test.

On the third postoperative day the concentrations of IL-8, IL-18 and IL-10 were higher ($P < 0.05$), and that of TNF-β was lower ($P < 0.01$) compared with the perioperative concentrations in both the SR and POAF groups (Paper II, Table 2). Additionally, in the SR group the postoperative concentration of IFN-γ was lower compared with the perioperative concentration ($P < 0.05$). Both the SR and POAF groups had higher postoperative concentrations of IL-6 and hs-CRP compared with the perioperative concentrations ($P < 0.01$) (Paper II, Figure 1). In addition, on the third postoperative day the concentration of IL-6 was higher in the POAF group than in the SR group ($P < 0.05$).

4.2.2 Fatty acid composition of RBC membrane lipids

The FA composition of RBC membrane lipids in the SR and POAF groups at baseline, preoperatively, and third postoperative day is shown in Appendix III, Table A3. To eliminate confounding resulting from different complexity of the surgical procedure, subjects undergoing open heart procedures other than CABG were excluded from all analysis concerning POAF. Table A4 in Appendix III shows the FA composition of RBC membrane lipids in the SR and POAF groups undergoing only CABG surgery at the three time points. Perioperatively, the SR and POAF groups differed in the n-6 and n-3 LC-PUFA levels of RBC membrane lipids (Paper II, Figure 2). The POAF group had lower levels of the n-6 LC-PUFA AA and higher levels of DHA, total n-3 LC-PUFA, and the Omega-3 Index compared with the SR group ($P < 0.05$). No difference in the EPA levels was found between the two groups ($P > 0.05$).

4.2.3 Incidence of POAF

In the logistic regression models adjusting for age, BMI and smoking as significant predictors of POAF there was no association observed between peri- or postoperative concentrations of any of the inflammatory mediators and POAF (Table 4).

Table 4. Multivariable logistic regression analysis of predictors of POAF.

Inflammatory mediators	OR (95% CI)	P-value
Perioperative		
TNF-α	0.989 (0.976-1,003)	0.124
TNF-β	0.999 (0.997-1.001)	0.360
IL-1β	0.990 (0.967-1.012)	0.373
IL-6	0.987 (0.968-1.008)	0.221
hs-CRP	1.028 (0.950-1.112)	0.500
IL-12	0.991 (0.978-1.005)	0.195
IL-18	0.999 (0.997-1.001)	0.607
IFN-γ	0.983 (0.953-1.015)	0.301
IL-8	0.982 (0.939-1.026)	0.417
MIP-1α	0.997 (0.993-1.001)	0.100
IL-10	0.992 (0.974-1.010)	0.378
TGF-β	0.999 (0.993-1.004)	0.591
Third postoperative		
TNF-α	0.987 (0.967-1.007)	0.204
TNF-β	0.999 (0.997-1.001)	0.466
IL-1β	1.006 (0.980-1.033)	0.648
IL-6	1.002 (1.000-1.003)	0.092
hs-CRP	1.004 (0.998-1.009)	0.237
IL-12	0.991 (0.972-1.010)	0.348
IL-18	1.000 (0.998-1.002)	0.834
IFN-γ	0.989 (0.947-1.033)	0.614
IL-8	0.996 (0.986-1.006)	0.390
MIP-1α	0.995 (0.991-1.000)	0.052
IL-10	1.000 (0.990-1.010)	0.998
TGF-β	1.001 (0.995-1.006)	0.841

See Table 1 for the abbreviations.
The inflammatory mediators are added individually to a model that Includes age, BMI and smoking.

However, in the logistic regression models adjusting for age, BMI, smoking and maximal CRP concentration as significant predictors of POAF, where each one of the FA was separately added to the model, the risk of POAF increased with higher perioperative level of DHA (OR (95% CI) = 1.506 (1.010-2.246)), and higher postoperative levels of EPA (OR (95% CI) = 1.952 (1.043-3.654)), DHA (OR (95% CI) = 1.983 (1.260-3.121)), total n-3 LC-PUFA (OR (95% CI) = 1.440 (1.119-1.853)), and the Omega-3 Index (OR (95% CI) = 1.399 (1.070-1.828)) in RBC membrane lipids ($P < 0.05$) (Paper II, Table 3).

The incidence of POAF according to quartiles of peri- and postoperative AA, EPA, DHA, total n-3 LC-PUFA levels, and the Omega-3 Index in RBC membrane lipids is shown in Paper II, Table 4 and Figure 3. Perioperatively, there was a significant trend for an increasing incidence of POAF with higher quartiles of the DHA and total n-3 LC-PUFA levels, and of the Omega-3 Index ($P = 0.006$, $P = 0.010$ and $P = 0.014$, respectively). Postoperatively, there was a significant difference in POAF incidence between quartiles of DHA and total n-3 LC-PUFA levels, and the Omega-3 Index ($P = 0.007$, $P = 0.034$ and $P = 0.018$, respectively), with a significant trend for an increasing incidence of POAF with higher quartiles ($P = 0.001$, $P = 0.002$ and $P= 0.001$, respectively).

5 Discussion

The results of the study presented in this thesis demonstrate, unexpectedly, that relatively high levels of DHA and EPA + DHA (the Omega-3 Index) in RBC membrane lipids were associated with increased risk of POAF. In addition, when adjusted for the confounding factors age, BMI and smoking, no association was found for inflammatory mediator concentrations and development of POAF in patients undergoing CABG surgery.

The results of this study suggest that the n-6 LC-PUFA AA in plasma PL and cell membranes may contribute to anti-inflammatory response following surgical injury, while the contribution of the n-3 LC-PUFA EPA and DHA are associated with an anti-inflammatory response. Further, this study show that relatively high levels of DHA and the Omega-3 Index in RBC membrane lipids are associated with increased risk of POAF. These results were unexpected, and suggest that this therapy is not as free of adverse effects as previously thought. In addition, we found no association between concentrations of perioperative or postoperative inflammatory mediators with development of POAF in patients following CABG surgery.

5.1 Anti-inflammatory effect of n-6 and n-3 LC-PUFA

Observational studies have demonstrated that dietary EPA and DHA reduce circulating concentrations of pro-inflammatory mediators, such as TNF-α, IL-6 and CRP (87). Ferrucci and coworkers (88) investigated the relationship between baseline levels of FA in plasma and pro-inflammatory and anti-inflammatory mediators in humans. This study (88) in the general population revealed that lower plasma levels of AA, EPA and DHA were independently associated with a higher concentration of IL-6 and a lower concentration of the anti-inflammatory mediator TGF-β. They also found that lower plasma levels of EPA and DHA were associated with a lower concentration of IL-10. Based on the associations between the levels of FA and inflammatory mediators one might expect that perioperative levels of FA in plasma PL and primarily in RBC membrane lipids might affect the inflammatory response to injury following open heart surgery.

We found that AA level in RBC at the time of surgery were associated with a higher postoperative level of the pro-inflammatory TNF-β and a lower postoperative level of the anti-inflammatory TGF-β. However, the perioperative plasma PL level of AA associated directly with the anti-inflammatory IL-10, as well as with a greater increase in IL-10 level following surgery. Thus, AA may have an important effect on both pro- and anti-inflammatory mediators, directly or indirectly. The aim is true for EPA level and the Omega-3 Index following surgery showing association with both pro- and anti-inflammatory mediators. On the other hand, DHA showed mainly an anti-inflammatory effect in response to surgery. Taken together these findings suggest that the n-6 and n-3 LC-PUFA are important contributors to the regulation of the inflammatory response following open heart surgery.

Studies have demonstrated that the production of inflammatory mediators is initiated immediately after tissue injury and that of others early in the postoperative course (16, 89-92). It has been suggested that early induction of the genes coding for the pro-inflammatory mediators TNF-α and IL-1β is important for normal repair and wound healing (89). TNF-α and IL-1β have been found to

peak earlier (16, 90-92), with IL-6 and IL-8 peaking later in the day of operation (16, 92, 93). Both TNF-α and TNF-β induce the expression of IL1-β (21), and IL1-β and TNF-α act synergistically to stimulate the production of IL-6 (17), which regulates the production of hs-CRP in liver (94). Moreover, TNF-α and IL-1β induced expression of IL-8 by endothelial cells may play an important role in reperfusion injury (92, 95). In the present study, where 80% of the patients underwent CPB with a median aortic cross-clamp time of 48 min, there was still a rise in the concentrations of hs-CRP, IL-6, IL-8, and IL-10 on the third postoperative day, while TNF-α, TNF-β and IL-12 concentrations were decreased. The clinical prognosis following CPB may depend on the balance between pro-inflammatory and anti-inflammatory mediators (1). The anti-inflammatory mediator IL-10 is a potent inhibitor of TNF-α, IL-1β and IL-8 expression (96), and IL-10 has been shown to be released together with pro-inflammatory mediators during and after CPB (97). In general, AA released from cell membrane PL is thought to have pro-inflammatory properties due to its role as precursor to oxygenated products that have potent vasoactive and chemotactic actions (98). During an acute inflammatory response, macrophages generate the potent anti-inflammatory and proresolving lipid mediator lipoxin A_4 from AA, which in turn stimulates the production of IL-10 (70). The relatively high level of AA in RBC membrane lipids observed in our study is also expected to be present in membranes of macrophages and other types of blood cells. In fact, the patients in our study had an elevated plasma concentration of IL-10 on the third postoperative day, while there was no association with the baseline level of AA in RBC membrane lipids. The production of IFN-γ from T and NK cells are stimulated by IL-12 along with IL-18 (34). When released into the circulation, IFN-γ is detecable in vivo by 6 hours and may be persistently elevated for as long as 8 days (13). IFN-γ has important roles in activating circulating and tissue macrophages. Injured tissues, such as operative wounds, also demonstrate that presence of IFN-γ production 5 to 6 days after injury. In the present study there was a decline in the concentration of IFN-γ on the third postoperative day, which is consistent with a previous report in patients undergoing cardiac surgery with CPB (99).

The biological function of CRP is not fully understood. However, evidence suggests that elevated hs-CRP concentration represents one of the strongest and most independent predictive risk factor for cardiovascular diseases (31, 100, 101). Elevated preoperative CRP level has been associated with development of POAF and also with AF in non-surgical patients (59, 102). The results, in our previous study (7), supported the notion that inflammation may be associated with pathogenesis of POAF as the peak postoperative levels of CRP were higher in the patients who developed POAF compared with those that did not. On the other hand, in the present study, we did not find such association between peri- or postoperative levels of hs-CRP or any of the other inflammatory mediators assessed. These results are in accordance with other controlled studies (103, 104). Nevertheless, further studies are required, as it has been concluded that the modulation of inflammation would probably represent a major therapeutic goal in the prevention of POAF.

Ferrucci et al. (88) concluded that their findings support the notion that AA and n-3 LC-PUFA may modulate the inflammatory response by acting both on the pro-inflammatory and anti-inflammatory arms of the cytokine network. In our study, we found that the baseline level of EPA + DHA in RBC membranes, a biomarker called Omega-3 Index and shown to be associated with cardioprotection

(105), may contribute to anti-inflammatory response, as it was inversely associated with IL-6, and also to pro-inflammatory response, as it was directly associated with IL-1β. Furthermore, Ferrucci et al. (106) have reported that the concentrations of IL-6, IL-18 and CRP increase with age in population low in n-3 PUFA. No such age-related effect was observed in our study, possibly due to the relatively high n-3 LC-PUFA levels in plasma PL and RBC membrane lipids of our patients. These high n-3 LC-PUFA levels in our patients are taken to reflect the dietary intake of the n-3 LC-PUFA found in fish and cod liver oil. On the other hand, our results are consistent with the findings that n-3 LC-PUFA supplements lead to a reduction in the plasma concentration of the pro-inflammatory mediator IL-18 in elderly men (107).

The participants in our study, mostly patients with coronary artery disease requiring revascularisation, are subjects in whom the association between the n-6 and n-3 LC-PUFA and inflammation is of great interest. In general, the n-3 LC-PUFA are thought to be beneficial for the disease course possibly based on their proposed anti-inflammatory properties, while the n-6 LC-PUFA AA has pro-inflammatory property. Our data indicate that, in patients undergoing open heart surgery, the n-3 LC-PUFA are associated with lower levels of pro-inflammatory mediators, in particular CRP, IL-12 and IL-18. Furthermore, our findings support the notion that n-6 and/or n-3 LC-PUFA in cell membrane lipids and/or plasma PL play an important role in modulating the inflammatory response following surgical injury. However, their effects may be complex. Additional studies are needed to investigate the relationship between plasma and membrane lipids and pro-inflammatory and anti-inflammatory mediators early following open heart surgery.

5.2 Anti-arrhythmic effect of n-6 and n-3 LC-PUFA

There has been a lot of interest in the potential anti-arrhythmic effect of the n-3 LC-PUFA EPA and DHA, which in part is considered to be due to their effects on human atrial electrophysiology (76-78). In spite of the beneficial effects of EPA and DHA on inflammation (4, 5, 72) and arrhythmia (76-78) in cell and animal models, the effect of n-3 LC-PUFA on the incidence of POAF in patients undergoing a CABG surgery or a more complex surgical intervention has been conflicting (6-10).

The findings of this study contradict the suggestion that elevated preoperative concentrations of CRP are predictive of POAF amongst patients undergoing CABG (59, 108) The most reliable and reproducible among the inflammatory mediators is thought to be hs-CRP (31). In the present study, we found no difference in the perioperative plasma concentrations neither of hs-CRP nor of the other inflammatory mediators between the SR and POAF group. It has been suggested that anti-arrhythmic effects of n-3 LC-PUFA supplementation could in part be attributed to anti-inflammatory actions (4, 5). A hypothesis of n-3 LC-PUFA anti-inflammatory and immunosuppressive properties is based on EPA and DHA in circulation and/or the cell membranes (72). The eicosanoids of EPA and docosanoids of DHA from membrane PL of inflammatory producing cells can inhibit the transcription of inflammatory mediators such as TNF-α, IL-1β, IL-6 and IL-8 by modulating nuclear translocation of NF-κB, and inhibit AA metabolism to pro-inflammatory AA-derived eicosanoids (5, 109, 110). In addition, EPA- and DHA-derived lipid mediators called resolvins and protectins stimulate anti-inflammatory, pro-resolving and protective signalling pathways (5). Bruins and coworkers (58) demonstrated that IL-6

concentration rises initially and peaks at six hours after surgery and that a second phase occurs in which the CRP concentration peak on postoperative day two. In the present study, the concentrations of hs-CRP and IL-6 were elevated in both SR and POAF groups on the third postoperative day compared with the perioperative day. Further, the hs-CRP concentration was higher in the POAF group compared with those in the SR group at baseline, and postoperatively the IL-6 concentration was higher in the patients of the POAF group who had higher levels of DHA in RBC membrane lipids compared with those in the SR group. Two studies demonstrated that use and duration of cardiopulmonary bypass (CPB) and aortic cross-clamp time has been correlated with degree of inflammatory response (111, 112), while a study (113) showed that elevation in hs-CRP and IL-6 concentrations on the fourth postoperative day were not affected by use of CPB in uncomplicated cardiac surgery. In the present study, no difference was in the use of CPB and aortic cross-clamp time between the two groups.

