Short term effect of omega-3 products on biomarkers of oxidative stress in healthy volunteers: a randomized controlled trial

Christina Ellefsen Hopland

Master Thesis in Clinical Nutrition

Department of Clinical Nutrition

Faculty of Medicine

University of Bergen

May 2021

Short term effect of omega-3 products on biomarkers of oxidative stress in healthy volunteers: a randomized controlled trial

Christina Ellefsen Hopland

Master Thesis in Clinical Nutrition

Department of Clinical Nutrition

Faculty of Medicine

University of Bergen

Institute of Marine Research

May 2021

Supervisors:

Dr. Scient Lisbeth Dahl

PhD Jannike Øyen

[1] Institute of Marine Research

Acknowledgements

First and foremost, I would like to express my gratitude to my supervisors Lisbeth Dahl and Jannike Øyen for their guidance, support and encouragement throughout this process. Thank you for finding the time to help and guide me in the right direction, and not to mention for reading through my thesis countless times and for giving me positive feedback and constructive criticism. I am immensely grateful.

I am very grateful for have been given the opportunity to complete my Master´s degree at the Institute of Marine Research, Section for Seafood and Nutrition. It has been inspiring to be included in such a great working environment housing excellent scientists.

Thank you to my fellow students at the Student Loft, whom all have contributed to making this year a positive experience. Thank you for the encouragement and support and for all fun discussions in the lunchbreaks.

Finally, I would like to thank my family and friends for their support. Although you may not always understand what I am talking about, you take the time to listen to my enthusiastic explanations of this thesis.

Christina Ellefsen Hopland

Bergen, May 2021

Abstract

Background: Intake of seafood and long-chain *n*-3 polyunsaturated fatty acid (*n*-3 LC PUFA) supplements has been associated with reduced cardiovascular disease risk. Recent evidence suggests that the cardioprotective effect may not be as prominent as earlier assumed, with studies showing little or no effect of *n*-3 LC PUFA supplements on cardiovascular risk factors. Due to their high number of double bonds, *n*-3 PUFAs are highly suspectable for lipid peroxidation. More knowledge is needed about the health effects of consumption of oxidized fish oil in human subjects, though it is suggested that oxidative metabolites of *n*-3 PUFAs may exert negative effects in physiological systems.

Aim: This study was set out to investigate the short-term effects of various *n*-3 products on markers of lipid peroxidation, *in vivo* oxidative stress and fatty acid composition in erythrocyte membranes in healthy subjects after three and 21 days of intervention.

Methods: In a randomized controlled study, healthy subjects (n=48) were assigned into one out of six groups receiving either fish, juice fortified with emulsified fish oil, capsule with non-oxidized fish oil (Non-OX), capsule with less oxidized fish oil (LowOX), capsule with highly oxidized fish oil (HighOX) or capsule with sunflower oil (Control). Dietary data was collected using a semi-quantitative food frequency questionnaire (FFQ) and markers of oxidative stress were measured in blood and urine samples at baseline and after three and 21 days.

Results: No significant between-groups changes in the lipid peroxidation markers plasma 4-hydroxyhexenal (HHE) and 4-hydroxynoneal (HNE) or in the oxidative stress marker urinary 8-iso-prostaglandin-$F_{2\alpha}$ (8-iso $PGF_{2\alpha}$) were observed. Serum vitamin E increased significantly from baseline to day three between intervention groups (p=0.009). All *n*-3 products increased the content of *n*-3 LC-PUFAs in erythrocyte plasma membranes. Dietary data showed that the majority of subjects had an intake of seafood, fruit and vegetables that were below the Norwegian dietary recommendations.

Conclusion: Findings in the present study do not indicate any harmful short-term effects of intake of oxidized fish oils on markers of *in vivo* oxidative stress and lipid peroxidation in healthy subjects. Since numerous humans use n-3 supplements, further investigation of possible long-term effects of consumption of oxidized products could be of interest.

Table of content

List of abbreviations ii

List of tables ii

List of figures iii

1 Introduction 1

1.1 Intake and recommendations for seafood and *n*-3 PUFA supplements 1

1.1.1 Recommendations for seafood and n-3 LC PUFA supplements 1

1.1.2 Seafood, n-3 LC PUFA supplements and cardiovascular disease 2

1.2 Lipids and fatty acids 3

1.2.1 Fatty acid structure 4

1.3 Essential fatty acids 4

1.3.1 Dietary sources of omega-3 and omega-6 fatty acids 5

1.3.2. Ratio between intake of n-3 and n-6 polyunsaturated fatty acids 6

1.3.3 Functions of essential fatty acids 6

1.4 Oxidative stress 7

1.4.1 Lipid peroxidation 7

1.4.2 Peroxidation of n-3 and n-6 polyunsaturated fatty acids 8

1.4.3 Oxidized fish oil and health outcomes 9

1.5 Oxidized fish oil supplements 10

1.6 Aim of study 11

2.1 Ethics 12

2.2 Study population 12

2.2.1 Recruitment 12

2.2.2 Inclusion and exclusion criteria 12

2.2.3 Blinding and randomization 13

2.3 Intervention 13

2.3.1 Study groups 13

2.3.2 Procedure 13

2.3.3 Oxidation of study products 14

2.3.4 Compliance and side-effects *15*

2.4 Data collection **15**

2.4.1 Sampling and analysis of blood and urine samples *15*

2.4.2 Anthropometric measurements *17*

2.4.3 Food frequency questionnaire *17*

2.5 Statistics **18**

3 Results **19**

3.1 Study population **19**

3.1.1 Characteristics of the study population *19*

3.1.2 Diet- and lifestyle habits in the study population *23*

3.2 Primary outcomes **24**

3.3 Secondary outcomes **26**

3.4 Fatty acids in erythrocyte plasma membrane **29**

3.5 Compliance and side-effects of study products *33*

4 Discussion **34**

4.1 Outcome variables *34*

4.1.1 Markers of lipid peroxidation and in vivo oxidative stress *34*

4.1.2 Serum vitamin E, triacylglycerol and lipoproteins *35*

4.1.3 n-3 polyunsaturated fatty acids in erythrocyte membranes *36*

4.1.4 Chemical form of n-3 polyunsaturated fatty acids and bioavailability *37*

4.2 Dietary data **38**

4.3 Methodological considerations **39**

4.4 Conclusions **41**

References **42**

Appendices **46**

List of abbreviations

AA: arachidonic acid

AHA: American Heart Association

ALA: α-linolenic acid

ALAT: alanine amino transferase

ASAT: aspartate amino transferase

AV: anisidine value

CHD: coronary heart disease

CRP: C-reactive protein

CVD: cardiovascular disease

DHA: docosahexaenoic acid

DPA: docosapentaenoic acid

EFA: essential fatty acid

EFSA: European Food Safety Authority

EPA: eicosapentaenoic acid

EE: ethyl ester

FA: fatty acid

FAO: Food and Agriculture Organization of the United Nations

FFQ: food frequency questionnaire

isoP: isoprostane

HETE: hydroxyeicosatetraenoic acid

HDL: high-density lipoprotein

IMR: Institute of Marine Research

LA: linoleic acid

LDL: low-density lipoprotein

LT: leukotrienes

MAG: monoacylglycerol

MUFA: monounsaturated fatty acid

n-3 LC PUFA: *n*-3 long-chain polyunsaturated fatty acid

PUFA: polyunsaturated fatty acid

PG: prostaglandin

PGI: prostacyclin

PUFA: polyunsaturated fatty acid

PV: peroxide value

ROS: radical oxygen species

SFA: saturated fatty acid

TAG.: triacylglycerol

TSH: thyroid stimulating hormone

T_4: thyroxine

TXA: thromboxane

VLDL: very low-density lipoprotein

4-HHE: 4-hydroxyhexenal

4-HNE: 4-hydroxynoneal

8-isoPGF: 8-iso-prostaglandin-$F_{2\alpha}$

25-OH D: 25-hydroxy vitamin D

List of tables

Table 1. Overview of study visits and biomarkers measured in blood-and urine samples at baseline and after three and 21 days. (p. 16)

Table 2. Baseline characteristics for all participants and intervention groups (*n*=48). Data presented as numbers (%) or mean values and standard deviations. (p. 20-21)

Table 3. Frequency of consumption of various foods for all participants and by intervention group (*n*=48). Data presented as numbers (%). (p. 21-22)

Table 4 Markers of lipid peroxidation at baseline and after three and 21 days of intervention (*n*=48). Data presented as mean values and standard deviations or mean values and 95% CI. (p. 25)

Table 5. Serum secondary outcomes (*n*=48). Data presented as mean values and standard deviations or mean values (95% CI). (p.27-28)

Table 6. Fatty acids in erythrocyte plasma membrane given as mg fatty acid/g erythrocyte (*n*=48). (p. 31-32)

List of figures

Figure 1: Illustration of lipid peroxidation process. (p. 8)

Figure 2: Illustration of *n*-3 products. (p.14)

Figure 3. Flowchart of the recruitment process. (p. 19)

Figure 4. Box plots presenting markers of lipid peroxidation and oxidative stress at baseline and after three and 21 days (*n*=48). Data are shown as mean values and standard deviations. (p. 24)

Figure 5. Box plot presenting serum vitamin E at baseline and after three days of intervention (*n*=48) given in µmol/L. Data are shown as mean values and standard deviations. (p. 27)

Figure 6. Box plots presenting fatty acids in erythrocyte plasma membrane given in mg fatty acid/g erythrocyte (*n*=48). Data are shown as mean values and standard deviations. (p.30)

1 Introduction

Cardiovascular disease (CVD) stands out as the major cause of morbidity and mortality globally, bearing the burden of 31% of annual deaths. CVDs are a group of disorders affecting the heart and blood vessels. Several risk factors have been associated with the development of CVDs, among them the most prominent dyslipidemia, hypertension, diabetes, obesity, unhealthy diet, tobacco smoking and physical inactivity (1). Consumption of seafood and *n*-3 long chain polyunsaturated fatty acids (*n*-3 LC PUFA) has been recognized as protective agents in CVDs (2). However, there is a potential drawback with consumption of *n*-3 LC PUFAs. Due to their chemical composition, *n*-3 LC PUFAs are unstable compounds and readily decompose to a range of oxidation products (3, 4). Along this line, it has been questioned if metabolites of *n*-3 LC PUFAs can exert negative effects in biological systems. Thus, a high intake of *n*-3 LC PUFAs might be a double-edged sword.

1.1 Intake and recommendations for seafood and *n*-3 PUFA supplements

Seafood is a source of several important macro-and micronutrients that have been reported to have several beneficial effects on human health. Including seafood in the diet provides the *n*-3 LC PUFAs eicosapentaenoic acid (EPA, 20:5 *n*-3), docosahexaenoic acid (DHA, 22:6 *n*-3) and docosapentaenoic acid (DPA, 22:5 *n*-3), high biological value proteins, vitamins, minerals and trace elements (5). According to the definition by the European Food Safety Authority (EFSA) seafood is defined as vertebrate and invertebrate aquatic animals of marine or freshwater origin, whether farmed or wild, with the exclusion of aquatic mammals (e.g. whales and dolphins), aquatic reptiles (e.g. turtles and crocodiles), echinoderms (e.g. starfish), jellyfish and aquatic plants (6).

1.1.1 Recommendations for seafood and *n*-3 LC PUFA supplements

At a global scope, health authorities recommend seafood as a part of a healthy and balanced diet. In a report from the Food and Agriculture Organization (FAO), an intake interval for total *n*-3 PUFAs was set to 0.5-2.0 energy percent (E%) of the total energy intake. Moreover, the minimum dietary requirement of the precursor *n*-3 PUFA α-linolenic acid (ALA, 18:3 *n*-3) necessary to prevent deficiency symptoms was estimated to 0.5 E%. FAO set the upper intake level of EPA+DHA to 2.0 mg/day due to experimental evidence suggesting that high supplemental intakes of *n*-3 PUFAs might lead to increased lipid peroxidation, reduced cytokine production and increased bleeding tendency. However, it was underlined that intake levels up to 3.0 mg EPA+DHA/day has been shown to reduce cardiovascular risk factors in randomized trials, and that some population with high seafood intake consume much higher levels without any apparent evidence of harm (7). In a scientific opinion on dietary reference values on fats, EFSA concluded that an intake of 0.5 E% ALA was adequate to prevent deficiency symptoms. Based on the

potential beneficial effects on cardiovascular health, an intake of 250 mg EPA+DHA was regarded as sufficient for primary prevention in healthy subjects (8). The American Heart Association´s (AHA) Diet and Lifestyle Recommendation on fish intake recommends at least 2 servings of fish per week to provide an average of 250 mg EPA+DHA per day (8).

In the Norwegian dietary recommendations, it is recommended to eat 2-3 dinner servings of fish per week, which equals 300-450 g of fish. Furthermore, it is underlined that one should consume at least 200 g fatty fish to provide sufficient EPA and DHA. Dinner servings can be replaced by fish as sandwich spread, where one dinner serving corresponds to six portions of fish spread. Those who do not have a sufficient fish intake or whom excludes fish from the diet are recommended to take *n*-3 LC PUFA supplements (10). However, the national dietary survey Norkost 3 shows that a scarce proportion of the Norwegian population meets the recommendation on fish intake. Norkost 3 assesses the habitual diet in representative groups of Norwegian adults between the age of 18-70 years, and in the last report from 2012 about 1/3 of the participants had a dietary intake within the recommended 300-450 g of fish per week and about 1/5 met the dietary recommendations on fatty fish intake. Furthermore, Norkost 3 shows that the intake of seafood is skewed in the population, demonstrated by men having an average intake of 79 ± 102 g/day and median intake of 38 g/day, while women had an average and median intake of 56 ± 72 g/day and 29 g/day, respectively (11).

1.1.2 Seafood, *n-3* LC PUFA supplements and cardiovascular disease

In the 1970s Danish researchers Bang et al. studied the low prevalence of ischemic heart disease in Greenlandic Inuit hunters and fishermen. Compared to Danish controls, the Inuit population showed significantly lower plasma lipid and lipoprotein levels. Bang et al. suggested that the Greenlandic diet rich in marine PUFAs from whale, seal, fish and sea birds were attributable to the low mortality from ischemic heart disease (12). This sparked the scientific interest in the role of *n*-3 LC PUFAs in CVD and since then the mechanism by which marine lipids might improve cardiovascular health has been extensively investigated. Although not fully understood, clinical evidence supports a cardioprotective effect, shown through reduced incidences of thrombotic disease, lower mortality from cardiac arrest and heart failure (2). On the contrary, some recent evidence has presented results that contradicts with consistent findings of an inverse association between both higher seafood intake and circulating EPA+DHA and lower risk of CVD in epidemiological studies. Meta-analyses of clinical trials investigating marine *n*-3 LC PUFA supplementation has shown little or none effect on risk of nonfatal and fatal coronary heart disease (CHD), major vascular events, all-cause mortality and CVD death. Furthermore, the effect of *n*-3 LC PUFA supplementation on CVDs in healthy population is somewhat unclear and primary and secondary prevention trials have shown inconsistent results (12-16).