Intake of n-3 LC-PUFA supplements and fish consumption have been associated with a decreased risk of sudden cardiac death in population based surveys and randomized clinical trials (114). However, the results of studies on the use of n-3 LC-PUFA to decrease events in high risk patients with implantable defibrillators have been disappointing (115). n-3 LC-PUFA have varied effects on atrial tissue which may account for potential anti-arrhythmic activity. This includes anti-inflammatory, anti-oxidant and anti-fibrotic effects (82). Additionally, n-3 LC-PUFA has direct, albeit modest, electrophysiologic effects on ion channels such as the sodium channel, potassium channels, including the the atrial selective I_{Kur} and I_{KAch}, the L type calcium channel and the Na/Ca exchanger (77). Indeed, a number of studies have shown promising results with n-3 LC-PUFA on AF in experimental animal models including decreasd inducibility of AF and shortened duration of induced AF (116).

The results of human studies for primary prevention have, on the other hand, not been as favourable. An initial, open label study, demonstrated a convincing reduction in the incidence of POAF, thus raising optimism that this simple therapy might be helpful in this situation (10). However, three subsequent randomized placebo controlled trials did not confirm these initial results (6-8). Another primary prevention study examining the effect of fish consumption on the occurrence of AF in a general population did not show any benefit (117). A similar pattern has been seen in studies where n-3 LC-PUFA have been investigated for secondary prevention of AF either paroxysmal or after cardioversion. In these studies n-3 LC-PUFA was used as monotherapy or in addition to anti-arrhythmic therapy. While one of these studies showed a benefit, four others did not (82). A recent meta-analysis, which included almost 1200 patients did not demonstrate any benefit of n-3 LC-PUFA in secondary prevention of AF (118).

Why have the results of primary and secondary prevention trials been so conflicting? While the answer is not clear speculation has pointed to different doses of n-3 LC-PUFA administered in these studies, possible delayed effects of n-3 LC-PUFA due to need for incorporation into atrial myocyte membranes and the ratio of EPA and DHA in the preparation used.

The dietary habit of our elderly patients of fish consumption and n-3 LC-PUFA supplement (cod liver oil, n-3 LC-PUFA capsules) is reflected in relatively high n-3 LC-PUFA levels, which is consistent with the general population in Iceland (119). Acute or short-term n-3 LC-PUFA administration

influences the FA composition of FA in plasma PL, that can be viwed as transporters of circulating FA (65). In contrast, the FA pattern of the RBC membrane lipids has been shown to be a good indicator of the FA composition of atrial tissue (66). The length of pre treatment may therefore be of importantance. The ratio of EPA and DHA might also play a role. EPA has a stronger potassium channel blocking capabilities while DHA has greater effects on the sodium channel (120). A recent study from Finland suggested that high serum levels of DHA, but not EPA, were associated with a significant decrease in incident of AF (121). This, however, is in conflict with a previous finding that DHA has no effect on POAF occurrence and that higher levels in plasma PL may actually increase the risk (122).

In that same previous study, there was a U-shaped relationship between n-3 LC-PUFA levels in plasma PL and POAF indicating that DHA may have a beneficial effect in reducing POAF at relatively low levels but not at higher levels (122). In conclusion it was suggested that baseline levels of n-3 LC-PUFA were of importance and in those populations were they are low, additional supplementation may be of help but in those with higher levels it may make matters worse. This has not been widely considered in the explanation of the discrepancy in the results of previously published studies. Also, if baseline levels matter confounding factors such as dietary fish intake may play an important role in the conflicting data.

In this current study we have convincingly demonstrated that the higher the RBC membrane levels of n-3 LC-PUFA and DHA the higher the risk of POAF. This might therefore suggest that higher levels may be pro-arrhythmic. The concept of n-3 LC-PUFA having a pro-arrhythmic effect is not entirely novel. This has been suggested before in a study on ventricular arrhythmias in patients with an inplantable cardioverter-defibrillator (115) and in a study where there was a strong trend toward AF among individuals who consumed $\geq$5 fish meals a week. This current study, however, is the first to link measured levels of n-3 LC-PUFA in RBC membrane lipids with an increased arrhythmia occurrence. What might cause n-3 LC-PUFA to be proarrhythmic? n-3 LC-PUFA increase parasympathetic tone and this is a known mechanism of triggering AF. Additionally, given the direct electrophysiologic effect of n-3 LC-PUFA, unfavourable actions on ion channels by high levels cannot be discounted.

The questions are raised, are n-3 LC-PUFA anti-arrhythmic, pro-arrhythmic, both or perhaps even neither? Despite a number of published studies this remains quite unclear. Our data that higher levels of n-3 LC-PUFA in RBC membrane, which reflect atrial myocyte membrane n-3 LC-PUFA levels, increase the risk of POAF adds an important piece of information to the ongoing debate on the merits of this treatment.

5.3 Limitations of the study

This study was carefully designed, but it has some limitations, including a relatively low number of participants. It is also worth noting that the peak concentration of some inflammatory mediators post surgery may have been missed, since blood samples were only collected at the third postoperative day. Further, late arrhythmia occurrences may have been missed, since the patients were not examined following discharge from the hospital. It has been well established that the incidence of POAF peaks in the first 2-4 days after surgery and this was also the case in the present study, in

which the average time from surgery to AF was approximately 2 days. Thus, it is unlikely that arrhythmia monitoring beyond discharge would have affected the results. The relatively high levels of n-3 LC-PUFA in RBC membrane lipids in our patiens may indicate high levels of these FA already in their cell membranes. The relative significance of cell membrane FA levels needs further investigation. Finally, the examination of association between the various factors and the risk of POAF cannot prove a cause-effect relationship but serve as a hypothesis generating exercise on which future studies may be founded.

6 Conclusion and future perspectives

This study shows that, while inflammation is clearly present in the first few days after CABG, this does not appear to be a strong risk factor for the development of POAF. Additionally, higher RBC membrane levels of n-3 LC-PUFA may be associated with increased risk of POAF, thereby suggesting a possible pro-arrhythmic action of these natural dietary constituents.

The study demonstrated an association between the baseline levels of AA, EPA, DHA and the Omega-3 Index in the RBC membrane lipids and the baseline concentrations as well as the changes in both pro-inflammatory and anti-inflammatory mediators following open heart surgery. Our findings therefore support the notion that n-6 and/or n-3 LC-PUFA in cell membrane lipids play an important role in modulating the inflammatory response following surgical injury. However, their effects on the inflammatory response may be complex. There is an ongoing debate on the difference between the effects of EPA and DHA through which DHA and EPA exert their action. It may be an over-simplification to generalize the effects of n-3 LC-PUFA on cell function as the multiple and complex mechanisms appear to be distinct but likely also complementary. Additional studies are needed to investigate the relationship between membrane FA and pro-inflammatory and anti-inflammatory mediators early following open heart surgery.

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Appendix I

Written informed consistent

Kynningarbréf fyrir þátttöku í vísindarannsókninni:
Ómega-3 fjölómettaðar fitusýrur við gáttatifi eftir opna hjartaaðgerð

Ábyrgðarmaður: Ólafur Skúli Indriðason, sérfræðingur, Lyflækningasvið LSH, Hringbraut, 101 Reykjavík, sími 543-1000, olasi@landspitali.is.

Aðrir rannsóknaraðilar: Bjarni Torfason, yfirlæknir Skurðlækningasvið LSH, Gizur Gottskálksson, yfirlæknir, Davíð Otto Arnar, yfirlæknir og Runólfur Pálsson, yfirlæknir, Lyflækningasvið LSH, Viðar Örn Eðvarðsson, sérfræðingur Barnasvið LSH og Guðrún Valgerður Skúladóttir, vísindamaður, Læknadeild HÍ. Lýsi hf mun gefa ómega-3 og lyfleysu.

Inntak rannsóknarinnar og markmið í hnotskurn: Gáttatif er einn algengasti fylgikvilli við opnar hjartaaðgerðir, svo sem kransæða- og lokuaðgerðir. Oftast er um tímabundið vandamál að ræða og orsakir þess eru ekki með öllu kunnar en afbrigðileg rafvirkni í gáttum hjartans leiðir til óreglulegs hjartsláttar. Eðlileg starfsemi hjartans truflast því og einstaklingar sem fá gáttaflökt eftir aðgerðir þessar eru í meiri hættu á að fá lágan blóðþrýsting og blóðtappa í heila. Dýratilraunir hafa sýnt að ómega-3 fjölómettaðar fitusýrur hafa áhrif á rafertingu í hjartavöðvafrumum og ein lítil rannsókn bendir til að þessar fitusýrur geti dregið úr tímabundnu gáttaflökti meðal manna. Í okkar rannsókn munum við skipta sjúklingum í tvo hópa og meðhöndla annan með ómega-3 fjölómettuðum fitusýrum en hinn með lyfleysu (alveg eins belgjum sem innihalda ólívuolíu). Síðan verður athugað hvort einstaklingar í ómega-3 fitusýruhópnum fái sjaldnar gáttaflökt. Hópaskiptingin verður tilviljunarkennd þannig að hlutkesti mun ráða í hvorum hópnum einstaklingar lenda og hvorki einstaklingarnir sem þátt taka né læknarnir sem umsjón hafa með rannsókninni munu vita hver fær hvaða lyf.

Einstaklingum sem eiga að gangast undir opna hjartaðgerð verður boðin þátttaka. Þeir sem ákveða að taka þátt svara stuttum spurningalista um fyrri sjúkdóma, lyf og mataræði, fara í blóðprufu og hjartalínurit. Þeir verða einnig beðnir að hætta neyslu lýsis og ómega-3 heilsubótarefna. Eftir það verður einstaklingum skipað í annan af tveimur hópum með slembidreifingu og meðhöndlaðir frá og með viku fyrir aðgerð og að útskrift eftir hana. Öll meðferð fyrir og í kringum aðgerðina verður að öðru leyti hefðbundin nema að aukablóðprufur (um 20 ml blóðs) verða teknar til að mæla ómega-3 fjölómettaðar fitusýrur og bólgusvörun fyrir aðgerðina og ef gáttaflökt á sér stað. Blóðsýnum verður fargað eftir rannsóknina.

Rannnsóknin hefur verið samþykkt af Lyfjastofnun, Persónuvernd, Vísindasiðanefnd og Siðanefnd LSH. Einstaklingum er frjálst að hafna þátttöku eða hætta í rannsókninni á hvaða stigi sem er, án útskýringa og án afleiðinga fyrir aðra meðferð sem þeir kunna að vera á.

Ómega-3 fjölómettaðar fitusýrur eru fitusýrur sem í flestum tilfellum eru unnar úr fiski, og er meðal annars mikið af þeim í lýsi. Gjöf þeirra hefur sýnt sig að vera mjög fylgikvillalítil en þó kvarta sumir um ropa og uppþembu, einnig getur blæðingartími lengst og því mun sértaklega verða fylgst með blæðingu í kringum aðgerðina. Önnur lyf sem áhrif hafa á blóðflögur og blæðingartíma svo sem magnýl og plavix eru reyndar gefin sem staðalmeðferð við aðgerðir sem þessar til að minnka líkur á að æðagræðlingar stíflist. Áhætta eða möguleg óþægindi af þátttöku eru því lítil. Ekki verða sérstakar greiðslur fyrir þátttöku í þessari rannsókn

_____

Undirskrift ábyrgðarmanns.

Ef þú hefur spurningar um rétt þinn sem þátttakandi í vísindarannsókn eða vilt hætta þátttöku í rannsókninni getur þú snúið þér til Siðanefndar Landspítala – háskólasjúkrahúss, Fossvogi, 108 Reykjavík.. Sími: 543 7465, fax: 543 2339, tölvupóstur: sidanefnd@landspitali.is.

Upplýst samþykki fyrir þátttöku í vísndarannsókninni:

Ómega-3 fjölómettaðar fitusýrur við gáttatifi eftir opna hjartaaðgerð

Með undirskrift minni hef ég samþykkt að taka þátt í rannsókninni **Ómega-3 fjölómettaðar fitusýrur til að fyrirbyggja gáttatif eftir opna hjartaaðgerð**. Ég hef fengið skriflegar upplýsingar um rannsóknina, hef lesið upplýsinga og kynningarbréfið og haft tækifæri til að spyrja um hana og framkvæmd hennar. Ég samþykki einnig að eftirlitsstofnanir, þar með talin Lyfjastofnun og Vísindasiðanefnd, fái aðgang að þeim rannsóknargögnum sem þurfa þykir, vegna framkvæmdar og eftirlits með rannsókninni.

Mér er ljóst að ég get neitað þátttöku og að ég get hætt þátttöku hvenær sem er án þess að það hafi áhrif á meðferð mína að öðru leyti.

Dagsetning: _____

_____

Undirskrift þátttakanda

_____

Undirskrift þess sem leggur yfirlýsinguna fyrir.

Kynningarbréf og upplýst samþykki eru í tvíriti og fær þátttakandi eitt eintak af hvoru en ábyrgðarmenn rannsóknarinnar hitt eintakið.

Appendix II

Questionnaires and progress records

Spurningalisti við upphaf rannsóknar

Rannsóknarnúmer:_____

Nafn sjúklings:_____

Kennitala:_____

Dagsetning (dd-mm-yy):_____

Kyn: M / K

Aldur: _____

Hæð: _____cm Þyngd: _____kg

Reykir þú? Já / Nei

Neytir þú áfengis? _____Aldrei

_____Einu sinni í viku eða sjaldnar

_____Oftar en einu sinni í viku

Tekur þú lýsi? Já / Nei

Tekur þú ómega-3 hylki? Já / Nei

Borðar þú fisk? _____Aldrei

_____Einu sinni í viku eða sjaldnar

_____Oftar en einu sinni í viku

Lyf: _____

_____

Spurningalisti við lok rannsóknar

Rannsóknarnúmer:_____

Dagsetning (dd-mm-yy):_____

Hefur þú fundið fyrir einhverjum óþægindum sem þú telur að rekja megi til rannsóknarlyfsins?
Já / Nei

Ef já, hverjum? _____

_____

_____

_____

Hefur þú haft eftirfandi einkenni:

Óeðlilega mikinn ropa		Já /Nei	
Lýsisbragð í munni	Já /Nei		
Uppþemba	Já /Nei		
Ógleði		Já /Nei	
Uppköst	Já /Nei		
Niðurgangur	Já /Nei		
Hægðatregða		Já /Nei	
Útbrot		Já /Nei	
Óeðlilega miklar blæðingar	Já /Nei		
Annað		Já /Nei	Hvað? _____

Hvort telur þú að þú sért í þeim hópi sem fékk ómega-3 fjölómettaðar fitusýrur eða lyfleysu?

Ómega-3 ___Lyfleysa

Framvinduskrá þátttakanda – fyrir aðgerð.

Rannsóknarnúmer:_____

Við slembiröðun:

Dagsetning (dd-mm-yy):_____

Undirliggjandi hjartasjúkdómur: _____

Útfallsbrot hjarta: _____%

Euroscore: _____

Aðrir sjúkdómar:

Háþrýstingur: já/nei

Sykursýki: já/nei

Heilablóðfall: já/nei

Útæðasjúkdómur: já/nei

Aðrir sjúkdómar:_____

_____

Rannsóknir:

CRP: _____ mg/l.

Framvinduskrá þátttakanda – Við innlögn

Rannsóknarnúmer:_____

Dagsetning (dd-mm-yy):_____

Rannsóknir:

Hemóglóbín: _____ g/l.

Kreatínín: _____ μmól/l.

Kalíum: _____ mmol/l.

CRP: _____ mg/l.