Current evidence for a cardioprotective effect suggests that *n*-3 LC PUFAs might act as pleiotropic agents in cardiovascular systems, showing anti-arrhythmic, anti-aggregatory, anti-inflammatory effects and triacylglycerol (TAG)-lowering effects, as well as improving endothelial function (17-19). EPA and DHA has been shown to be preferentially shunted into phospholipid synthesis pathways compared to other fatty acids (FA) and following consumption an increased incorporation in phospholipid membranes occurs. An increased incorporation of *n*-3 LC PUFAs at the expense of *n*-6 PUFAs may be of relevance for cardiovascular health. Certain metabolites of *n*-6 PUFAs are associated with unfavorable effects on cardiovascular systems, hence, a shift towards *n*-3 LC PUFAs might inhibit the negative effects of *n*-6 PUFA metabolites (17, 20, 21).

n-3 LC PUFAs are shown to have a lowering effect on TAG levels, though through which molecular mechanisms not fully understood. Potential pathways are a combination of reduced hepatic secretion of very low-density lipoproteins (VLDL) and enhanced clearance of circulating TAGs in VLDL and chylomicrons. The hepatic enzymes that regulate the metabolism of FAs and TAGs are under transcriptional control. Dietary lipids can modify expression of these enzymes by interaction with various nuclear receptors and transcription factors, which might be the key to the effect of *n*-3 LC PUFAs on VLDL. *n*-3 LC PUFAs are ligands for transcription factors and nuclear receptors involved in regulation of hepatic TAG- and phospholipid metabolism. Implicated mechanisms are inter alia decreased *de novo* lipogenesis and subsequent reduced hepatic availably of FAs for TAG synthesis, increased FA beta-oxidation, reduced delivery of non-esterified FAs to the liver, reduced activity in hepatic enzymes involved in TAG synthesis and increased activity in enzymes involved in synthesis of phospholipids rather than TAGs. Ultimately, these mechanistical effects might contribute to decreased hepatic secretion of VLDL (20, 22, 23).

1.2 Lipids and fatty acids

Lipids are a diverse group of hydrophobic organic compounds comprised of hydrogen atoms attached to a carbon backbone with a small number of oxygen atom substitutions (25). Lipids serve a plethora of important functions within the body. TAG in the adipose tissue are energy-depots, plus provides thermal and mechanical insulation. FA are essential components of the phospholipid molecules that comprise the lipid bilayer surrounding all cells and organelles. These membranes separate the cell from its environment and compartmentalize the cell interior into different structures that carry out specialized functions. Different lipids are precursors for hormones and other signaling molecules that serves as chemical intracellular and extracellular messengers in cells, tissues and organs. Also, lipids are components in the fat-soluble vitamin A, D, E and K (26).

Lipids are energy dense compounds, with an energy content of 9 kcal per 1 g of lipid. Dietary lipids are primarily present in the form of TAG, which accounts for about 95% of the dietary fat content, while the remaining mainly comprise cholesterol and phospholipids (27). Major sources of lipids in the diet include oils, butter, dairy products and meat and meat products. Furthermore, the main sources of saturated fat are dairy products, butter, meat products, sweet bakery products and confectionary, whereas vegetable oils, soft margarines and fish are the main sources of mono-and polyunsaturated fat (28). It is recommended that total fat should account for 25-40 E% in the diet, whereof saturated fat should be limited to 10 E% and the intake of PUFAs should make up 5-10 E%, where *n*-3 PUFA should comprise at least 1 E% (10, 28).

1.2.1 Fatty acid structure

A FA is a chain of hydrocarbons that terminate with a carboxylic acid group. Although the molecule as a whole is water-insoluble, the composition of the FA gives polar properties in the hydrophilic carboxylic acid end and nonpolar properties in the hydrophobic methyl end. The hydrocarbon chains vary in length and degree of saturation, with a normal range between four and 22 carbon atoms and up to six double bonds. The simplest form of FAs are unbranched chains of hydrocarbons bound by carbon-carbon single-bonds and terminate with a carboxylic acid group. These compounds are termed saturated fatty acids (SFA), indicating that the maximum number of hydrogen atoms are bound to each carbon in the chain. Unsaturated FAs have one or more carbon-carbon double-bonds in the hydrocarbon chain; monounsaturated fatty acids (MUFA) have one double-bond, while PUFAs have two or more double-bonds (17, 24, 25). *n*-3 PUFAs are a group of heterogenous PUFAs with the first double bond between carbon number three and four from the carboxylic acid end of the molecule. α-linoleic acid (ALA, 18:3 n-3) is the parent 18 carbon three double-bonds *n*-3 PUFAs, which are desaturated and elongated to longer-chain EPA, DPA and DHA. Their large number of double bonds makes *n*-3 PUFAs highly prone to oxidation. This is due to the carbon atoms situated at each side of the double-bonded carbons have low activation energy for loss of hydrogen and radical formation (25, 26).

1.3 Essential fatty acids

An essential fatty acid (EFA) is a PUFA that cannot be synthesized within the organism and must be provided through the diet. Humans can *de novo* synthesize SFAs and MUFAs from acetate, but not *n*-3 and *n*-6 PUFAs. This is due to the lack of enzymes called Δ^{12} and Δ^{15} desaturases, which are necessary for synthesis of FAs with double-bonds beyond the Δ^{9} site. Linoleic acid (LA, 18:2 n-6) and ALA are the only FAs that are known to be truly essential for complete nutrition in human beings. Within human tissues ALA and LA are converted to longer chain *n*-3 and *n*-6 PUFAs .

1.3.1 Dietary sources of omega-3 and omega-6 fatty acids

Plant cells have Δ^{12} and Δ^{15} desaturases, thus LA and ALA are acquired from plant sources in the diet. LA is widespread in near all foods containing plant fats, though especially in vegetable oils from soybean, safflower, corn, nuts, seeds and products of these. ALA is present in green plant tissue and important dietary sources include common vegetables oils such as soybean, canola and rapeseed and some nuts and flaxseed. Also, a growing number of foods are being fortified with *n*-3 PUFAs, like eggs, butter, margarine, bread, yoghurt and milk (8). Human subjects convert ALA to *n*-3 PUFAs at a low rate due to enzymes favorizing *n*-6 PUFAs and low enzymatic efficiency. Studies has shown that the endogenous conversion rate of ALA to EPA is between 0.2% to 8.0% and for ALA to DHA between 0% to 4.0% (30). Preformed *n*-3 LC PUFAs are much more accessible for the body, and tissue and circulating levels of EPA and DHA are thus predominantly determined by their direct dietary consumption. Preformed EPA and DHA are obtained through marine lipids found in seafood, especially fatty fish like salmon, herring, mackerel, sardines and smelt. Marine *n*-3 LC PUFAs are synthetized in the bottom of the food chain by marine algae and phytoplankton. Fish are not capable to *de novo* produce *n*-3 PUFAs, but obtain these through algae, phytoplankton and krill in the diet. Thus, fish, especially fatty fish, provide EPA, DHA and DPA in the human diet (27). In fish, and in the unrefined oils derived from them, *n*-3 LC PUFAs primarily exist in the form of TAGs and to a lesser extent as free FAs. Natural fish oils contain about 18% EPA and 12% DHA, but the amount and proportion can be modified to a higher proportion of EPA and DHA in concentrates of marine oils (30).

Various technological steps are involved in obtaining oils for use as dietary supplements and processing considerably affects composition and chemical bonds in the *n*-3 LC PUFAs. Concentrates of marine oils used in liquid and capsule supplements contain *n*-3 LC PUFAs in the form of free FAs, ethyl esters (EE) and re-esterified TAG. In the refining processes, TAGs can be trans-esterified with ethanol, producing FA-EE, which then again can be distilled to an EPA+DHA content up to 90% (31, 32). The processing of marine oils introduces steric changes in the TAG molecule. In dietary lipids, the *n*-3 PUFAs are usually esterified in the *sn*-2 position. *Sn*-2 denotes that the FA is bound to "seat" number 2 in the glycerol backbone. The two remaining seats in the *sn* 1-and 3-position are usually occupied by short- and medium-chained FAs. Upon the hydrolytic activities of pancreatic lipases, dietary lipids are mainly absorbed in the form of *sn*-2 monoacylglycerol (MAG). This means that the *n*-3 PUFA keeps its "seat" in the glycerol backbone, while the FAs in the *sn*-1-and -3 position are released from the molecule and substituted with endogenous FAs. Within the enterocyte, *sn*-2-MAGs are re-esterified to TAGs and incorporated into chylomicrons. By contrast, re-esterified TAG molecules often contain additional *n*-3 PUFAs in the 1-and 3-postitions, allowing a higher content of EPA and DHA in concentrates of marine oils (30). It has been hypothesized if these steric differences may affect the bioavailability of the *n*-3 LC PUFAs. This could

potentially be explained by EPA, DHA and DPA mainly being esterified in the *sn*-2 position of TAGs and glycerophospholipids. Pancreatic lipases favorizes cleaving FAs in the *sn*-1 and 3-position, thus the arrangement in the *sn*-2 position to a great extent preserves the *n*-3 LC PUFAs from hydrolysis during digestion and absorption of exogenous fat (34).

1.3.2. Ratio between intake of *n*-3 and *n*-6 polyunsaturated fatty acids

LA and ALA are metabolized in metabolic pathways that involves the same enzymes (e.g., desaturases, elongases and cyclo-oxygenases). These pathways are involved in hemostasis, vascular reactivity and inflammation and the fact that *n*-3 and *n*-6 PUFAs compete as substrate for the same enzymes has led to an opinion of an optimal balance between these FAs in the diet and in the blood. Historically, the ratio between these two PUFAs have been around 1:1. Following the introduction of cooking oils and margarines in the second half of the 20th-century, the dietary intake of LA has increased in the Western societies. Hence, the ratio between *n*-6 and *n*-3 is markedly shifted towards *n*-6 PUFAs (34, 35), normally ranging between 5 to 20 in most Western societies (37). The concern that imbalance between intake of *n*-3 and *n*-6 PUFAs might promote development of chronic diseases have led to several health authorities reviewing the issue. FAO concluded that there is no rational for a specific recommendation for *n*-6 to *n*-3 ratio, given that the intake of *n*-3 and *n*-6 PUFAs lies within the recommended levels (7).

1.3.3 Functions of essential fatty acids

PUFAs and their metabolites are bioactive nutrients that serves a plethora of biological functions, inter alia as source of energy, constituent of membranes, regulators of gene transcription and as precursors for potent lipid mediators (38). *n*-3 PUFAs are selectively incorporated into membrane lipids in various cell types, especially those endowed with specialized functions like cardiac, nerve, muscle and immune cells (38). DHA is the most prominent *n*-3 PUFA in membranes, whereas the concentration of membrane bound EPA is much lower. Due to their flexible chemical structure, *n*-3 PUFAs are important for membrane fluidity. Membrane fluidity is essential for optimal function in the proteins embedded in the lipid bilayer, such as ion channels, receptors, enzymes and transporters. Thus, any changes in the fatty acid composition of the membrane might affect the activity of the proteins, consequently leading to altered cellular metabolism and signal transduction (2).

Membrane *n*-3 and *n*-6 PUFAs are involved in cell signaling pathways upon activation and mediate their functions through enzymatically oxygenated metabolites named eicosanoids (38). The term eicosanoids cover the bioactive compounds prostaglandins (PG), prostacyclins (PGI), thromboxanes (TXA), leukotrienes (LT) and hydroxyeicosatetraenoic acid (HETE). These compounds are involved in several physiological systems and pathologic processes, and exerts their actions through autocrine, paracrine and endocrine signaling pathways. The eicosanoids are not stored within the cells but are rapidly synthesized

in response to hormonal stimuli, thence rapidly degraded (25, 27). Synthesis starts with liberation of AA, EPA and DHA from the membrane by phospholipases and further metabolization in synthesis pathways that involves lipoxygenases, cyclooxygenases and cytochrome P450. *n*-3 and *n*-6 PUFAs give rise to different series of eicosanoids, and *n*-6 PUFA derived series of eicosanoids generally exerts more proinflammatory effects, whereas the *n*-3 PUFA derived series of eicosanoids exerts less proinflammatory effects (21). Depending on cell types, eicosanoids execute various biological functions in pathways involved in hemostasis, vascular reactivity and inflammation (39, 40).

1.4 Oxidative stress

Oxidative stress is defined as a condition with an imbalance between production of reactive oxygen species (ROS) and free radicals and the ability of a biological system to neutralize these compounds. Overproduction of ROS and free radicals is the hallmark of oxidative stress. The high reactivity of ROS and free radicals is explained by a free unpaired electron in the outer orbital. This unpaired electron readily reacts with other molecules and may induce chain reactions that damage biomolecules. Harmful effects on biomolecules like nucleic acids, proteins and structural carbohydrates and lipids becomes apparent as the levels of ROS and free radicals increases. Superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH) and singlet oxygen ($^1O_2$) are ROS which are produced as metabolic by-products in biological systems. ROS are products of normal oxidative cellular metabolism, with the mitochondria, endoplasmic reticulum and the peroxisomes being the major producers. Exogenous stimuli like ionizing radiation, ultraviolet radiation, tobacco, environmental toxins and pathogen infections contribute to *in vivo* ROS production .

At low concentration rates, ROS takes part in cellular processes like proliferation, hormone biosynthesis, chemotaxis, aggregation and apoptosis (38). The body possess several protective systems that hinder oxidative damage by elimination or neutralization of ROS. However, when the defense systems are unable to sufficiently eliminate pro-oxidant species, oxidative stress occurs and causes damage to cells, tissues and organs. Increased oxidation of biomolecules is associated with the development of various pathologies, e.g., cardiovascular disease (CVD), diabetes, obesity, cancer and Alzheimer's disease . At present, no single marker of *in vivo* oxidative stress exists, and different methods are utilized to assess this condition. However, it seems like oxidation products of PUFAs might be the most reliable marker to assess oxidative stress (43, 44).

1.4.1 Lipid peroxidation

Briefly, lipid peroxidation can be described as the oxidative degradation of lipids. In this process free radicals or non-radical species attack lipids with carbon-carbon double-bonds, resulting in the abstraction of a hydrogen atom from a carbon and the insertion of an oxygen atom. The primary oxidations products

of these reactions are unstable lipid peroxyls and lipid hydroperoxides. Subsequently, these unstable products are converted to more stable secondary oxidation products such as aldehydes. PUFAs are prone to lipid peroxidation due to their high number of carbon-carbon double-bonds and methylene groups with hydrogen atoms that are especially reactive with ROS .

Like any other radical reaction, lipid peroxidation consists of three major steps: initiation, propagation and termination. Initiation is the formation of a lipid radical (L˙) catalyzed by an initiator, most notably ROS but could also be triggered by energy or transition metals. Lipid radicals are not stable compounds and readily reacts with molecular oxygen in the propagation step, resulting in lipid peroxyl radicals (LOO˙). These radicals are unstable and quickly abstracts a hydrogen atom from an adjacent FA and generates a new lipid radical (L˙) and a lipid hydroperoxide (LOOH). Thus, the peroxidation of a lipid creates a self-propagating cycle where each new lipid radical reacts in the same manner, creating a chain reaction with increasing concentrations of radical species. The radical reaction is terminated when two radical species react and creates a non-radical species. Termination occurs when the concentration of radical species is high enough for it to be highly likely of two radicals colliding. However, in living organisms there are several protective mechanisms that speed up termination by neutralizing free radicals. These mechanisms involve several molecules that bind and quench free radicals to protect lipids from oxidation (45, 46). To exemplify, the antioxidant vitamin E will donate a hydrogen atom to a lipid peroxyl, thence neutralizing the radical. The resulting vitamin E radical can then react with a new lipid peroxyl and form a non-radical compound (42).