Cholesteról _____

HDL _____

Þríglíseríðar _____

Hjartarafrit: _____

Blóðþrýstingur:_____

Þyngd:_____kg

Hæð:_____cm

Framvinduskrá þátttakanda – í aðgerð.

Rannsóknarnúmer:_____

Dagsetning (dd-mm-yy):_____

Tegund aðgerðar:_____

Lengd aðgerðar:_____

Tími á hjarta-lungnavél:_____

Blóðtap: _____ml

Fjöldi blóðeininga:_____

Fjöldi blóðflögueininga_____

Fjöldi plasmaeininga:_____

Gáttatif: já/nei

Aðrar hjartsláttartruflanir: já/nei Hverjar:_____

Fylgikvillar í aðgerð:

_____

_____

_____

_____

Framvinduskrá þátttakanda – á gjörgæslu.

Rannsóknarnúmer:_____

Dagsetning (dd-mm-yy):_____

Gáttatif: já/nei

Aðrar hjartsláttartruflanir: já/nei Hverjar:_____

Blóð í dren fyrsta sólarhring:_____ml

Blóð í dren samtals:_____ml

Hemóglóbín gildi: 1._____ 2._____ 3._____ 4._____ 5._____

Fjöldi blóðeininga:_____

Tími á öndurnavél:_____klst

Lægsti blóðþrýstingur:

Serum kreatínín hækkar um meira en 50% miðað við gildi fyrir aðgerð: já/nei

Serum kreatínín hækkar um meira en 100% miðað við gildi fyrir aðgerð: já/nei

Skilunarmeðferð: já/nei

Sýkingar

Í húð:	já/nei
Tengt dreni:	já/nei
Tengt æðaleggjum	já/nei
Sepsis	já/nei

Tími á gjörgæslu: _____klst

Aðrir fylgikvillar:

Framvinduskrá þátttakanda – á legudeild/lok rannsóknar.

Rannsóknarnúmer:_____

Dagsetning (dd-mm-yy):_____

Gáttatif > 5 mín: já/nei

Tímasetning gáttatifs: _____ klst e. aðgerð

Meðferð gáttatifs: Eingin
Lyf
Rafvending

Aðrar hjartsláttartruflanir: já/nei Hverjar:_____

Lægsti blóðþrýstingur:

Fyrsta þyngd á deild: _____ kg

Serum kreatínín hækkar um meira en 50% miðað við gildi fyrir aðgerð: já/nei

Serum kreatínín hækkar um meira en 100% miðað við gildi fyrir aðgerð: já/nei

Skilunarmeðferð: já/nei

Sýkingar
- Í húð: já/nei
- Tengt dreni: já/nei
- Tengt æðaleggjum já/nei
- Sepsis já/nei

Heilablóðfall: já/nei ef já, tappi eða blæðing?

Tími á legudeild: _____dagar

Aðrir fylgikvillar:

Dagsetning útskriftar/rannsóknarloka (dd-mm-yy):_____

Appendix III

Table A1. Plasma levels of inflammatory mediators at baseline, perioperatively and on the third postoperative day in the sinus rhythm (SR) and postoperative atrial fibrillation (POAF) groups.

Inflammatory markers	Baseline		Perioperative		Third postoperative day	
	SR group (n=63)	POAF group (n=62)	SR group (n=63)	POAF group (n=62)	SR group (n=63)	POAF group (n=62)
TNF-α (pg/mL)	4 (4-210)	4 (4-138)	4 (4-181)	4 (4-147)	4 (4-224)	4 (4-76)
TNF-β (pg/mL)	104 (4-1520)	139.0 (4-733)	99 (4-2083)	111.0 (4-750)	50 (4-1545)	89.5 (4-390)
IL-1β (pg/mL)	4 (4-108)	4 (4-85)	8 (4-110)	4 (4-76)	8 (4-60)	4 (4-90)
IL-6 (pg/mL)	16.5 (4-143)	13.5 (4-50)	9.0 (4-114)	10.0 (4-112)	82.0 (13-1369)	122.0 (4-2132)*
hs-CRP (mg/L)	5.87 (0.58-18.80)	3.10 (0.67-19.08)*	2.66 (0.52-16.20)	2.19 (0.55-144.9)	168.8 (15.20-284.8)	171.7 (7.69-463)
IL-8 (pg/mL)	4 (4-86)	4 (4-51)	4 (4-47)	4 (4-36)	4 (4-356)	4 (4-206)
IL-12 (pg/mL)	9 (4-252)	9.5 (4-129)	10 (4-275)	4.0 (4-132)	9 (4-124)	10.0 (4-118)
IL-18 (pg/mL)	406.5 (184-1129)	351.5 (65-1000)	322.0 (104-1033)	290.5 (52-936)	366.0 (111-1043)	347.5 (62-1175)
IFN-γ (pg/mL)	4 (4-146)	4 (4-117)	4 (4-125)	4 (4-77)	4 (4-61)	4 (4-71)
MIP-1α (pg/mL)	50 (10-577)	35.0 (10-596)	56 (10-587)	35.5 (10-603)	51 (10-468)	31.5 (10-404)
IL-10 (pg/mL)	17.50 (4-109)	14.0 (4-97)	14.0 (4-131)	12.0 (4-95)	29.0 (4-210)	29.5 (4-221)
TGF-β (pg/mL)	60 (36-403)	39 (17-328)	85 (39-330)	39 (11-402)	39 (39-415)	75 (17-405)

Data are expressed as median (range). *P<0.05 compaired with SR group, Wilcoxon-Mann-Whitney test.

Table A2. Fatty acid composition (% of total FA) of RBC membrane lipids at baseline, perioperatively and on the third postoperative day[a].

Fatty acids	Baseline (n=158)	Preoperative (n=160)	Third postoperative day (n=160)
∑SFA	39.86±0.13	40.01±0.11	40.16±0.12
14:00	0.12±0.01	0.12±0.01	0.08±0.01
16:00	19.29±0.08	19.34±0.08	19.63±0.08
18:00	15.04±0.07	15.10±0.07	15.04±0.06
20:00	0.25±0.01	0.24±0.01	0.23±0.01
22:00	1.34±0.02	1.35±0.01	1.35±0.01
24:00	3.94±0.03	3.97±0.03	3.91±0.02
∑MUFA	16.90±0.09	16.87±0.09	16.99±0.07
16:1n-7	0.13±0.01	0.11±0.01	0.12±0.01
18:1n-7	1.01±0.02	1.02±0.01	1.05±0.02
18:1n-9	12.04±0.07	12.02±0.07	12.09±0.06
20:1n-9	0.08±0.01	0.08±0.01	0.09±0.01
24:1n-9	3.73±0.03	3.72±0.04	3.73±0.03
∑PUFA	34.31±0.11	34.37±0.09	33.99±0.09
∑n-6 PUFA	21.89±0.18	21.73±0.17	21.86±0.15
18:2-n6	7.72±0.09	7.58±0.09	7.72±0.07
20:3n-6	0.12±0.01	0.10±0.01	0.10±0.01
20:4n-6 (AA)	12.14±0.14	12.12±0.13	12.08±0.11
22:4n-6	1.91±0.05	1.93±0.05	1.96±0.04
∑n-3 PUFA	12.42±0.17	12.64±0.17	12.13±0.15
20:5n-3 (EPA)	1.94±0.07	2.09±0.06	1.87±0.06
22:5n-3 (DPA)	3.08±0.03	3.09±0.03	3.05±0.03
22:6n-3 (DHA)	7.40±0.10	7.46±0.10	7.20±0.08
n-6 /n-3 PUFA	1.84±0.04	1.79±0.04	1.86±0.03
Omega-3 Index (EPA+DHA)	9.34±0.15	9.55±0.15	9.13±0.13

[a]Values are mean ± SEM.

SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Table A3. Fatty acid composition (% of total FA) of RBC membrane lipids in the SR and POAF groups at baseline, perioperatively and on the third postoperative day[a].

Fatty acids	Baseline		Perioperative		Third postoperative day	
	SR group (n=72)	POAF group (n=86)	SR group (n=73)	POAF group (n=87)	SR group (n=73)	POAF group (n=87)
∑SFA	39.70±0.12	40.00±0.21	40.08±0.15	39.94±0.16	40.17±0.15	40.15±0.18
14:0	0.11±0.02	0.13±0.02	0.10±0.02	0.14±0.02	0.09±0.02	0.07±0.01
16:0	19.18±0.10	19.39±0.13	19.35±0.12	19.34±0.10	19.60±0.11	19.66±0.11
18:0	14.96±0.08	15.10±0.10	15.14±0.10	15.06±0.09	15.00±0.09	15.08±0.09
20:0	0.25±0.02	0.25±0.02	0.23±0.02	0.26±0.19	0.25±0.02	0.21±0.02
22:0	1.34±0.02	1.34±0.02	1.35±0.02	1.36±0.02	1.35±0.02	1.34±0.01
24:0	3.96±0.05	3.92±0.04	4.02±0.04	3.93±0.04	3.97±0.03	3.86±0.03*
∑MUFA	16.86±0.13	16.94±0.12	16.72±0.13	17.00±0.11	16.82±0.10	17.14±0.09*
16:1n-7	0.16±0.02	0.10±0.02*	0.12±0.02	0.10±0.02	0.15±0.02	0.11±0.02
18:1n-7	1.00±0.02	1.02±0.03	1.02±0.02	1.02±0.02	1.05±0.02	1.04±0.03
18:1n-9	11.94±0.10	12.12±0.10	11.85±0.10	12.17±0.10*	11.87±0.08	12.27±0.08**
20:1n-9	0.10±0.02	0.07±0.01	0.07±0.01	0.09±0.02	0.10±0.02	0.09±0.02
24:1n-9	3.76±0.05	3.70±0.04	3.74±0.06	3.71±0.06	3.75±0.04	3.72±0.04
∑PUFA	34.21±0.15	34.39±0.15	34.40±0.15	34.35±0.12	33.98±0.13	34.00±0.13
∑n-6 PUFA	22.10±0.26	21.70±0.25	22.14±0.25	21.39±0.23*	22.24±0.22	21.55±0.20*
18:2-n6	7.62±0.13	7.81±0.11	7.54±0.14	7.61±0.11	7.70±0.10	7.74±0.10
20:3n-6	0.12±0.02	0.11±0.02	0.11±0.02	0.10±0.02	0.11±0.02	0.10±0.02
20:4n-6 (AA)	12.38±0.21	11.95±0.19	12.47±0.20	11.83±0.17*	12.36±0.17	11.84±0.14*
22:4n-6	1.99±0.07	1.84±0.06	2.03±0.07	1.85±0.08	2.07±0.06	1.87±0.04**
∑n-3 PUFA	12.11±0.25	12.68±0.24	12.26±0.24	12.96±0.23*	11.74±0.22	12.46±0.21*
20:5n-3 (EPA)	1.87±0.10	2.00±0.09	2.00±0.10	2.17±0.09	1.76±0.08	1.97±0.08
22:5n-3 (DPA)	3.02±0.04	3.14±0.04	3.01±0.04	3.16±0.04*	2.98±0.04	3.11±0.04*
22:6n-3 (DHA)	7.22±0.14	7.54±0.13	7.25±0.13	7.64±0.14*	7.00±0.12	7.38±0.11*
n-6 /n-3 PUFA	1.91±0.06	1.79±0.05	1.88±0.05	1.72±0.05*	1.96±0.05	1.79±0.04*
Omega-3 Index (EPA+DHA)	8.96±0.25	9.44±0.24	9.24±0.21	9.81±0.21	8.86±0.20	9.36±0.18

[a]Values are mean ± SEM.

SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. **P<0.05, *P<0.001, compared with SR group. Independent samples t-test.

Table A4. Fatty acid composition (% of total FA) of RBC membrane lipids in the SR and POAF groups undergoing only CABG at baseline, perioperatively and on the third postoperative day[a].

Fatty acids	Baseline		Perioperative		Third postoperative day	
	SR group (n=59)	POAF group (n=58)	SR group (n=59)	POAF group (n=58)	SR group (n=62)	POAF group (n=60)
∑SFA	39.63±0.12	39.94±0.26	39.99±0.17	39.87±0.19	40.14±0.17	39.96±0.15
14:0	0.11±0.02	0.12±0.02	0.10±0.02	0.12±0.02	0.08±0.02	0.05±0.01
16:0	19.13±0.11	19.30±0.15	19.29±0.13	19.17±0.10	19.58±0.12	19.51±0.11
18:0	14.90±0.09	15.09±0.12	15.05±0.11	15.11±0.11	14.97±0.10	15.01±0.08
20:0	0.25±0.02	0.27±0.02	0.24±0.02	0.28±0.02	0.26±0.02	0.22±0.02
22:0	1.34±0.03	1.34±0.03	1.36±0.03	1.37±0.02	1.34±0.02	1.35±0.02
24:0	4.00±0.05	3.94±0.05	4.06±0.05	3.96±0.05	3.99±0.03	3.87±0.04*
∑MUFA	16.80±0.14	16.97±0.15	16.76±0.14	17.09±0.14	16.83±0.11	17.15±0.11
16:1n-7	0.17±0.03	0.09±0.02*	0.13±0.02	0.07±0.02	0.15±0.02	0.09±0.02*
18:1n-7	1.00±0.02	1.02±0.04	1.03±0.02	1.03±0.02	1.07±0.02	1.05±0.03
18:1n-9	11.90±0.11	12.11±0.13	11.83±0.11	12.15±0.13	11.85±0.09	12.22±0.11*
20:1n-9	0.10±0.02	0.06±0.02	0.08±0.02	0.09±0.02	0.12±0.02	0.09±0.02
24:1n-9	3.73±0.06	3.76±0.05	3.77±0.06	3.84±0.05	3.76±0.04	3.78±0.04
∑PUFA	34.09±0.17	34.16±0.19	34.21±0.17	34.33±0.15	33.83±0.14	33.97±0.14
∑n-6 PUFA	22.06±0.30	21.48±0.28	21.98±0.28	21.13±0.27*	22.13±0.24	21.29±0.25*
18:2-n6	7.56±0.14	7.68±0.14	7.41±0.15	7.40±0.13	7.62±0.10	7.52±0.12
20:3n-6	0.14±0.02	0.12±0.03	0.12±0.02	0.10±0.02	0.11±0.02	0.09±0.02
20:4n-6 (AA)	12.38±0.24	11.85±0.23	12.44±0.23	11.75±0.21*	12.32±0.19	11.83±0.18
22:4n-6	1.99±0.08	1.83±0.07	2.02±0.08	1.89±0.10	2.08±0.07	1.85±0.05**
∑n-3 PUFA	12.03±0.28	12.68±0.28	12.22±0.27	13.19±0.27*	11.70±0.23	12.69±0.24**
20:5n-3 (EPA)	1.83±0.11	1.98±0.11	1.99±0.11	2.20±0.11	1.74±0.09	2.00±0.09
22:5n-3 (DPA)	3.02±0.05	3.11±0.05	3.01±0.05	3.16±0.05*	2.97±0.04	3.12±0.04*
22:6n-3 (DHA)	7.18±0.16	7.59±0.15	7.23±0.16	7.84±0.15**	6.99±0.13	7.57±0.13**
n-6 /n-3 PUFA	1.92±0.06	1.76±0.06	1.87±0.06	1.67±0.06*	1.95±0.05	1.73±0.05**
Omega-3 Index (EPA+DHA)	8.86±0.29	9.41±0.29	9.22±0.25	10.04±0.24*	8.78±0.21	9.55±0.21*

[a]Values are mean ± SEM.

SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated fatty acids; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. **P<0.05, *P<0.001, compared with SR group. Independent samples t-test.

Appendix IV

Paper I and II to be submitted

Association of n-6 and n-3 long-chain polyunsaturated fatty acids in plasma phospholipids and red blood cell membrane lipids with circulating inflammatory mediators in patients undergoing open heart surgery

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ABSTRACT

Background: A systemic inflammatory response occurs in patients undergoing cardiac surgery. The balance between pro- and anti-inflammatory mediators may partly depend on the status of n-6 and n-3 long-chain polyunsaturated fatty acids (LC-PUFA).

Objective: To examine the relationship of n-6 and n-3 LC-PUFA in plasma phospholipids (PL) and red blood cell (RBC) membrane lipids with inflammatory mediators before and following open heart surgery.

Design: Blood samples from patients undergoing open heart surgery (n = 168) were collected at baseline, immediately before surgery (perioperatively), and on the third postoperative day for fatty acid analysis and assessment of inflammatory mediators.

Results: Plasma levels of interferon-γ (IFN-γ) and tumor necrosis factor β (TNF-β) were lower and those of interleukin-6 (IL-6), IL-8, IL-10, IL-18 and high-sensitivity C-reactive protein (hs-CRP) were higher on the third postoperative day than perioperatively. At baseline, higher plasma PL level of EPA was associated with lower concentration of hs-CRP, and higher DHA level was associated with lower IL-12 and IL-18 concentrations. In RBC, higher AA level was associated with higher concentration of TNF-β, whereas higher DHA level was associated with lower IL-18 concentration. In perioperative plasma PL, higher level of AA was associated with more increase in IL-10 and lesser increase in TGF-β, whereas higher level of EPA was associated with lesser increase in IL-10 following surgery. In perioperative RBC lipids, higher AA level was associated with more pronounced decrease in TNF-β, and lesser increase in TGF-β, whereas higher level of EPA was associated with more increase in IL-1 β and TGF-β.

CONCLUSIONS: Our findings support the notion that n-6 and/or n-3 LC-PUFA in plasma and/or cell membrane lipids play an important role in modulating the inflammatory response following surgery.

Introduction

Open heart surgery is commonly performed to treat a variety of cardiac diseases, including coronary artery disease and valvular disorders. Cardiac surgery provokes a vigorous inflammatory response that propagates within the injured tissue to initiate the healing process (1). However, excessive systemic inflammation can have severe adverse effects during the postoperative period (1). The severity of the systemic inflammatory response has predominantly been attributed to the exposure of the patient's blood with the artificial surface of the extracorporeal cardiopulmonary bypass (CPB) circuit (2). However, activation of inflammatory pathways is also observed when open heart surgery is performed without CPB (off-pump), suggesting that alternative factors, such as surgical injury, blood loss, blood transfusion and hypothermia, may play a role (1, 3). Furthermore, the inflammatory response may be modulated by a balance between pro-inflammatory and anti-inflammatory mediators, synthesized by different cell types, including activated monocytes, tissue macrophages, lymphocytes and endothelial cells (1, 4, 5).

The n-6 long-chain polyunsaturated fatty acid (LC-PUFA) arachidonic acid (AA) and the n-3 LC-PUFA eicosapentaenoic acid (EPA) are precursors of potent bioactive eicosanoids that confer differential effects on leukocytes and other cell types (6, 7). Although both types of LC-PUFA are metabolized by the same enzyme systems, eicosanoids derived from the AA are more potent mediators of inflammation than those derived from EPA. It has also been reported that eicosanoids derived from EPA and docosanoids derived from the n-3 LC-PUFA docosahexaenoic acid (DHA), i.e. resolvins, protectins, maresins, as well as the lipoxins derived from AA, are potent anti-inflammatory lipid mediators (8). Recently, Chapkin and coworkers (9) proposed a molecular model that may explain, in part, the pleiotropic anti-inflammatory and immunosuppressive properties of the n-3 LC-PUFA. The hypothesis is based on the notion that n-3 LC-PUFA in cell membranes and/or in the circulation suppress

nuclear receptor activation and thereby nuclear factor (NF)-κB that controls the transcription of genes involved in synthesis of many pro-inflammatory mediators, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and IL-8 (10, 11). Therefore, the relative balance between pro-inflammatory and anti-inflammatory mediators may partly depend on the perioperative levels of AA, EPA and DHA in plasma phospholipids (PL) and in cell membrane PL (12).

The fatty acid composition of plasma PL has been used to reflect the overall fatty acid state in an individual. However, plasma PL are merely transporters of circulating fatty acids, and the types and quantity of n-6 and n-3 PUFA consumed during the previous week influence the fatty acid composition of plasma PL (13). In contrast, the composition of n-6 and n-3 PUFA in human red blood cell (RBC) membrane lipids is a good indicator of dietary n-6 and n-3 PUFA intake over the preceding three months (13, 14), and the fatty acid pattern of the RBC membrane lipids has been shown to be a good indicator of the fatty acid composition of atrial tissue (15).

The aim of this study was to investigate the association of n-6 and n-3 LC-PUFA in plasma PL and RBC membrane lipids with circulating inflammatory mediators in patients with coronary artery and/or valvular disease, both at baseline and in response to open heart surgery.

SUBJECTS AND METHODS

Subjects and study design

The study was part of a prospective, randomized, double-blinded, placebo-controlled clinical trial on the use of n-3 LC-PUFA for a week prior to open heart surgery to prevent postoperative atrial fibrillation. Details of the study design have been published previously (16, 17). In brief, all patients scheduled for elective or semi-emergent open heart surgery

between August 2007 and May 2009 were evaluated for participation. Of the total of 170 patients enrolled in the study, two patients had their surgery cancelled after randomization leaving 168 patients for analysis. Patients, younger than 40 years of age, with a history of any form of supraventricular arrhythmias or taking the anti-arrhythmic medications amiodarone and/or sotalol, and those undergoing an emergent operation were excluded. Prior to surgery, all participants answered a questionnaire on lifestyle issues, including consumption of fish and cod liver oil, intake of n-3 LC-PUFA capsules, smoking habit, height, body weight, and medication use. All patients gave written informed consent. Those who consented to participation were asked to discontinue intake of cod liver oil and n-3 LC-PUFA capsules if they were taking such supplements but were otherwise advised to remain on their usual diet. The patients were then randomly assigned to one of two study groups initiating the study treatment one week prior to surgery. Active treatment (n-3 LC-PUFA group) consisted of a total of 1240 mg of EPA and 1000 mg of DHA as ethyl esters daily and placebo consisted of 2000 mg olive oil in identical capsules. The n-3 LC-PUFA capsules are commercially available in Iceland (Omega Forte, Lysi Inc, Reykjavík, Iceland). The study was approved by the Bioethics Committee of Landspitali – The National University Hospital of Iceland, and the Icelandic Data Protection Authority.

Blood sampling

Venous blood samples obtained from the patients before initiating the study medication (baseline), immediately before the surgery (perioperative) and on the third postoperative day (postoperative). The blood samples were collected into disodium EDTA tube and the plasma separated from RBC by immediate centrifugation at 1000 *g* for 10 min. The RBC's were washed three times with an isotonic saline solution and the antioxidant butylated hydroxytoluene (BHT), dissolved in methanol, was added to the cells at a final concentration

of 50 mg/L. The plasma and RBC samples were frozen at -76°C and stored until the analysis of the inflammatory mediators and the fatty acids was carried out.

Assessment of inflammatory mediators

The plasma samples were analyzed for the concentration of the pro-inflammatory mediators IL-1β, IL-6, IL-8, IL-12, IL-18, interferon-γ (IFN- γ), TNF-α, and TNF-β, and macrophage inflammatory protein-1α (MIP-1α), and the anti-inflammatory mediators IL-10 and transforming growth factor-β (TGF-β) as described by Skogstrand et al. (18). The samples were diluted 1:10 in phosphate buffered saline (PBS) containing 0.5% Tween 20 and 1% bovine serum albumin (BSA). A 50 µL aliquot was mixed 1:1 with a suspension of capture-antibody-conjugated beads in multiwell plates. After incubation for 1½ hour at room temperature with gentle shaking, the beads were washed twice with PBS and subsequently reacted for 1½ hour with a 50 µL mixture of corresponding biotinylated antibodies, each diluted 1:1000. Streptavidin-phycoerythrin (50 µL) was added to the wells and the incubation continued for additional 30 min. Finally, the beads were washed twice and resuspended in 125 µL of PBS and analyzed on the Luminex 100™ platform (Luminex Corp, TX, USA). Samples were measured in duplicates and standard curves were fitted with a five parameter logistic equation (Logistic-5PL) using BioPlex™ Manager 5.0 (Bio-Rad Laboratories, CA, USA).

Plasma high sensitivity C-reactive protein (hs-CRP) was measured on a Hitachi 911 analyzer using a commercially available latex-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). The lower detection limit of the assay was 0.1 mg/L. The total coefficient of variation for CRP measurements of the internal controls was 1.1% at a concentration of 3.73 mg/L and 1.9% at a concentration of 0.68 mg/L.

Analysis of fatty acids in plasma phospholipids and RBC membrane lipids

The method of the fatty acid analysis in plasma phospholipids (PL) has been described previously (16, 17). The lipid fraction was extracted from RBC membrane lipids as described by Bligh & Dyer (19) exept isopropanol was used instead of methanol (isopropanol/chloroform 2:1, v/v). BHT (50 mg/L) was added to the extraction medium. The fatty acids in RBC total lipids were transmethylated for 45 min at 110°C using 14% boron trifluoride/methanol (Sigma Chemical Co., St. Louis, MO). Fatty acid methyl esters (FAME) of plasma PL and RBC membrane lipids were prepared and analyzed by gas-liquid chromatography as described earlier (15). The RBC FAME were analyzed by gas chromatography (Agilent 6890 N, Agilent, Palo Alto, CA) using a Chrompack CP-SIL 8CB column (25 m x 250 µm i.d. x 0.12 µm film thickness). The oven was programmed to provide an initial temperature of 150°C for 4 min, then increasing tempereature by 4°C per min to 230°C and then by 20°C per min to 280°C, and was finally held isothermal for 4 min. The injector and detector temperatures were maintained at 280°C and 300°C, respectively. Hydrogen was used as the carrier gas. The FAME peaks were identified and calibrated against those of commercial standards (Sigma Chemical Co.; Nu-Chek-Prep, Elysian, MN). Fatty acid values in RBC membrane lipids and plasma PL are presented as % weight of total fatty acids with chain length from C_{14} to C_{24}. Instrumental control and data handling was done using HP 3365 Chemstation, Version A.02.12. (Hewlett Packard Co., Palo Alto, CA).

Statistical analysis

In this study the main focus was to examine the association between fatty acids and inflammatory mediators at baseline, between fatty acids measured perioperatively and inflammatory mediators on the third postoperative day as well as between the perioperative fatty acid levels and the changes in inflammatory mediators from the perioperative time

period to the third postoperative day. In this analysis we ignored the n-3 LC-PUFA treatment assignment. Wilcoxon signed rank test was used to compare the difference in inflammatory mediators between time points. Paried t-test was used to compare the difference in fatty acid composition between plasma PL and RBC membrane lipids at different time points. Spearman‘s correlation coefficient was used to examine the relationship between continuous variables and Wilcoxon-Mann-Whitney test to compare groups. Multivariable linear regression was used to estimate the relationship between levels of fatty acids and concentrations of inflammatory mediators at baseline, adjusting for age, BMI and smoking. Multivariable linear regression was also used to estimate the relationship between perioperative levels of fatty acids and the postoperative levels of inflammatory mediators as well as the changes in concentrations of inflammatory mediators from the perioperative time point to the third postoperative day, adjusting for age, BMI and smoking.

Data are presented as median (range), percentages or mean ± standard error of the mean (SEM), unless otherwise noted. A two-sided P value < 0.05 was considered statistically significant. All statistical analyses were carried out using SPSS software (version 17.0).

RESULTS

The baseline characteristics of the patients who underwent open heart surgery are shown in Table 1. The patients were elderly with a median age of 67 (range, 43-82) years, 79.2% were men, and their median BMI was 27.4 (17.2-41.3) kg/m^2. Seventy two percent of the patients consumed fish once or more a week, 55% took cod liver oil and one-quarter n-3 LC-PUFA capsules as daily supplements.

Figure 1 shows the median plasma concentrations of selected pro-inflammatory and anti-inflammatory mediators perioperatively and on the third day after cardiac surgery. On the third postoperative day, the plasma concentrations of IFN-γ and TNF-β were significantly

lower, and those of hs-CRP, IL-6, IL-8, IL-10 and IL-18 were significantly higher compared with perioperative concentrations. No statistical difference was observed in the median concentrations of TNF-α, IL-1β, IL-12, MIP-1α and TGF-β between the perioperative and the third postoperative day, 4 (4-181) vs. 4 (4-224) pg/mL, 4 (4-110) vs. 4 (4-90) pg/mL, 9 (4-275) vs. 10 (4-199) pg/mL, 46 (10-603) pg/mL vs. 38.5 (10-468), and 39 (11-402) pg/mL vs. 55 (17-415), respectively.

There was no change in fatty acid levels of plasma PL or RBC between the baseline and perioperative time points and the difference in fatty acid composition between plasma PL and RBC membrane lipids was similar at baseline and perioperatively (Table 2). RBC contained higher levels of total monounsaturated fatty acids (MUFA), oleic acid (OA), AA, total n-3 LC-PUFA, docosapentaenoic acid (DPA) and DHA, and higher ratio of AA to EPA + DHA than plasma PL ($P < 0.001$). In contrast, the levels of total saturated fatty acids (SFA), total n-6 PUFA, linoleic acid (LA) and EPA were lower in RBC than in plasma ($P < 0.001$). The levels of all the fatty acids in RBC correlated directly with the corresponding plasma fatty acids at both time points (Spearman's correlation, $P < 0.05$).

A multivariable linear regression model was used to assess the relationship between the concentrations of circulating inflammatory mediators and levels of AA, EPA and DHA in plasma PL and RBC membrane lipids at baseline (Table 3). In plasma PL, higher level of EPA was associated with lower concentration of hs-CRP, and higher DHA level was associated with lower IL-12 and IL-18 concentrations ($P < 0.05$). In RBC, higher AA level was highly associated with higher concentration of TNF-β ($P < 0.01$), whereas higher DHA level was associated with lower IL-18 concentration, and higher level of EPA + DHA (The Omega-3 Index) was associated with higher IL-1β, and lower IL-6 and IL-18 concentrations ($P < 0.05$). The relationship between the concentrations of circulating inflammatory mediators and levels of AA, EPA and DHA in plasma PL and RBC membrane lipids on the day of

surgery differed from that at baseline, (Table 4). In plasma PL, higher level of AA was associated with higher concentration of IL-10, higher levels of EPA and DHA were associated with lower concentration of IL-18, and higher level of DHA was associated with higher concentration of TGF-β ($P < 0.05$). In RBC, higher AA level was associated with higher concentration of TNF-β and lower concentration of TGF-β, higher level of EPA was associated with higher concentrations of IL-1β and TGF-β, higher DHA level was associated with lower IL-18 concentration and higher EPA + DHA was associated with higher concentration of IL-1β and lower concentration of IL-18 ($P<0.05$).