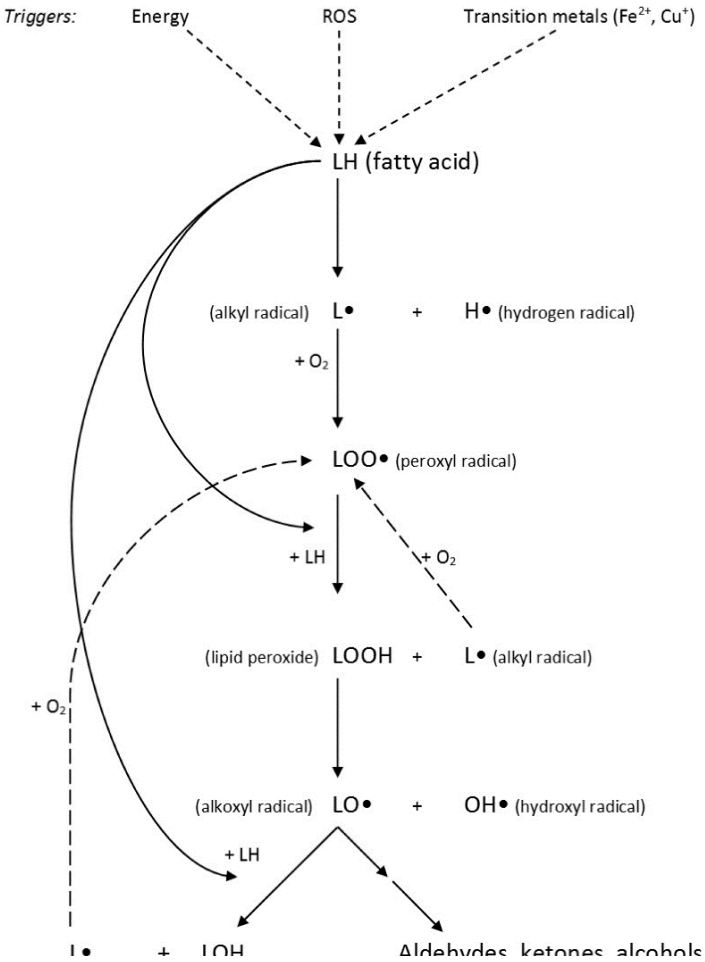


Figure 1. Illustration of lipid peroxidation process. Derived from Maehre et al.

1.4.2 Peroxidation of *n*-3 and *n*-6 polyunsaturated fatty acids

PUFAs probably pose the highest sensitivity to oxidative damage among cellular macromolecules, and it seems like peroxidation predominantly occurs in membrane phospholipids. Lipid peroxidation has two major outcomes: damage of membranes and the generation of secondary products. Peroxidation of

membrane lipid bilayer can greatly alter its physiochemical properties, leading to severe cellular dysfunction with changes in permeability, fluidity and ion transport activity and inhibition of metabolic processes (47). Moreover, several different compounds are produced as biproducts of peroxidation in a process where lipid hydroperoxides breaks down to various aldehydes. Some of these aldehydes are highly reactive and might exert both adverse and beneficial biological effects (29). Ultimately, the major end-products of *n*-3 and *n*-6 PUFA peroxidation are highly reactive aldehydes 4-hydroxyhexenal (4-HHE) and 4-hydroxynoneal (4-HNE), respectively . By contrast to the volatile free radicals, these aldehydes are more stable and can diffuse intracellularly and extracellularly, hence capable of attacking targets far from their place of origin. 4-HHE and 4-HNE are highly reactive with different chemical groups and can induce structural changes in biomolecules (38). These effects are suggested to be involved in pathological conditions, inter alia through modification of cell signaling, damaging of proteins and DNA, enzymatic deactivation, cytotoxicity and induction of apoptosis .

Free radical peroxidation of *n*-3 and *n*-6 PUFAs can also generate a series of prostaglandin-like compounds termed isoprostanes (isoP) and neuroprostanes (49). These compounds are produced *in situ* while the PUFA still is esterified to the phospholipid layer, and subsequently released into the circulation by the actions of phospholipases. *n*-6 AA generates a series of F_2-isoPs, while EPA and DHA produces various F_3-isoPs and F_4-neuroprostanes. These compounds are regarded as nonclassical eicosanoids, and their biological activities might exert adverse and beneficial effects within the body. Generally, evidence supports that *n*-3 PUFA derived isoPs and neuroprostanes exhibits beneficial actions in biological systems (49). On the other hand, *n*-6 PUFA derived isoPs are associated with adverse effects and elevated urine and plasma levels have been observed in the pathophysiology of several diseases associated with oxidative stress, including diabetes, atherosclerosis and neurodegenerative conditions (50-52). Thoroughly investigated the past decades, isoPs have been established as reliable markers of *in vivo* oxidative stress (52, 53). Especially 8-iso-prostaglandin-$F_{2\alpha}$ (8-isoPGF) has been under scientific scrutiny (43). 8-isoPGF are generated from peroxidation of AA and elevated urine levels have been observed in inflammation and oxidative stress-related diseases, including atherosclerosis, hypercholesterolemia, diabetes and smoking .

1.4.3 Oxidized fish oil and health outcomes

Fish oil and *n*-3 LC PUFAs have shown beneficial effects on cardiovascular health in epidemiological and clinical studies and in animal studies. Paradoxically, their high content of double bonds makes them prone to lipid peroxidation, which then again is associated with numerous diseases. Whether consumption of oxidized *n*-3 LC PUFA oils can contribute to unfavorable effects on cardiovascular health compared with less oxidized oils is under scrutiny. Relatively few studies have been conducted on the topic and

evidence on the implications oxidized *n*-3 LC PUFA oils on cardiovascular health is inconsistent (3, 34, 55).

Atherosclerosis, the narrowing of arteries due to build-up of atherosclerotic plaques, is one of the major driving forces behind the development of CVDs. Over time, the narrowing of the arteries can affect the blood flow, consequently giving a mismatch between the oxygen demand in the supplying tissues and the arteries ability to deliver oxygen. Atherosclerosis is a multifactorial and progressive inflammatory condition, where modification of low-density lipoproteins (LDL) in the vascular endothelium seems to be essential in its development. The formation of an atherosclerotic plaque starts with circulating lipids and immune cells breaking through the artery wall. Activation of the endothelia induces signaling cascades in endothelial and blood cell, resulting in recruitment of monocytes and other immune cells to the endothelial surface. Once inside the intima layer of the endothelial wall, monocytes differentiate to macrophages. Macrophages are phagocytic cells with scavenger-receptors for modified LDL on its surface. Normal mechanisms for handling and transport of LDL are impaired in these activated macrophages and accumulation of cholesterol within the cell eventually leads to foam cell formation. Stimulation of proliferation and migration in smooth muscle cells leads to encapsulation of the excessive lipids within the intima, resulting in the formation of an atherosclerotic lesion (34, 56, 57). The high reactivity of 4-HNE and 4-HHE with other chemical groups might be of relevance in atherosclerosis. 4-HHE and 4-HNE can contribute to oxidative modification of LDL by forming covalent bonds with protein constituents in the molecule (34). Protein-bound 4-HHE in modified LDL has been identified in atherosclerotic lesions (58). Also, ingested lipid peroxides are transported in LDL, VLDL and chylomicrons (59). Lipid peroxides accelerate oxidation of other FAs; thus, their way of transport may be of importance in modification of LDL. Following this thought, one can speculate if consumption of oxidized marine oils might exert atherogenic effects in humans (3).

1.5 Oxidized fish oil supplements

As thoroughly described, *n*-3 PUFAs are chemically unstable compounds. Marine oils used as dietary supplements readily oxidize during processing and storage, rendering a complex soup of lipid peroxides and secondary oxidation products, and a diminishing amount of unoxidized PUFAs (3). The degree of total primary and secondary oxidation products in refined *n*-3 oils is measured through the peroxide value (PV) and the anisidine value (AV). Measurements of PV is considered as the gold standard for assessment of the oxidative status of an oil. The PV measures primary oxidation, that is the level of lipid hydroperoxides as the PUFA oxidizes. Initially, the PV will increase as the PUFAs are exposed to oxidative species and the level of lipid hydroperoxides increases. Eventually, the PV value decreases as the hydroperoxides convert in further oxidative reactions. Thus, the PV might not reflect the rancidity of

the oil. The AV is more unspecific and gives a measure of secondary oxidation products, primarily aldehydes from degradation of peroxides (60). As of today, there is no consensus for an official limit of lipid peroxidation in *n*-3 oils used in dietary supplements and acceptable levels are defined by different monographs. The European Pharmacopeia states that PV and AV values should not exceed 10 and 20 mEq/kg, respectively (61). That said, these industry indices are primarily based on palatability, whereas there is insufficient data to set standards based on health effects (6).