A multivariable linear regression model was also used to assess the relationship between the perioperative levels of AA, EPA and DHA in plasma PL and RBC membrane lipids and the changes in the levels of circulating inflammatory mediators from perioperative to the third postoperative day (Table 5). In plasma PL, higher level of AA was associated with a more increase in IL-10 and a lesser increase in TGF-β ($P < 0.01$), whereas higher level of EPA was associated with lesser increase in IL-10 ($P < 0.05$). In RBC, higher AA level was associated with a more pronounced decrease in TNF-β, and lesser increase in TGF-β, whereas a higher level of EPA and the Omega-3 Index (EPA + DHA) were associated with more increase in IL-1β and TGF-β.

Age-related associations were observed in concentrations of mediators. The perioperative concentration of TGF-β was negatively associated ($r = -0.165$, $P = 0.036$), the postoperative concentrations of IL-6 and IL-8 were positively associated ($r = 0.179$, $P = 0.023$, and $r = 0.180$, $P = 0.022$, respectively), and that of TGF-β negatively associated ($r = -0.168$, $P = 0.033$) with age. No associations were found between BMI and the concentrations of inflammatory mediators at either time-point.

DISCUSSION

The results of this study suggest that the n-6 LC-PUFA AA in plasma PL and cell membranes may contribute to both pro- and anti-inflammatory response following surgical injury, while the n-3 LC-PUFA EPA and DHA may be mainly involved in the anti-inflammatory response.

The participants in our study, mostly patients with coronary artery disease requiring revascularisation, are subjects in whom the association between the n-6 and n-3 LC-PUFA and inflammation is of great interest. In general, the n-3 LC-PUFA are thought to be beneficial for the disease course possibly based on their proposed anti-inflammatory properties, while the n-6 LC-PUFA AA has pro-inflammatory property. Our data indicate that, in patients undergoing open heart surgery, the n-3 LC-PUFA are associated with lower levels of pro-inflammatory mediators, in particular CRP, IL-12 and IL-18. Observational studies have demonstrated that dietary EPA and DHA reduce circulating concentrations of pro-inflammatory mediators, such as TNF-α, IL-6 and CRP (20). One such study showed that n-3 LC-PUFA supplements lead to a reduction in the plasma concentration of the pro-inflammatory mediator IL-18 in elderly men (21). Our findings confirm this inverse association betweeen levels of DHA in plasma PL and RBC membrane lipids as well as the Omega-3 Index and IL-18 concentration. Another study in a general population, based on fatty acid levels of total plasma lipids (i.e. triacylglycerols, PL, nonesterified or free fatty acids and cholesterolesters) revealed that plasma levels of EPA and DHA were inversely associated with the concentration of IL-6 and other pro-inflammatory mediators, but directly associated with the anti-inflammatory mediator TGF-β (22). In that study AA was inversely related to IL-6 but also correlated possetively with other pro-inflammatory mediators. The authors concluded that AA and n-3 LC-PUFA might modulate the inflammatory response by acting both on the pro-inflammatory and anti-inflammatory arms of the cytokine network. In the present study, however, AA showed limited association with inflammatory mediators at

baseline as the only positive association was between its level in RBC and the pro-inflammatory mediator TNF-β. Our baseline study therefore shows a relationship between inflammatory mediators and LC-PUFA levels that are consistent with the proposed anti-inflammatory effects of n-3 CL-PUFA and a possible pro-inflammatory effect of AA.

It has been suggested that early induction of the genes coding for the pro-inflammatory mediators TNF-α and IL-1β is important for normal repair and wound healing (23). TNF-α and IL-1β have been found to peak earlier (4,24-26), with IL-6 and IL-8 peaking later in the day of operation (4,26,27). TNF-α and TNF-β induce the expression of IL1-β (28), and IL1-β and TNF-α act synergistically to stimulate the production of IL-6 (29). During an acute inflammatory response the production of hs-CRP in liver is regulated by IL-6 (30). Moreover, TNF-α and IL-1β induced expression of IL-8 by endothelial cells may play an important role in reperfusion injury (26, 31). The anti-inflammatory mediator IL-10 is a potent inhibitor of TNF-α, IL-1β and IL-8 expression (32), and IL-10 has shown to be released together with pro-inflammatory mediators during and after CPB (33). In this present study the concentration of IL-10 was elevated as well as the concentrations of the pro-inflammatory mediators IL-6, IL-8, IL-18 and hs-CRP on the third day after the open heart surgery. These observations and the proposed influence of the n-3 and n-6 LC-PUFA in the inflammatory response lead us to examine the relationship between the perioperative levels of the different fatty acids in plasma PL or RBC membrane lipids and the various inflammatory mediators following surgery. Consistent with baseline findings, AA level in RBC at the time of surgery was associated with a higher postoperative level of the pro-inflammatory TNF-β and a lower postoperative level of the anti-inflammatory TGF-β. However, the perioperative plasma PL level of AA associated directly with the anti-inflammatory IL-10, as well as with a greater increase in IL-10 level following surgery. Thus, AA may have an important effect on both pro- and anti-inflammatory mediators, directly or indirectly. The aim is true for EPA

level and the Omega-3 Index following surgery showing association with both pro- and anti-inflammatory mediators. On the other hand, DHA showed mainly an anti-inflammatory effect in response to surgery. Taken together these findings suggest that the n-6 and n-3 LC-PUFA are important contributors to the regulation of the inflammatory response following open heart surgery.

Unfortunately we only have measurements of the inflammatory mediators on the third postoperative day. It is known that the inflammatory cascade is initiated immediately after tissue injury and some of the inflammatory mediators may peak early in the postoperative course (4,23-26). Furthermore, due to stimulatory and/or feedback inhibitory effect of various pro- or anti-inflammatory mediators on each other, an initial pro-inflammatory response may at a given time result in a prevalence of anti-inflammatory mediators. Future studies need to examine how n-6 and n-3 LC-PUFA may be associated with inflammatory mediators in the first hours and days following surgery.

In general, AA released from cell membrane PL is thought to have pro-inflammatory properties due to its role as precursor to oxygenated products that have potent vasoactive and chemotactic actions (34). During an acute inflammatory response, macrophages generate the potent anti-inflammatory and proresolving lipid mediator lipoxin A_4 from AA, which in turn stimulates the production of the anti-inflammatory mediator IL-10 (8). The relatively high level of AA in RBC membrane lipids observed in our study subjects is also expected to be present in macrophages and other types of blood cells. In fact, the patients in our study had an elevated plasma concentration of IL-10 on the third postoperative day, but only a positive association was observed with AA in plasma PL.

In conclusion, our study demonstrated an association between the levels of AA, EPA, DHA and the Omega-3 Index and the concentrations at baseline following open heart surgery. Furthermore our findings support the notion that n-6 and/or n-3 LC-PUFA in cell membrane

lipids and/or plasma PL play an important role in modulating the inflammatory response following surgical injury. However, their effects may be complex. Additional studies are needed to investigate the relationship between plasma and membrane fatty acids and pro-inflammatory and anti-inflammatory mediators early following open heart surgery.

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Conflict of interest

The authors declare no conflict of interest

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TABLE 1

Baseline and operative characteristics of the study subjects (n=168)

Characteristic	Value
Age (years)	67 (43-82)[1]
BMI (kg/m^2)	27.4 (17.2-41.3)
Gender (% men)	79.2
Smoking (%)	19.0
Fish intake (%, >1x a week)	72.0
Liquid cod liver oil (%)	54.8
n-3 LC-PUFA capsules (%)	26.8
Use of statins (%)	80.4
ECC time (min)	96 (0-261)
On-pump surgery (%)	88.1
Blood volume in drains (mL)	765 (96-4980)
Aortic cross-clamp time (min)	48 (0-208)
Diabetes (%)	14.9

[1]Data are expressed as median (range).

LC-PUFA, long-chain polyunsaturated fatty acids;

ECC, extracorporeal circulation.

TABLE 2

Fatty acid composition (% of total fatty acids) of plasma phospholipids (PL) and red blood cell (RBC) membrane lipids at baseline and perioperatively[1].

	Baseline		Perioperatively	
Fatty acids	Plasma PL (n=157)	RBC lipids (n=157)	Plasma PL (n=157)	RBC lipids (n=157)
∑SFA	44.52±0.27	39.85±0.13*	44.82±0.24	40.01±0.11*
∑MUFA	14.45±0.25	16.89±0.09*	14.40±0.23	16.87±0.09*
OA (18:1n-9)	9.12±0.13	12.03±0.07*	9.00±0.14	12.02±0.07*
∑n-6 PUFA	29.78±0.23	21.88±0.18*	28.84±0.24	21.77±0.17*
18:2n-6 (LA)	17.42±0.21	7.72±0.09*	16.30±0.22	7.58±0.09*
20:4n-6 (AA)	8.76±0.18	12.14±0.14*	9.02±0.18	12.14±0.13*
∑n-3 LC-PUFA	9.85±0.21	12.43±0.17*	10.96±0.22	12.61±0.17*
20:5n-3 (EPA)	2.61±0.11	1.95±0.07*	3.33±0.13	2.07±0.06*
22:5n-3 (DPA)	1.09±0.02	3.09±0.03*	1.16±0.02	3.09±0.03*
22:6n-3 (DHA)	6.11±0.11	7.39±0.10*	6.44±0.10	7.45±0.10*
AA / EPA+DHA	1.11±0.04	1.39±0.04*	1.01±0.03	1.35±0.03*

[1]Data are expressed as mean ± SEM. ∑SFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; OA, oleic acid; ∑n-6 PUFA, total n-6 polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid; ∑n-3 LC-PUFA, total n-3 long-chain PUFA; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.*P<0.001 compared with plasma, Paired Samples t-test.

TABLE 3

Relationship between levels of fatty acids in plasma phospholipids (PL) and red blood cell (RBC) membrane lipids and inflammatory mediators at baseline, multivariable linear regression[1].

Inflammatory mediators	AA		EPA		DHA		EPA + DHA
	Plasma PL	RBC lipids	Plasma PL	RBC lipids	Plasma PL	RBC lipids	RBC lipids
TNF-α (pg/mL)	0.076	0.072	0.108	-0.023	-0.023	0.026	0.094
TNF-β (pg/mL)	0.074	0.261**	-0.060	-0.099	-0.119	-0.129	-0.038
IL-1β (pg/mL)	-0.022	-0.007	0.018	0.007	-0.016	0.086	0.212*
IL-6 (pg/mL)	-0.034	0.026	-0.061	0.001	-0.0092	-0.064	-0.175*
hs-CRP (mg/L)	-0.023	-0.144	-0.191*	0.011	-0.131	-0.114	-0.031
IL-8 (pg/mL)	0.034	-0.087	-0.017	0.080	0.003	0.011	-0.035
IL-12 (pg/mL)	-0.024	0.077	-0.085	-0.011	-0.185*	-0.062	-0.104
IL-18 (pg/mL)	0.040	0.086	-0.109	-0.098	-0.211*	-0.236**	-0.180*
IFN-γ (pg/mL)	0.034	0.112	-0.020	-0.085	-0.006	0.055	-0.002
MIP-1α (pg/mL)	-0.034	-0.022	0.060	0.075	-0.051	0.023	-0.005
IL-10 (pg/mL)	-0.075	-0.136	0.056	0.087	0.134	0.134	-0.016
TGF-β (pg/mL)	-0.043	-0.054	0.041	0.038	-0.047	-0.038	0.119

[1]Data are expressed as the standardized beta coefficient. Statistics for the association of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with selected inflammatory mediators are adjusted for age, BMI and smoking. *$p<0.05$, **$p<0.01$.

TABLE 4

Relationship between perioperative levels of fatty acids in plasma phospholipids (PL) and red blood cell (RBC) membrane lipids and the concentrations of inflammatory mediators on the third postoperative day, multivariable linear regression[1].

Inflammatory	AA		EPA		DHA		EPA+DHA
mediators	Plasma PL	RBC lipids	Plasma PL	RBC lipids	Plasma PL	RBC lipids	RBC lipids
TNF-α (pg/mL)	0.000	0.009	0.058	0.078	0.061	0.063	0.076
TNF-β (pg/mL)	0.050	0.188*	0.003	-0.045	-0.004	-0.014	-0.030
IL-1β (pg/mL)	-0.046	-0.105	0.115	0.212*	0.084	0.174	0.208*
IL-6 (pg/mL)	0.042	0.027	-0.122	-0.109	-0.168	-0.177	-0.161
hs-CRP (mg/L)	-0.046	-0.035	-0.088	-0.034	0.044	-0.005	-0.019
IL-8 (pg/mL)	0.095	0.061	-0.069	-0.038	-0.064	-0.030	-0.037
IL-12 (pg/mL)	0.020	0.095	-0.049	-0.053	-0.160	-0.081	-0.076
IL-18 (pg/mL)	0.031	0.033	-0.186*	-0.160	-0.225**	-0.227*	-0.216*
IFN-γ (pg/mL)	0.015	0.112	0.115	-0.011	0.021	0.058	0.032
MIP-1α (pg/mL)	0.064	0.055	-0.117	-0.047	-0.143	-0.092	-0.079
IL-10 (pg/mL)	0.164*	0.034	-0.122	-0.047	0.011	0.026	-0.006
TGF-β (pg/mL)	-0.033	-0.175*	0.062	0.166*	0.197*	0.108	0.145

[1]Data are expressed as the standardized beta coefficient. Statistics for the association of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with selected inflammatory mediators are adjusted for age, BMI and smoking. *p<0.05, **p<0.01.

TABLE 5

Relationship between perioperative levels of fatty acids in plasma phospholipids (PL) and red blood cell (RBC) membrane lipids and the change in the concentrations of inflammatory mediators perioperatively to the third postoperative day, multivariable linear regression[1].

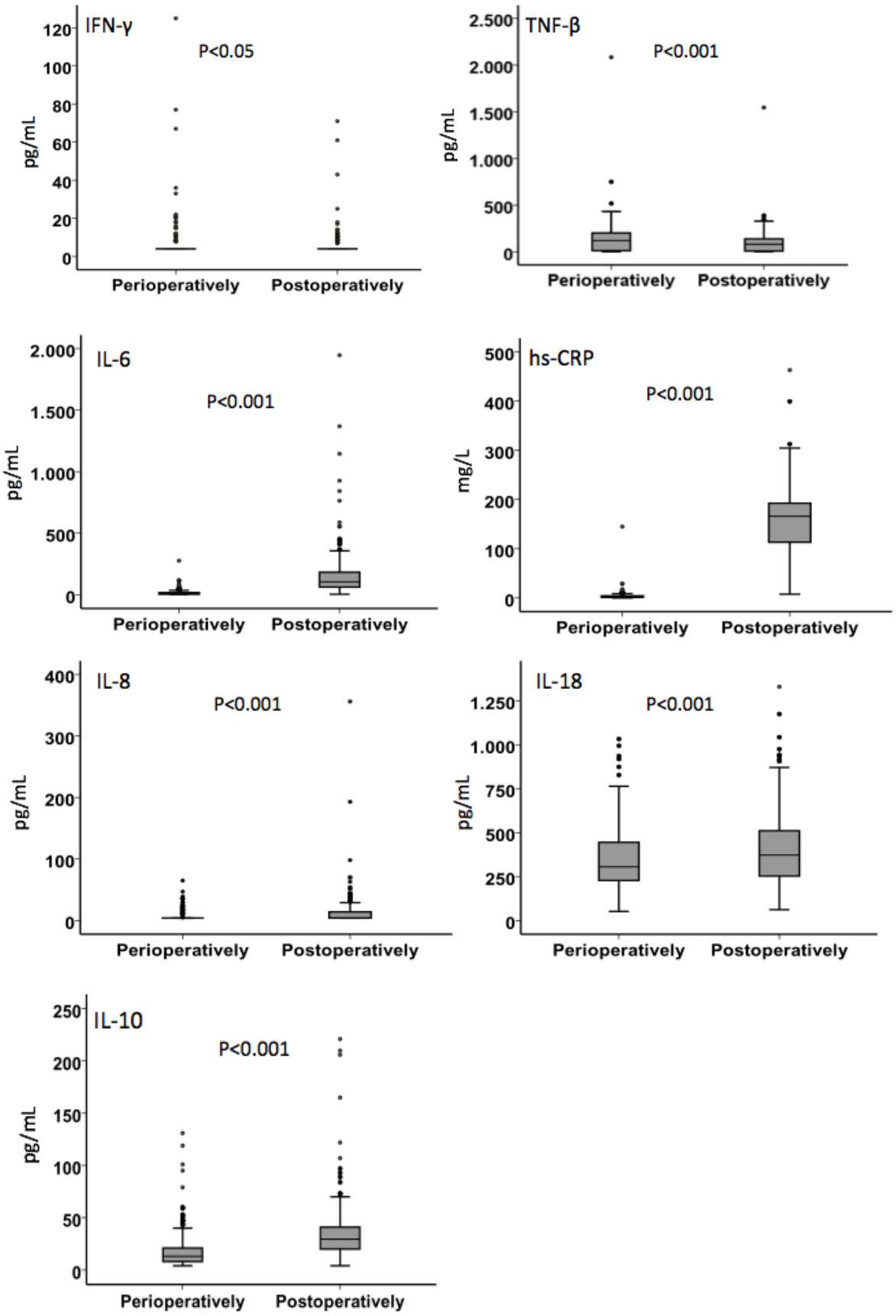
Inflammatory	AA		EPA		DHA		EPA+DHA
mediators	Plasma PL	RBC lipids	Plasma PL	RBC lipids	Plasma PL	RBC lipids	RBC lipids
TNF-α (pg/mL)	-0.032	-0.113	-0.014	0.086	0.133	0.095	0.100
TNF-β (pg/mL)	-0.013	-0.240**	-0.016	0.072	0.130	0.144	0.124
IL-1β (pg/mL)	-0.101	-0.154	0.058	0.221**	0.159	0.121	0.180*
IL-6 (pg/mL)	0.041	0.013	-0.105	-0.092	-0.137	-0.147	-0.135
hs-CRP (mg/L)	-0.063	-0.076	-0.078	-0.002	0.046	0.030	0.018
IL-8 (pg/mL)	0.091	0.050	-0.086	-0.038	-0.047	0.010	-0.012
IL-12 (pg/mL)	-0.131	-0.117	-0.062	-0.069	0.081	0.050	-0.001
IL-18 (pg/mL)	-0.052	-0.097	-0.118	0.021	-0.020	0.072	0.055
IFN-γ (pg/mL)	0.000	-0.110	0.127	0.182	0.158	0.011	0.092
MIP-1α (pg/mL)	-0.027	-0.092	-0.082	0.027	0.077	0.103	0.077
IL-10 (pg/mL)	0.163*	0.072	-0.191*	-0.064	-0.034	-0.035	-0.052
TGF-β (pg/mL)	-0.217**	-0.264**	-0.013	0.198*	0.101	0.165	0.197*

[1]Data are expressed as the standardized beta coefficient. Statistics for the association of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) with selected inflammatory mediators are adjusted for age, BMI and smoking. *p<0.05, **p<0.01.

Figure legend

FIGURE 1 Median plasma concentrations of anti-inflammatory and pro-inflammatory mediators perioperatively and on the third postoperative day. $P<0.05$, compared with perioperative concentrations. Wilcoxon signed rank test.

FIGURE 1


IFN-γ
P<0.05
pg/mL
120
100
80
60
40
20
0
Perioperatively
Postoperatively
TNF-β
P<0.001
pg/mL
2.500
2.000
1.500
1.000
500
0
Perioperatively
Postoperatively
IL-6
P<0.001
pg/mL
2.000
1.500
1.000
500
0
Perioperatively
Postoperatively
hs-CRP
P<0.001
mg/L
500
400
300
200
100
0
Perioperatively
Postoperatively
IL-8
P<0.001
pg/mL
400
300
200
100
0
Perioperatively
Postoperatively
IL-18
P<0.001
pg/mL
1.250
1.000
750
500
250
0
Perioperatively
Postoperatively
IL-10
P<0.001
pg/mL
250
200
150
100
50
0
Perioperatively
Postoperatively

Higher levels of n-3 polyunsaturated fatty acids in red blood cells are associated with an increased risk of postoperative atrial fibrillation: a possible pro-arrhythmic effect?

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ABSTRACT

Background: Open heart surgery leads to both local and systemic inflammation. This inflammation has been associated with an increased risk for complications such as postoperative atrial fibrillation (POAF). n-3 Polyunsaturated fatty acids (n-3 LC-PUFA) are know to have both anti-inflammatory and direct anti-arrhythmic effects, Thus they may be useful for prevention of POAF. However, the results of clinical trials using n-3 LC-PUFA for this purpose have been conflicting.

Methods: A total of 125 patients undergoing CABG were enrolled in the study. Inflammatory mediators were assessed in plasma and fatty acids analyzed in red blood cell (RBC) membrane lipids perioperatively and on the third postoperative day. The endpoint was defined as POAF lasting ≥ 5 min.

Results: Sixty-two CABG patients (49.6%) developed POAF. No difference was in perioperative inflammatory mediators between the POAF and sinus rhythm (SR) groups. In both groups postoperative IL-6, IL-8, IL-18, IL-10 and hs-CRP were higher ($P<0.05$), and TNF-β was lower ($P<0.01$) than those perioperatively. Only postoperative IL-6 was higher in POAF group compared with the SR group. Multivariable logistic regression analysis did not reveal inflammatory mediators as independent predictors of POAF, while higher peri- and postoperative quartiles of DHA, total n-3 LC-PUFA and Omega-3 Index in RBC had higher predictors of POAF.

Conclusions: The study indicates that higher n-3 LC-PUFA levels in RBC membrane lipids are associated with an increased risk for AF in CABG patients. Additionally, these results suggest that inflammatory mediators are not strongly associated with the development of POAF.

INTRODUCTION

Postoperative atrial fibrillation (POAF) is common following open heart surgery, associated with an increased length of hospital stay and resource utilization (1-3). The pathophysiological basis for POAF is multi-factorial and includes both factors related to the surgery and others including the presence of significant structural heart disease and advanced age (3-5). One of the surgery related factors, which has been implicated as predisposing to POAF, is inflammation (6,7). Both the use of cardiopulmonary bypass and the cardiac surgery itself are associated with an acute systemic inflammatory reaction (6,8,9). The temporal association of the onset of POAF, typically on postoperative day two, coincides with the time course of elevation of C reactive protein (CRP), and the release of pro-inflammatory mediator interleukin-6 (IL-6) (1,10,11). This suggests that inflammation may be a component in triggering POAF. Furthermore, our previous study (12) has demonstrated higher peak CRP values among those who developed POAF than those who remained in sinus rhythm.

n-3 Long-chain polyunsaturated fatty acids (n-3 LC-PUFA) have anti-inflammatory effects (13-15) and direct electrophysiologic actions on several ion channels (16-18), which make them attractive as a potential intervention to decrease the risk of POAF. There has been a lot of interest in the potential clinical application of n-3 LC-PUFA to reduce the incidence of POAF. However, the results of published studies have been surprisingly inconsistent, with some showing benefit but others not (19-23). The same holds true for a number of studies on the potential benefit of n-3 LC-PUFA for atrial fibrillation (AF) not associated with cardiac surgery (24). A more detailed analysis of our data demonstrated that the association between n-3 LC-PUFA in plasma phospholipids and POAF followed a U-shaped curve (12). We interpreted this as such that the administration of n-3 LC-PUFA to prevent POAF may benefit those with a low baseline level of the n-3 LC-PUFA but be potentially harmful in those with higher baseline levels.

While our previous studies measured the n-3 LC-PUFA levels in plasma phospholipids, the fatty acid composition of the red blood cell (RBC) membrane lipids has been shown to be a good indicator of the fatty acid composition of atrial tissue (25). Additionally, the measurement of n-3 LC-PUFA levels in RBC membrane lipids is a better indicator of long term composition than plasma phospholipids (26), which may be acutely affected by short term administration (27).

The purpose of this investigation was twofold. First, to examine the relationship between inflammatory mediators and the risk of POAF in patients undergoing open heart surgery. Secondly, since our previous observation indicated that higher levels in plasma phospholipids may actually be harmful, we examined the relationship between POAF and n-3 LC-PUFA levels in RBC membrane lipids.

SUBJECTS AND METHODS

Subjects and study design

The study was part of a prospective, randomized, double-blinded, placebo-controlled clinical trial on the use of n-3 LC-PUFA for a week prior to open heart surgery to prevent postoperative atrial fibrillation. Details of the study design have been published previously (12,22). In brief, all patients scheduled for elective or semi-emergent open heart surgery between August 2007 and May 2009 were evaluated for participation. Of the total of 170 patients enrolled in the study, two patients had their surgery cancelled after randomization leaving 168 patients for analysis. Patients, younger than 40 years of age, with a history of any form of supraventricular arrhythmias or taking the anti-arrhythmic medications amiodarone and/or sotalol, and those undergoing an emergent operation were excluded. In the current study we also excluded those subjects who underwent other surgery than CABG in order to limit confounding by different complexity of the surgical procedure. Prior to surgery, all

participants answered a questionnaire on lifestyle issues, including consumption of fish and cod liver oil, intake of n-3 LC-PUFA capsules, smoking habit, height, body weight, and medication use. All patients gave written informed consent. The study was approved by the Bioethics Committee of Landspitali – The National University Hospital of Iceland, and the Icelandic Data Protection Authority.

Blood sampling

Venous blood samples obtained from the patients immediately before the surgery and on the third postoperative day were collected into disodium EDTA tube and the plasma separated from RBC by immediate centrifugation at 1000 *g* for 10 min. The RBC were washed three times with an isotonic saline solution and the antioxidant butylated hydroxytoluene (BHT), dissolved in methanol, was added to the cells at a final concentration of 50 mg/L. The plasma and RBC samples were frozen at -76°C and stored until the analysis of the inflammatory mediators and the fatty acids was carried out.

Assessment of inflammatory mediators

The plasma samples were analyzed for the concentration of the pro-inflammatory mediators IL-1β, IL-6, IL-8, IL-12, IL-18, interferon-γ (IFN- γ), TNF-α, and TNF-β, and macrophage inflammatory protein-1α (MIP-1α), and the anti-inflammatory mediators IL-10 and transforming growth factor-β (TGF-β) as described by Skogstrand et al. (28). The samples were diluted 1:10 in phosphate buffered saline (PBS) containing 0.5% Tween 20 and 1% bovine serum albumin (BSA). A 50 µL aliquot was mixed 1:1 with a suspension of capture-antibody-conjugated beads in multiwell plates. After incubation for 1½ hour at room temperature with gentle shaking, the beads were washed twice with PBS and subsequently reacted for 1½ hour with a 50 µL mixture of corresponding biotinylated antibodies, each

diluted 1:1000. Streptavidin-phycoerythrin (50 µL) was added to the wells and the incubation continued for additional 30 min. Finally, the beads were washed twice and resuspended in 125 µL of PBS and analyzed on the Luminex 100™ platform (Luminex Corp, TX, USA). Samples were measured in duplicates and standard curves were fitted with a five parameter logistic equation (Logistic-5PL) using BioPlex™ Manager 5.0 (Bio-Rad Laboratories, CA, USA).

Plasma high sensitivity C-reactive protein (hs-CRP) was measured on a Hitachi 911 analyzer using a commercially available latex-enhanced immunoturbidimetric assay (Roche Diagnostics, Mannheim, Germany). The lower detection limit of the assay was 0.1 mg/L. The total coefficient of variation for CRP measurements of the internal controls was 1.1% at a concentration of 3.73 mg/L and 1.9% at a concentration of 0.68 mg/L. In addition, CRP was measured by a conventional assay daily following the surgery as part of the regular care of the patients. We used these measurements do define the maximal (peak) CRP in the postoperative phase.

Analysis of fatty acids in RBC membrane lipids

The lipid fraction was extracted from RBC membrane lipids as described by Bligh & Dyer (29) exept isopropanol was used instead of methanol (isopropanol/chloroform 2:1, v/v). BHT (50 mg/L) was added to the extraction medium. Fatty acid methyl esters (FAME) of plasma PL and RBC membrane lipids were prepared and analyzed by gas-liquid chromatography as described earlier (15). The RBC FAME were analyzed by gas chromatography (Agilent 6890 N, Agilent, Palo Alto, CA) using a Chrompack CP-SIL 8CB column (25 m x 250 µm i.d. x 0.12 µm film thickness). The oven was programmed to provide an initial temperature of 150°C for 4 min, then increasing temperature by 4°C per min to 230°C and then by 20°C per min to 280°C, and was finally held isothermal for 4 min. The injector and detector

temperatures were maintained at 280°C and 300°C, respectively. Hydrogen was used as the carrier gas. The FAME peaks were identified and calibrated against those of commercial standards (Sigma Chemical Co.; Nu-Chek-Prep, Elysian, MN). Fatty acid values in RBC membrane lipids and plasma PL are presented as % weight of total fatty acids with chain length from C_{14} to C_{24}. Instrumental control and data handling was done using HP 3365 Chemstation, Version A.02.12. (Hewlett Packard Co., Palo Alto, CA).

Statistical analysis

As there was no difference in the incidence of POAF between the n-3 LC-PUFA and placebo groups (22), the treatment assignment was ignored in this analysis. Differences in baseline and operative characteristics between the patients with POAF (POAF group) and those who remained in sinus rhythm (SR group) were compared with Wilcoxon–Mann–Whitney test for continuous variables and the chi squared test or Fisher's exact test for categorical variables. Wilcoxon-Mann-Whitney test was also used to compare the groups with regard to the inflammatory mediators and Wilcoxon signed ranks test to compare the difference in the inflammatory mediators between time points. An independent samples t-test was used to compare the fatty acid composition in RBC between the groups. To examine the association between the levels of individual fatty acids and POAF, we compared the rate of POAF between quartiles of the fatty acid levels using chi squared test and the Somers'd for ordinal variables. To examine independent association between the level of each fatty acid and/or inflammatory mediator and POAF, a logistic regression analysis was used with POAF as the dependent variable, adjusting for age, BMI and smoking and peak postoperative CRP, although CRP was excluded in the analysis of inflammatory markers. Data are presented as median (range), percentages or mean ± standard error of mean (SEM), unless otherwise noted. Two-sided P value < 0.05 was considered statistically significant. All statistical

analyses were carried out using SPSS software (version 17.0, IBM Corporation, Somers, NY, USA).

RESULTS

A total of 125 patients underwent CABG, of whom 62 patients (49.6%) developed POAF. Eleven patients with missing data on fatty acid levels in RBC, eight perioperatively and three postoperatively were not included in the respective analysis.