In 2012, NOFIMA investigated the oxidative status in 56 *n*-3 LC PUFA products available on the Norwegian market. A total of 28 out of 56 products had PV exceeding the levels stated in several European Pharmacopeia monographs. Compared with the monographs given by the Global Organization for EPA and DHA (GOED), 52 out of 56 products had PV exceeding the limit value (60). Similarly, reviews of studies investigating the oxidative status in over-the-counter *n*-3 LC PUFA supplements shows that oxidation levels exceeding the recommended levels are highly common, affecting between 11 % to 62 % of investigated products (3).

1.6 Aim of study

Whether regular consumption of oxidized *n*-3 oils might be associated with unfavorable health outcomes remains unclear. Hence, the present study was conducted with aim to investigate the short-term effect of various *n*-3 products on markers of lipid peroxidation and oxidative stress in healthy subjects after three and 21 days of intervention. The hypothesis was that the most oxidized *n*-3 products will lead to a larger increase in markers of lipid peroxidation and oxidative stress compared with control and non-oxidized products.

2 Methods

The "Health effect of oxidized fish oil"- study was a randomized controlled trial conducted in Bergen from January to May 2013. The study was organized and led by the Institute of Marine Research[1] (IMR) in cooperation with the Center for Clinical Trials, Bergen, Norway. The overall aim of the study was to investigate how different *n*-3 products effected markers of lipid peroxidation and oxidative stress after three and 21 days of intervention.

2.1 Ethics

The study protocol was approved by the Regional Committee for Medical Research Ethics (2011/1264/REK Vest) in Norway and was conducted in accordance with the guidelines stated in the Declaration of Helsinki and Good Clinical Practice (GCP) routines at the Center for Clinical Trials, Bergen. Written informed consent was obtained from all participants before start of intervention and participants were free to withdraw at any time without giving a reason for dismissal.

2.2 Study population

2.2.1 Recruitment

Healthy men and women between the age of 18-65 years were invited to participate and were recruited through newspaper advertisements and information elsewhere (e.g., individuals listed as interested based on previously participations in an intervention study at the Institute of Marine Research). A total of 150 men and women were assessed for eligibility, of which 59 were excluded due to not meeting the inclusion criteria (*n*=17), declining to participate (*n*=38) and other reasons (*n*=4). The 91 eligible participants were randomly allocated into one of six intervention groups: Fish, Juice, Control, non-oxidized fish oil (Non-OX), fish oil with low oxidation grade (LowOX) and fish oil with high oxidation grade (HighOX). Additional 36 were excluded due to no-show at the baseline visit (*n*=15) and withdrawal (*n*=21). A total of 55 participants started the intervention and additional seven withdrew during follow-up (Figure 2).

2.2.2 Inclusion and exclusion criteria

Interested participants were included if they had a body mass index (BMI) of 18.5-34.9 kg/m^2 and could confirm one or more of the following questions: 1. Eat less than 5 portions of fruit or vegetables daily, 2. Eat one or less portion of fish weekly, 3. Less than 30 minutes of physical activity daily, 4. Smoker and 5. BMI over 24.9 kg/m^2 and below 35 kg/m^2. The exclusion criteria were as follows: pregnant or lactating, allergy or intolerance for study products, known HIV or hepatitis, use of medication that impact the

[1] In 2013 the Institute of Marine Research went under the name National Institute for Nutrition and Seafood Research (NIFES)

biomarkers of interest, bleeder or use of blood thinners, planed weight reduction and consuming fatty fish for dinner or as spread more than 2 times a week or equivalent amount as *n*-3 PUFA supplements.

2.2.3 Blinding and randomization

Subjects in the four capsule groups were blinded by identical appearance of capsules and capsule containers. The study investigators who conducted the study and data collection were not blinded. However, the randomization code was kept concealed for the master student performing the statistics until all statistical analyses were completed. Randomization was performed by one of the researchers using Microsoft Excel and its random generator. The randomization was run before participants were recruited by assuming 120 participants and later re-run since the number of participants was lower.

2.3 Intervention

2.3.1 Study groups

The Fish-group received frozen portion packed farmed salmon fillets with an EPA+DHA content of ~3.7 g per portion. The Juice-group received a juice which contained emulsified salmon oil with an EPA+DHA content of 2.0 g per container of 200 ml. The weight of each capsule was ~0.5 g and the amount of EPA +DHA was ~0.09 g per capsule with the exception of the control group which received capsules containing sunflower oil. Non-OX group received capsules with good quality salmon oil. LowOX group received a capsule with salmon oil with a PV and AV of 3 and 27, respectively. HighOX group got a capsule with salmon oil with PV 15 and AV 14. All participants with the exception of the control group received approximately 1.6 g of EPA+DHA per day.

The study included four visits at Center for Clinical Trials, Bergen. At the first visit before inclusion in the study, the volunteers interested to participate in the study went through a medical examination by a general practitioner and answered a food frequency questionnaire (FFQ). Participants included in the study then went through a two-week run-in period where they were requested to not consume any fish or *n*-3 PUFA supplements. At the baseline visit, participants were randomly allocated to one of six intervention groups; this is day one of the intervention. Remaining visits were after three and 21 days. Fasting blood samples and morning urine were taken at each visit during the intervention period.

2.3.2 Procedure

The Fish-group were asked to consume salmon fillets of ~ 170 g three times a week (a total of ~500 g salmon per week). The first week of intervention participants were instructed to eat salmon for three consecutive days. For the remaining intervention period participants ate three portions of salmon a week at optional days. For the Juice-group, participants were instructed to drink two juices a day for three consecutive days and none the remaining days of the first intervention week. From week two to end of

study participants drank one juice a day at six optional days a week. The four capsule groups received 18 capsules daily which were to be distributed in even amounts 3-4 times per day. During the first week for intervention, participants were instructed to consume 44 capsules per day for three consecutive days and then none the remaining four days. Starting from intervention week two to end of study participants consumed capsules daily. All participants were asked to continue to eat their habitual diet, however they were not allowed to consume any fish, seafood or *n*-3 LC PUFA supplements other than the designated study products during the intervention period.

Figure 2. Illustration of *n*-3 products.
Photo: Institute of Marine Research

2.3.3 Oxidation of study products

The salmon oil used for the capsules was produced by Fortuna Oils AS and purchased from GC Rieber Oils AS, Norway. The salmon oil batch was divided in into three parts by collaborating researchers at NOFIMA prior to oxidation. The Non-OX oil was flushed with nitrogen (N_2) and stored dark at 4ºC pending the encapsulation. The LowOX oil and HighOX oil were oxidized by sparkling pure oxygen through the oil for 30 minutes twice a day at room temperature until the used AV and PV in the present study, then flushed with N_2 and stored dark at 4°C. Vitamin E (tocopherols) were added to obtain similar concentration in all oils. High oleic sunflower oil (control) and the fish oils were encapsulated in 0.5 g softgel capsules made of bovine gelatin at Pharmatec AS, Oslo, Norway. All capsules were stored in closed dark containers at 4°C until the start of the study.

2.3.4 Compliance and side-effects

All participants registered intake of designated product in a diary during the intervention period. At end of study, participants were requested to return the schema, which then was used to assess compliance. Side-effects and palatability were evaluated by Visual Analogous Scale (VAS) at the last study visit. VAS is a method of quantifying subjective experience of a symptom, where subjects are asked to evaluate their perception of the symptom on a continuous 10 cm line. To exemplify, if one are to evaluate stomach pain, 0 is no pain at all and 10 is worst thinkable pain (62). Subjects were asked to evaluate experience of flatulence, stomach pain, diarrhea, nausea and belching. Also, subjects were asked to report their experience of taste, smell and appearance of study products.

2.4 Data collection

2.4.1 Sampling and analysis of blood and urine samples

Blood and urine samples were taken at baseline, after three days and 21 days of intervention. Blood samples was performed by authorized personnel at Center for Clinical Trials, Bergen. Participants were requested to avoid consumption of alcohol and doing hard physical training the day before blood sampling. Venous blood from the participants elbow cavity were drawn after an overnight fast (≤ 12 h). For preparation of plasma and erythrocytes, blood was collected in EDTA vacutainers vials and centrifugated 10 minutes at 1500g at room temperature within 30 minutes. Blood samples for serum preparation were collected in SST Vacutainers and set to coagulate for a minimum of 30 minutes before centrifuging at 1500g in 10 minutes at room temperature within 60 minutes after extraction.