The baseline and operative characteristics of the patients in the POAF and the SR groups are shown in Table 1. The patients in the POAF group were older (P = 0.003), their body mass index (BMI) was lower (P = 0.026), and their median peak CRP concentration was higher (P = 0.042) compared with the SR group. The patients in the POAF group were more likely to consume fish once or more a week than those in the SR group (P = 0.013). No difference was in use of cardiopulmonary bypass (CPB) between the SR and POAF groups (P = 0.349).

Perioperatively, no difference in the plasma concentrations of any of the inflammatory mediators was found between the SR and POAF groups (Table 2 and Figure 1). On the third postoperative day the concentrations of the pro-inflammatory mediators IL-8 and IL-18 and the anti-inflammatory mediator IL-10 were higher (P < 0.05), and that of the pro-inflammatory mediator TNF-β was lower (P < 0.01) compared with the perioperative concentrations in both the SR and POAF groups. Additionally, the postoperative concentration of the pro-inflammatory mediator IFN-γ was lower compared with the perioperative concentration in the SR group (P < 0.05). Both the SR and POAF groups had higher postoperative concentrations of the pro-inflammatory mediators IL-6 and hs-CRP compared with the perioperative concentrations (P < 0.01) (Figure 1). In the SR group the IL-6 concentrations were 82.0 (13-1369) vs. 9.0 (4-114) pg/mL (median (range)) and those of the

hs-CRP were 168.8 (15.20-284.8) vs. 2.66 (0.52-16.20) mg/L, post- vs perioperatively. In the POAF group the IL-6 concentrations were 122.0 (4-2132) vs. 10.0 (4-112) pg/mL and those of the hs-CRP were 171.7 (7.69-463) vs. 2.19 (0.55-144.9) mg/mL, post- vs perioperatively. On the third postoperative day, the concentrations of IL-6 were higher in the POAF group than in the SR group ($P < 0.05$) (Figure 1), while no difference was found between the groups with regard to the other inflammatory mediators (Table 2).

Perioperatively, the SR and POAF groups differed in the n-6 and n-3 LC-PUFA levels of RBC membrane lipids (Figure 2). The POAF group had lower levels of the n-6 LC-PUFA arachidonic acid (AA) and higher levels of total n-3 LC-PUFA, DHA and EPA + DHA (Omega-3 Index) compared with the SR group (11.75 ± 0.21% vs.12.44 ± 0.23%, 13.19 ± 0.27% vs. 12.22 ± 0.27%, 7.84 ± 0.15% vs. 7.23 ± 0.16%, and 10.04 ± 0.24% vs. 9.22 ± 0.25%, respectively, $P < 0.05$). No difference in the EPA levels was found between the two groups ($P > 0.05$). Similar differences were found in postoperative levels of these fatty acids as in perioperative levels between the two groups (data not shown).

In the logistic regression models adjusting for age, BMI and smoking as significant predictors of POAF there was no association observed between peri- or postoperative concentrations of any of the inflammatory mediators and POAF (data not shown). However, in the logistic regression models adjusting for age, BMI, smoking and maximal CRP concentration as significant predictors of POAF where each one of the fatty acids was separately added to the model, the risk of POAF increased with higher perioperative level of DHA (OR (95% CI) = 1.506 (1.010-2.246)), and higher postoperative levels of EPA (OR (95% CI) = 1.952 (1.043-3.654)), DHA (OR (95% CI) = 1.983 (1.260-3.121)), and total n-3 LC-PUFA (OR (95% CI) = 1.440 (1.119-1.853)), and Omega-3 Index (OR (95% CI) = 1.399 (1.070-1.828)) in RBC membrane lipids ($P < 0.05$) (Table 3).

Table 4 shows the incidence of POAF according to quartiles of peri- and postoperative AA, EPA, DHA and total n-3 LC-PUFA levels, and Omega-3 Index (EPA + DHA) in RBC membrane lipids. Perioperatively, there was a significant linear trend for an increasing incidence of POAF with higher quartiles of the DHA (Figure 1) and total n-3 LC-PUFA levels, and of the Omega-3 Index (Figure 1) ($P = 0.006$, $P = 0.010$ and $P = 0.014$, respectively). Postoperatively, there was a significant difference in POAF incidence between quartiles of DHA and total n-3 LC-PUFA levels, and Omega-3 Index ($P = 0.007$, $P = 0.034$ and $P = 0.018$, respectively), with a significant linear trend for an increasing incidence of POAF with higher quartiles ($P = 0.001$, $P = 0.002$ and $P= 0.001$, respectively).

DISCUSSION

This study show that relatively high levels of DHA and EPA + DHA (the Omega-3 Index) in RBC membrane lipids are associated with increased risk of POAF. These results were unexpected, and suggest that this therapy is not as free of adverse effects as previously thought. In addition, we found no association between concentrations of perioperative or postoperative inflammatory mediators with development of POAF in patients following CABG surgery.

Intake of n-3 LC-PUFA supplements and fish consumption have been associated with a decreased risk of sudden cardiac death in population based surveys and randomized clinical trials (16,30). However, the results of studies on the use of n-3 LC-PUFA to decrease events in high risk patients with implantable defibrillators have been dissappointing (31). n-3 LC-PUFA have varied effects on atrial tissue which may account for potential anti-arrhythmic activity (32-37). This includes anti-inflammatory and anti-fibrotic effects (38). Additionally, n-3 LC-PUFA have direct, albeit modest, electrophysiologic effects on ion channels such as the sodium channel, potassium channels, including the atrial selective I_{Kur} and I_{KAch}, the L

type calcium channel and the Na/Ca exchanger (39). Indeed, a number of studies have shown promising results with n-3 LC-PUFA on AF in experimental animal models including decreased inducibility of AF and shortened duration of induced AF (40).

The results of human studies for prevention on POAF development have, on the other hand, not been as favourable (19,21-23,41). An initial, open label study, demonstrated a convincing reduction in the incidence of POAF, thus raising optimism that this simple therapy might be helpful in this situation (19). However, three subsequent randomized placebo controlled trials did not confirm these initial results (21-23). Another primary prevention study examining the effect of fish consumption on the occurrence of AF in a general population did not show any benefit (42). A similar pattern has been seen in studies where n-3 LC-PUFA have been investigated for secondary prevention of AF either paroxysmal or after cardioversion (43). In these studies n-3 LC-PUFA was used as monotherapy or in addition to antiarrhythmic therapy. While one of these studies showed a benefit, four others did not (40). A recent meta-analysis, which included almost 1200 patients did not demonstrate any benefit of n-3 LC-PUFA in secondary prevention of AF (39).

Why have the results of primary and secondary prevention trials been so conflicting? While the answer is not clear speculation has pointed to different doses of n-3 LC-PUFA administered in these studies, possible delayed effects of n-3 LC-PUFA due to need for incorporation into atrial myocyte membranes and the ratio of EPA and DHA in the preparation used. Acute or short-term n-3 LC-PUFA administration influences the fatty acid composition of fatty acids in plasma PL, which are merely transporters of circulating fatty acids (26). In contrast, the fatty acid pattern of the RBC membrane lipids has been shown to be a good indicator of the fatty acid composition of atrial tissue (43). The length of treatment may therefore be of importance and the ratio of EPA to DHA might also play a role. In vitro study has shown that EPA has a stronger potassium channel blocking capabilities while DHA

has greater effects on the sodium channel (44). A recent study from Finland suggested that high serum levels of DHA, but not EPA, were associated with a significant decrease in incident AF (45). This is in conflict with our previous finding (12), where relatively high level as well as low level of DHA in plasma PL actually increased the risk of POAF and intermediate levels were associated with reduced incidence of POAF. We suggested that baseline levels of n-3 LC-PUFA were of importance and in those populations were they are low, additional supplementation may be of help but in those with higher levels it may make matters worse. This has not been widely considered in the explanation of the discrepancy in the results of previously published studies. Also, if baseline levels matter confounding factors such as dietary fish intake and fish oil consumption may play an important role in the conflicting data. In the present study, we assessed the fatty acid composition of RBC membrane lipids, which is expected to reflect the dietary lipid intake for several weeks.

hs-CRP has been thought to be the most reliable and reproducible among the inflammatory mediators (46), and it has been implied that the causal role of CRP is to drive inflammation as well as thrombosis (47).Our findings contradict the suggestion that elevated preoperative concentrations of inflammatory mediators such as CRP are predictive of POAF amongst patients undergoing CABG (48,49), whereas we found no difference in the perioperative plasma concentrations neither of hs-CRP nor of the other inflammatory mediators between the SR and POAF group

A hypothesis of n-3 LC-PUFA anti-inflammatory and immunosuppressive properties is based on EPA and DHA in circulation and/or the cell membranes (15). It is indicated that the eicosanoids of EPA and the docosanoids of DHA from membrane PL of inflammatory producing cells can inhibit the transcription of inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IL-8 by modulating nuclear translocation of nuclear factor (NF)-κB, and inhibit AA metabolism to pro-inflammatory AA-derived eicosanoids (14,50,51). In addition, EPA- and

DHA-derived lipid mediators called resolvins and protectins stimulate anti-inflammatory, pro-resolving and protective signalling pathways (14). Bruins and coworkers (10) demonstrated that IL-6 concentration rises initially and peaks at six hours after surgery and that a second phase occurs in which the CRP concentration peak on postoperative day two. Two studies demonstrated that use and duration of cardiopulmonary bypass (CPB) and aortic cross-clamp time has been correlated with degree of inflammatory response (52,53), while another study (54) showed that elevation in hs-CRP and IL-6 concentrations on the fourth postoperative day were not affected by use of CPB in uncomplicated cardiac surgery. In the present study, no difference was in the use of CPB and aortic cross-clamp time between the two groups, where the concentrations of hs-CRP and IL-6 were elevated in both SR and POAF groups on the third postoperative day compared with those perioperatively. Furthermore, the relatively higher levels of DHA in RBC membrane lipids of the patients in the POAF group seem not to have preventing effect on their IL-6 concentration, since it was higher compared with those in the SR group.

In the present study we have convincingly demonstrated that the higher the RBC membrane levels of DHA and n-3 LC-PUFA the higher the risk of POAF. This might therefore suggest that higher levels of n-3 LC-PUFA may be pro-arrhythmic. The concept of n-3 LC-PUFA having a pro-arrhythmic effect is not entirely novel. This has been suggested before in a study on ventricular arrhythmias in ICD patients (31) and in a study where there was a strong trend toward AF among individuals who consumed >5 fish meals a week (55). The present study, however, is the first to link measured levels of n-3 LC-PUFA in RBC membrane lipids with an increased arrhythmia occurrence. What might cause n-3 LC-PUFA to be pro-arrhythmic? The parasympathetic tone is increased by n-3 LC-PUFA and this is a known mechanism of triggering AF (40). Additionally, given the direct electrophysiological

effect of n-3 LC-PUFA, unfavourable actions on ion channels by high n-3 LC-PUFA levels cannot be discounted.

The questions are raised, are n-3 LC-PUFA anti-arrhythmic, pro-arrhythmic, both or perhaps even neither? Despite a number of published studies this remains quite unclear. Our data that higher levels of n-3 LC-PUFA in RBC membrane, which reflect atrial myocyte membrane n-3 LC-PUFA levels, increase the risk of POAF add an important piece of information to the ongoing debate on the merits of this treatment.

In summary this study shows that while inflammation is clearly present in the first few days after CABG, this does not appear to be a strong risk factor for the development of POAF. Additionally, higher RBC membrane levels of DHA and n-3 LC-PUFA increase the risk of POAF, thereby suggesting a possible pro-arrhythmic action of these natural dietary constituents.

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Conflict of interest

The authors declare no conflict of interest.

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TABLE 1 Baseline and operative characteristics of the patients who did (POAF group) or did not (SR group) develop POAF.

	SR group (n=63)	POAF group (n=62)	P value
Age (years)	66 (45-79)	69 (45-82)*	**0.003**
BMI (kg/m^2)	28.4 (20.9-41.3)	27.0 (19.1-35.0)*	**0.026**
Gender (% men)	84.1	79.0	0.462
Smoking (%)	27.0	14.5	0.086
Fish intake (%, >once a week)	62.3	82.3*	**0.013**
Cod liver oil intake (%)	54.0	56.5	0.367
n-3 LC-PUFA intake (%)	25.4	27.4	0.364
Use of β blockers (%)	84.1	77.4	0.341
Use of statins (%)	90.5	83.9	0.270
Blood volume in drains (mL)	700 (110-3070)	775 (96-4980)	0.664
Peak postoperative CRP level (mg/L)	197.0 (34.0-370.0)	216.5 (36.0-416.0)*	**0.042**
On pump surgery (%)	81.0	87.1	0.349
Hypertension (%)	65.1	64.5	0.947
Diabetes (%)	19.0	12.9	0.349

Data are expressed as median (range) or percentage of subjects. POAF, postoperative atrial fibrillation; SR, sinus rhythm; BMI, body mass index; LC-PUFA, long-chain polyunsaturated fatty acids.

*P < 0.05, compared with the SR group, Wilcoxon-Mann-Whitney or chi-squared tests.

TABLE 2

Plasma levels of inflammatory mediators in the sinus rhytm (SR) and the POAF groups at perioperative day and on the third postoperative day.

	Perioperative day		Third postoperative day	
Inflammatory markers	SR group (n = 63)	POAF group (n = 62)	SR group (n = 63)	POAF group (n = 62)
TNF-a (pg/mL) Pro	4 (4-181)	4 (4-147)	4 (4-224)	4 (4-76)
TNF-b (pg/mL) Pro	99 (4-2083)	111.0 (4-750)	50 (4-1545)**	89.5 (4-390)**
IL-1b (pg/mL) Pro	8 (4-110)	4 (4-76)	8 (4-60)	4 (4-90)
IL-8 (pg/mL) Pro	4 (4-47)	4 (4-36)	4 (4-356)**	4 (4-206)*
IL-12 (pg/mL) Pro	10 (4-275)	4.0 (4-132)	9 (4-124)	10.0 (4-118)
IL-18 (pg/mL) Pro	322.0 (104-1033)	290.5 (52-936)	366.0 (111-1043)**	347.5 (62-1175)**
IFN-y (pg/mL) Pro	4 (4-125)	4 (4-77)	4 (4-61)*	4 (4-71)
MIP-1a (pg/mL) Pro	56 (10-587)	35.5 (10-603)	51 (10-468)	31.5 (10-404)
IL-10 (pg/mL) Anti	14.0 (4-131)	12.0 (4-95)	29.0 (4-210)**	29.5 (4-221)**
TGF-b (pg/mL) Anti	85 (39-330)	39 (11-402)	39 (39-415)	75 (17-405)

Data are expressed as median (range). *P<0.05, **P<0.01, compared with the perioperative levels, Wilcoxon signed ranks test.

TABLE 3 Multivariable logistic regression analysis of predictors of postoperative atrial fibrillation (POAF).

Fatty acids	OR (95% CI)	P-value
Perioperative		
AA (20:4n-6)	0.783 (0.602-1,018)	0.068
EPA (20:5n-3)	1.382 (0.821-2.327)	0.224
DHA (22:6n-3)	1.506 (1.010-2.246)	**0.045**
n-3 LC-PUFA	1.229 (0.991-1.525)	0.061
Omega-3 Index	1.248 (0.979-1.591)	0.073
Third postoperative day		
AA (20:4n-6)	0.802 (0.595-1.082)	0.149
EPA (20:5n-3)	1.952 (1.043-3.654)	**0.037**
DHA (22:6n-3)	1.983 (1.260-3.121)	**0.003**
n-3 LC-PUFA	1.440 (1.119-1.853)	**0.005**
Omega-3 Index	1.399 (1.070-1.828)	**0.014**

See Figure 2 for the abbreviations.
The fatty acids are added individually to a model that includes age, BMI, smoking and maximal CRP concentration following surgery.

TABLE 4 Incidence of postoperative atrial fibrillation (POAF) according to quartiles of arachidonic acid and n-3 LC-PUFA levels in RBC membrane lipids at perioperative day and on the third postoperative day*

	Quartiles				P value	
	1	2	3	4	χ2†	Somers´d‡
Perioperative day						
AA level (%)§	8.66-10.92	10.96-12.12	12.26-13.10	13.16-17.19		
Incidence (%)	58.1	54.8	50.0	34.5	0.275	0.062
EPA level (%)	0.62-1.46	1.47-1.93	1.99-2.58	2.59-4.88		
Incidence (%)	44.8	48.4	48.1	56.7	0.824	0.386
DHA level (%)	4.72-6.49	6.61-7.52	7.56-8.39	8.46-10.72		
Incidence (%)	33.3	42.4	53.3	66.7	0.076	**0.006**
n-3 LC-PUFA level (%)	7.95-10.96	11.11-12.67	12.68-13.99	14.18-19.68		
Incidence (%)	35.7	41.4	54.8	65.5	0.101	**0.010**
EPA + DHA level (%)	5.50-7.99	8.08-9.61	9.69-10.66	10.77-15.60		
Incidence (%)	37.0	43.3	48.3	67.7	0.099	**0.014**
Third postoperative day						
AA level (%)	8.68-11.08	11.14-12.08	12.09-13.01	13.01-16.62		
Incidence (%)	56.3	50.0	50.0	40.0	0.644	0.221
EPA level (%)	0.84-1.31	1.33-1.69	1.70-2.32	2.33-4.36		
Incidence (%)	36.4	46.7	57.1	58.1	0.269	0.051
DHA level (%)	5.05-6.48	6.50-7.14	7.21-7.87	7.89-10.00		
Incidence (%)	23.3	48.3	61.3	62.5	**0.007**	**0.001**
n-3 LC-PUFA level (%)	8.15-10.72	10.73-11.81	11.83-13.06	13.26-17.23		
Incidence (%)	29.0	46.4	56.3	64.5	**0.034**	**0.002**
EPA + DHA level (%)	5.98-7.88	7.88-8.80	8.87-10.16	10.26-13.52		
Incidence (%)	27.6	43.3	59.4	64.5	**0.018**	**0.001**

* See Figure 2 for the abbreviations.
†Pearson´s chi square statistics.
‡Somers´ d statistics for trend in association between ordinal variables.
§Levels are expressed as a percentage of total fatty acids.

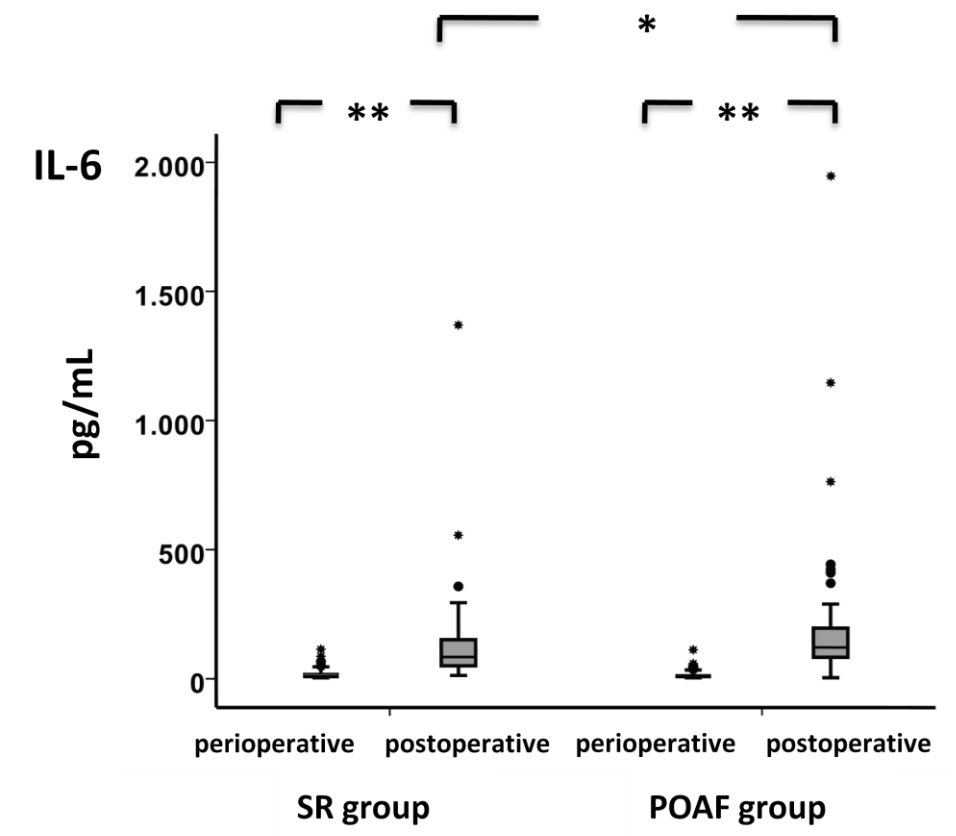
FIGURE LEGENDS

FIGURE 1 Perioperative and third postoperative day plasma concentrations of IL-6 and hs-CRP in the sinus rhythm (SR) and postoperative atrial fibrillation (POAF) groups.
**P < 0.001 compared with the perioperative concentrations, Wilcoxon signed rank test.
*P < 0.05 compaired with the SR group, Wilcoxon-Mann-Whitney test.

FIGURE 2 Perioperative RBC membrane lipid levels (% of total fatty acids) of arachidonic acid (AA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total n-3 long-chain polyunsaturated fatty acid (LC-PUFA), and Omega-3 Index (EPA + DHA) in the sinus rhythm (SR) and postoperative atrial fibrillation (POAF) groups.
Values are mean ± SEM. *P < 0.05, **P < 0.01, independent samples *t*-test.

FIGURE 3 Frequency of postoperative atrial fibrillation (POAF) based on peri- and postoperative quartiles of docosahexaenoic acid (DHA) and Omega-3 Index (EPA+DHA). See Table 4 for the range of fatty acid levels (% of total fatty acids) in each quartile.

FIGURE 1


IL-6
pg/mL
2.000
1.500
1.000
500
0
*
**
**
perioperative
postoperative
perioperative
postoperative
SR group
POAF group

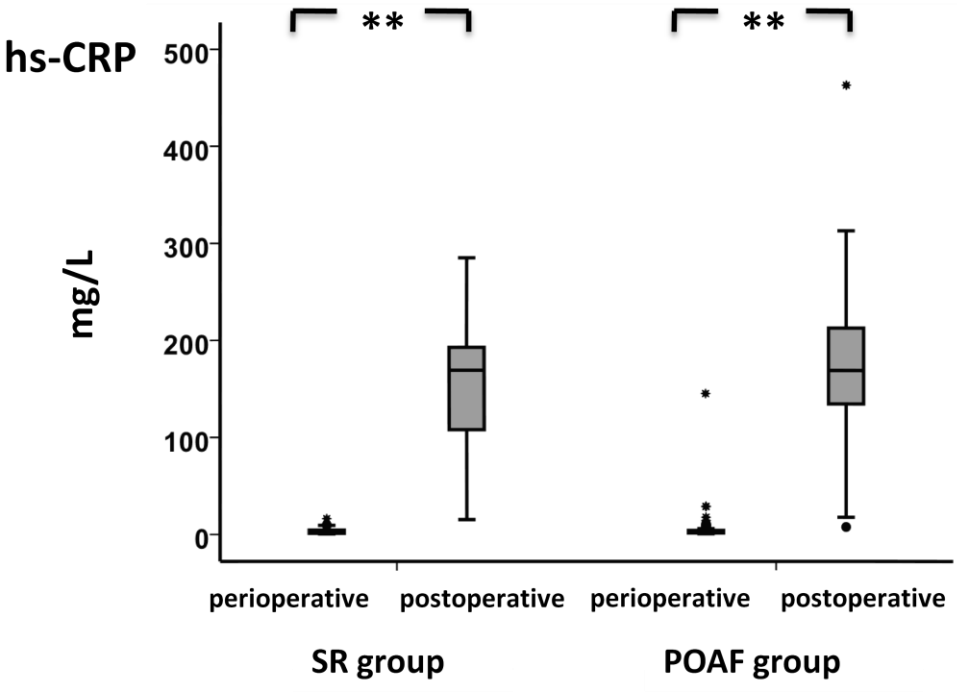

hs-CRP
mg/L
500
400
300
200
100
0
**
**
perioperative
postoperative
perioperative
postoperative
SR group
POAF group

FIGURE 2

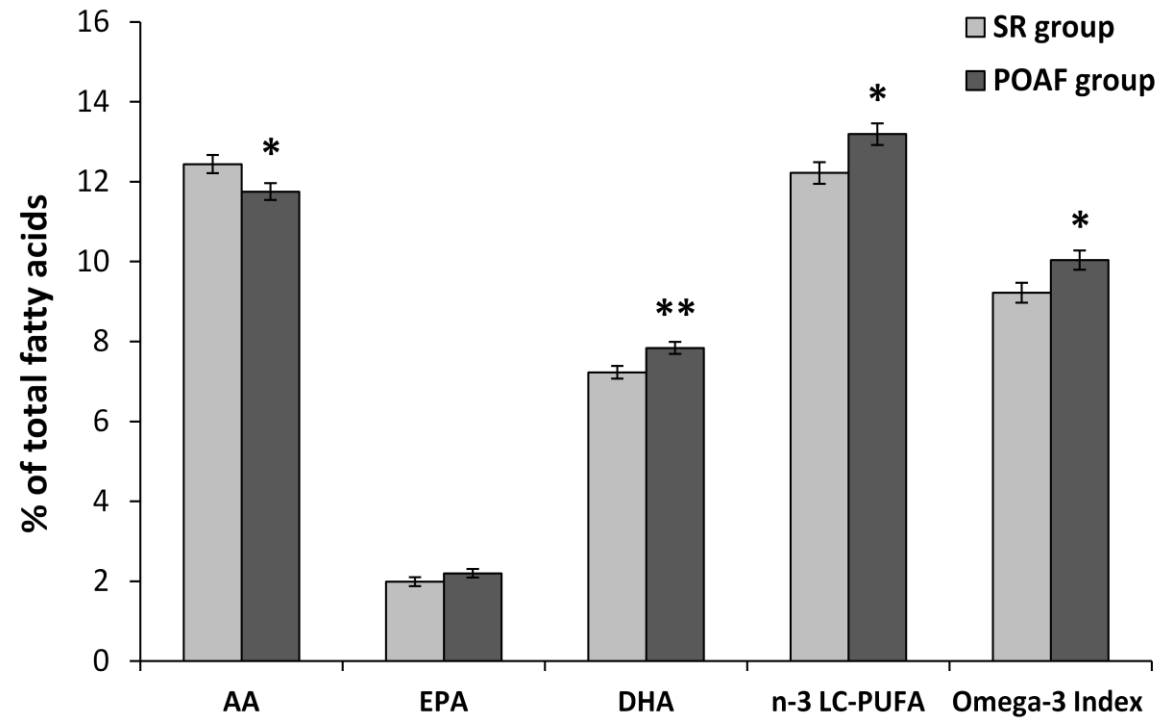
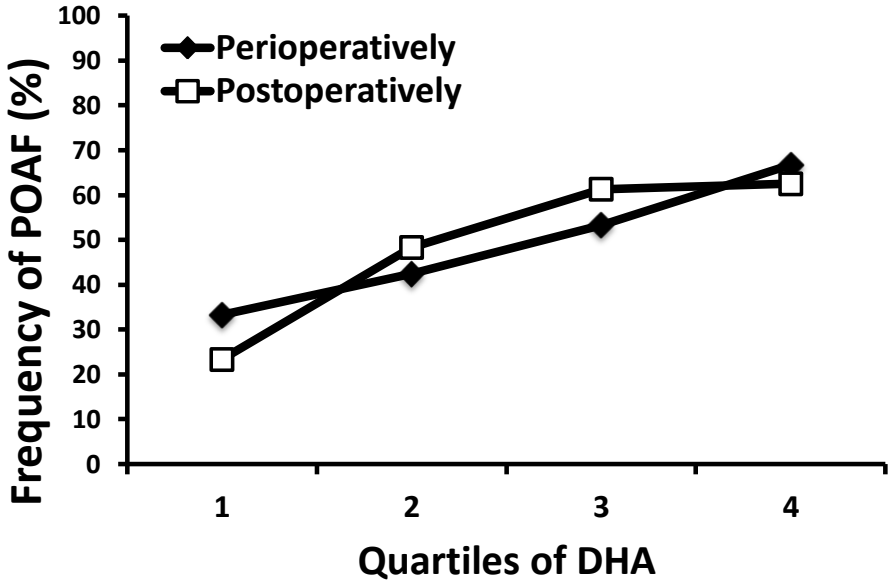
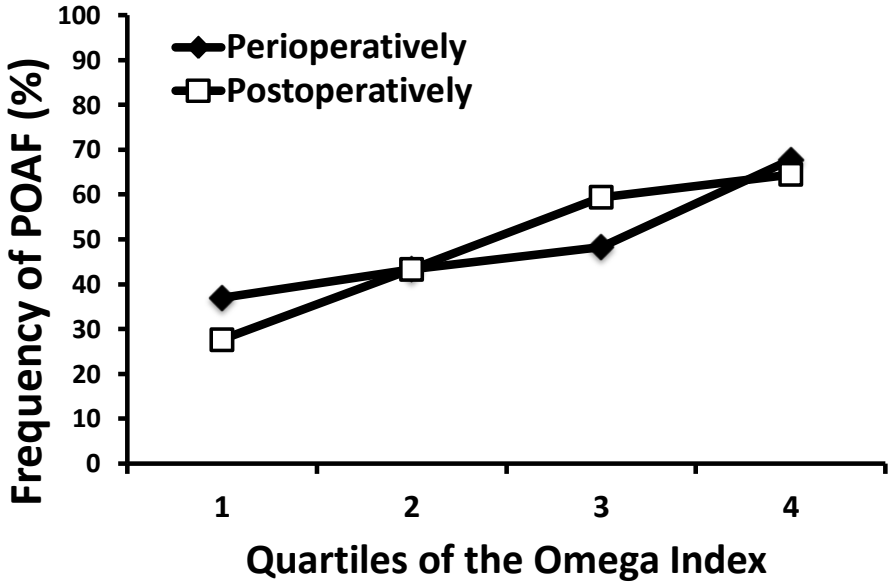

SR group
POAF group
% of total fatty acids
16
14
12
10
8
6
4
2
0
*
*
**
*
AA
EPA
DHA
n-3 LC-PUFA
Omega-3 Index

FIGURE 3


Perioperatively
Postoperatively
Frequency of POAF (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Quartiles of DHA


Perioperatively
Postoperatively
Frequency of POAF (%)
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Quartiles of the Omega Index