The various markers measured in blood and urine during intervention are outlined in Table 1. The primary outcome variables in the study were urinary 8-isoPGF and serum 4-HHE and 4-HNE. 8-isoPGF and creatinine were measured by collaborating researchers at the Department of Public health and Science, Uppsala University, Sweden. Serum 4-HHE and 4-HNE were measured by collaborating researchers at NOFIMA, Ås, Norway. Secondary outcome variables were serum vitamin E, TAGs, total cholesterol, HDL-cholesterol, LDL-cholesterol and FAs in erythrocyte membrane. These serum analyses were performed at Fürst Medical Laboratory, Bergen Norway using standard analytical laboratory methods. A total of 38 FAs were measured through analysis of erythrocyte membrane fatty acid composition at IMR. Of these the *n*-3 fatty acids EPA, DPA, DHA and total *n*-3 PUFAs and the *n*-6 fatty acids LA, AA and total *n*-6 PUFAs were selected for evaluation of effect of intervention. *n*-3 PUFA ALA was also of interest in this regard but were below the limit of quantification (LOQ).

Table 1. Overview of study visits and biomarkers measured in blood- and urine samples at baseline and after three and 21 days.

Visit	**Assessments**
Visit 1	Medical check Food frequency questionnaire
Visit 2 (day 0)	Urine sample - 8-isoPGF - Creatinine Blood sample - 4-HHE - 4-HNE - Vitamin E - 25-OH D - TAG - Total cholesterol - HDL-cholesterol - LDL-cholesterol - Erythrocyte fatty acid composition - Hb - Micro-CRP - ASAT - ALAT - TSH - Free T_4
Visit 3 (day 3)	Urine sample - 8-isoPGF - Creatinine Blood sample - Vitamin E
Visit 4 (day 21)	Urine sample - 8-isoPGF - Creatinine Blood sample - 4-HHE - 4-HNE - Vitamin E - TAG - Total cholesterol - HDL-cholesterol - LDL-cholesterol - Erythrocyte fatty acid composition

Abbreviations: 4-HHE, 4-hydroxy hexenal; 4-HNE, 4-hydroxynoneal, 8-isoPGF, 8-isoprostaglandin$_{2\alpha;}$25-OH D, 25-hydrooxy vitamin D; ALAT, alanine amino transferase; ASAT, aspartate amino transferase; CRP, micro C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TAG, tricacylglycerol; TSH, thyroid stimulating hormone; T_4, thyroxine

2.4.2 Anthropometric measurements

The height of the subjects was measured without shoes on a standing height scale. Height was measured in cm to the nearest whole number. Weight was measured on a scale weight in kg and rounded to the nearest whole number. The same standing height scale and weight scale was used for all subjects. BMI was calculated by dividing the weight in kg by the square of the height in m. Hip circumference was measured around the largest circumference around the buttocks and waist circumference was measured halfway between the lower rib and the iliac crest, both measures to the nearest 0.1 cm.

2.4.3 Food frequency questionnaire

All participants answered a FFQ about their habitual intake of seafood, various food groups and supplements, plus sunbathing habits, physical activity level and socioeconomic status. The semi-quantitative FFQ included 15 questions and was especially developed to capture the participants' seafood intake, here defined as fish, fish products and other seafood products such as shell and shellfish. The questionnaire was developed by Dahl et al. and questions on seafood intake and *n*-3 LC PUFA supplements have been validated against relevant biomarkers (63). Participants were asked to report their average intake with the last three months in mind. Seafood questions were divided into intake as dinner and as sandwich spread, and the two main sections were again divided into subsections which captures consumption of various seafood species and products. Frequency of consumption for seafood was recorded as follows: Never, less than once a month, one to three times a month, once a week, two to three times a week, four or more times a week. Other food groups included in the FFQ were fruit and vegetables, eggs, dairy products, bread, fats in cooking and dietary supplements. With regards to vitamin D status participants were asked to report use of solarium, vacation habits and outdoor activity in the summertime. Participants were asked to evaluate their emphasis of having a healthy diet and assess physical activity level by answering questions about how often the participants engaged different activities for more than 30 minutes. Socioeconomic status was mapped by questions about education level and personal economy.

The dietary data retrieved from the FFQs was compared against relevant dietary recommendations from the Norwegian Health Department. Generally, it is recommended to have a diet with a high content of vegetables, fruit, fish and wholegrain and limited processed meat, red meat, salt and sugar. As described earlier, the recommendation on fish intake is to consume fish for dinner two or three times a week, which corresponds to 300-450 g seafood per week. Fish as sandwich spread can also be part of the intake, whereof six portions as sandwich spread corresponds to one dinner portion. The recommendation on fruit and vegetables states that one should eat at least five portions of fruit, vegetables and berries a day. One should engage in at least 150 minutes of moderate physical activity or 75 minutes of strenuous physical activity a week (10).

2.5 Statistics

All raw data was first processed in Microsoft Excel Version 2016. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for windows (IBM SPSS Statistics 25, Chicago, IL, USA, (www.spss.com)). GraphPad Prism 8 was used for box plots. The level of significance was set to *p*-value <0.05.

Baseline variables were confirmed normally distributed with a Shapiro-Wilk test. Continuous variables are presented as mean values and standard deviations and categorical variables as numbers and percentages. A Chi-square test was used for analysis of categorical variables and an one-way ANOVA was used for continuous variables. In statistical handling of the dietary data retrieved from the FFQ, frequency of consumption categories was modified. The FFQ included more answer alternatives than those presented in Table 3. To enable statistical analysis, plus a more orderly presentation of data, answer alternatives were merged into fewer categories. As an example, the FFQ included the following question about dairy intake: "How many portions of dairy products do you eat a day?" Five alternatives were possible for this question: 1. One portion a day, 2. 2-3 portions a day, 3. 4-6 portions a day, 4. 7-9 portions a day and 5. 10 portions a day. No participants reported higher consumption than 2-3 times a day, thus the frequency of consumption was merged into two categories: 1. One portion a day and 2. Two or more portions a day. This was done for all questions that had answer alternatives with a response rate of null. Data retrieved from the FFQ were analyzed by a Chi-square test.

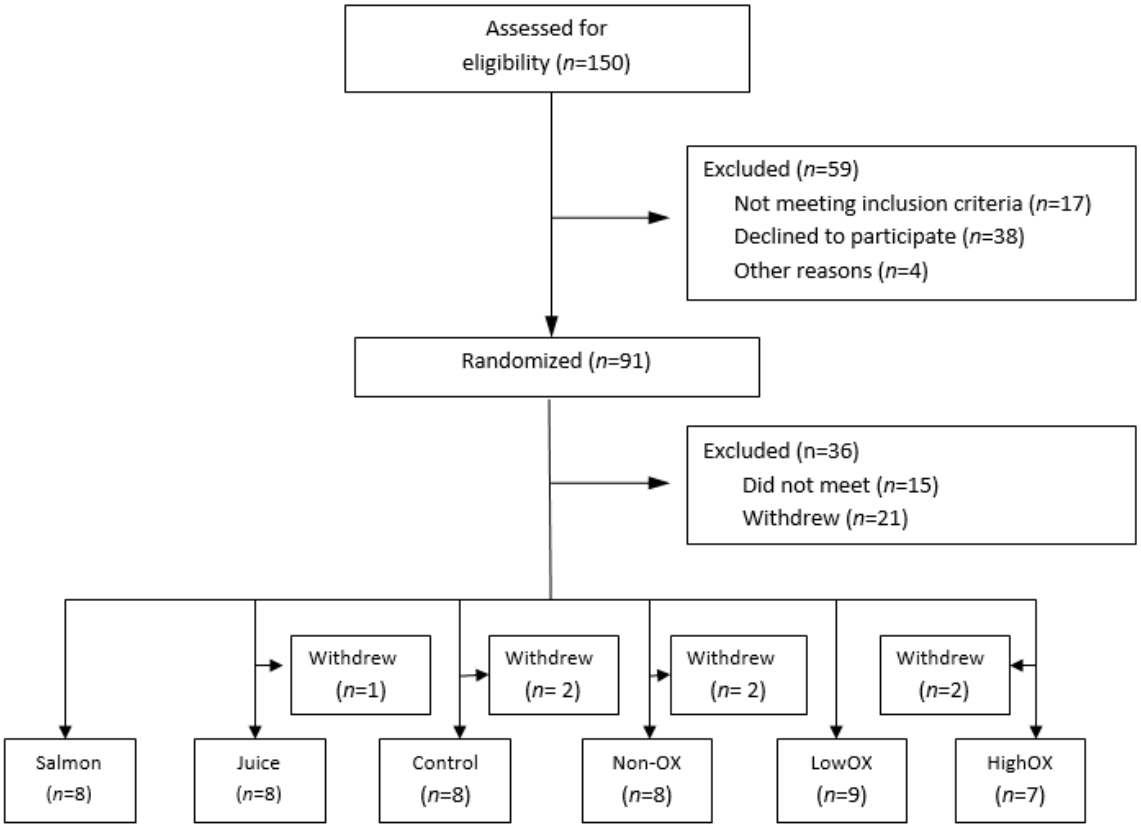
Primary and secondary outcomes are presented as mean values and standard deviations. Delta-variables (V_4-V_2) were computed for all outcome variables. A General linear model analysis with adjustment for the pre-variable were performed for the delta results in comparison between the intervention groups. If the overall *p*-value was significant, a pairwise group comparison with Bonferroni correction was performed. For analysis of changes within groups from baseline to end of study a paired-samples t-test was utilized. In addition to the paired-samples t-test, eta squared effects sizes were calculated. For the two variables that were measured at the three days follow-up, that is Vitamin E and 8-iso-$PGF_2\alpha$, changes were tested from baseline to three days follow-up and from baseline to end of study. Erythrocyte FAs were measured both in relative amounts, that is the percentage content of the FA out of the total contents of FAs, and in absolute amounts, that is the weight of the FA per total weight of the sample. Absolute amounts were chosen in statistical handling.

3 Results

3.1 Study population

A total of 48 subjects completed the intervention: eight in the Fish group, eight in the Juice group, eight in the Control group, eight in the Non-OX group, nine in the LowOX group and seven in the HighOX group. Details on the number of subjects included in statistical analysis are outlined in Figure 3.

Figure 3. Flow chart of the recruitment process.



3.1.1 Characteristics of the study population

The study population comprised 19 men and 29 women with a mean (SD) age of 49 ± 15 years. The subjects had a mean BMI of 25.1 ± 3.7 kg/m^2 and mean hip and waist circumference of 104.1 ± 8.6 cm and 93.4 ± 15.0 cm, respectively. Mean serum levels of ALAT, ASAT, creatinine, micro-CRP, (25-OH) D, TSH, T_4 and total cholesterol were within the laboratory's reference range for each specific analyte at baseline. At baseline, no significant differences were detected between the intervention groups with regards to sex, age, BMI, hip and waist circumference, biochemical markers and education level (Table 2).

Table 2. Baseline characteristics for all participants and intervention groups (*n*=48). Data are presented as numbers (%) or mean values and standard deviations.

Variables		***n***	**All (*n*=48)**	**Fish (*n=8)***	**Juice (*n=8)***	**Control *(n=8)***	**Non-OX (*n=8)***	**LowOX (*n=9)***	**HighOX (*n=7)***	***p-value*[1]**
Sex		48								
	Male		19 (39.6)	2 (25.0)	3 (37,5)	4 (50.0)	6 (75.0)	2 (22.2)	2 (28.6)	0.238
	Female		29 (60.4)	6 (75.0)	5 (62.5)	4 (50.0)	2 (25.0)	7 (77.8)	5 (71.4)	
	Age, yrs	48	49.3 ± 14.9	48.1 ± 15.0	49.6 ± 18.7	52.5 ± 16.5	42.3 ± 15.0	50.7 ± 16.4	53.3 ± 6.6	0.755
Anthropometrics										
	BMI (kg/m^2)	48	25.1 ± 3.7	24.6 ± 3.8	24.8 ± 3.3	26.3 ± 3.6	27.7 ± 4.3	24.0 ± 4.2	25.7 ± 2.2	0.383
	Hip circumference, cm	48	104.1 ± 8.6	104.9 ± 8.2	101.1 ± 9.1	107.4 ± 10.7	105.5±10.5	101.4 ± 7.7	104. 4 ± 4.8	0.683
	Waist circumference, cm	48	93.4 ± 15.0	90. 9± 13.2	87.8 ± 15.0	95.9 ± 16.5	100.3±21.3	88.2 ± 12.2	91.6 ± 9.7	0.545
Biochemistry										
	ALAT[2] U/L	44	28 ± 19	22 ± 6	34 ± 40	27 ± 10	35 ± 19	25 ± 12	23 ± 6	0.686
	ASAT[2] U/L	44	22 ± 24	20 ± 5	40 ± 58	20 ± 8	19 ± 5	20 ± 4	17 ± 5	0.505
	Micro-CRP mg/L	43	1.66 ± 1.86	0.79 ± 0.66	0.96 ± 0.73	1.02 ± 1.23	2.86 ± 3.31	2.10 ± 1.56	1.99 ± 0.91	0.130
	25-OH D[2] nmol/L	48	62 ± 24	59 ± 35	70 ± 20	61 ± 30	63 ± 21	64 ± 22	57 ± 14	0.932
	Creatinine mmol/L	48	9.57± 4.37	9.70 ± 5.11	10.3 ± 6.09	9.43 ± 3.51	12.5 ± 1.32	8.27 ± 4.09	7.12 ± 3.97	0.238
	TSH mU/L	48	2.10 ± 1.09	1.95 ± 0.71	1.62 ± 0.60	2.60 ± 1.74	2.53 ± 1.15	2.01 ± 1.14	1.70 ± 0.55	0.386
	Free T_4 pmol/L	48	16.8 ± 2.73	17.5 ± 3.92	16.4 ± 1.49	16.5 ± 3.06	16.4 ± 2.19	16.5 ± 1.56	17.2 ± 4.03	0.233

Education level		45							
	≤13 yrs		13 (28.9)	2 (28.6)	2 (25.0)	2 (25.0)	2 (25.0)	3 (37.5)	2 (33.3)	0.999
	13-16 yrs		15 (33.3)	3 (42.9)	3 (37.5)	3 (37.5)	2 (25.0)	2 (25.0)	2 (33.3)	
	>16 yrs		17 (37.8)	2 (28.6)	3 (37.5)	3 (37.5)	4 (50.0)	3 (37.5)	2 (33.3)	

Abbreviations: 25-OH D, 25 hydroxy vitamin D; ALAT, alanine amino transferase; ASAT, aspartate amino transferase; micro-CRP, micro C-reactive protein; TSH, thyroid stimulating hormone; free T_4, thyroxin

[1] Statistical analysis for differences between intervention group was performed by chi-square test for categorical variables and by one-way ANOVA for continuous variables.

[2] Analytes measured as whole numbers.

Table 3. Frequency of consumption of various foods for all participants and by intervention group (*n*=48). Data are presented as numbers (%).

Food	**Frequency of consumption**	***n***	**All (*n*=47)[2]**	**Fish (*n=8)***	**Juice (*n=8)***	**Control (*n=8)***	**Non-OX (n=*8)***	**LowOX (n=*8)***	**HighOX (*n=7)***	***p-value[1]***
Fish (dinner)		47								
	≤ 3 times a month		13 (27.7)	2 (25)	2 (25)	4 (50.0)	7 (87.5)	4 (50.0)	1 (14.3)	0.842
	Once a week		16 (34.0)	3 (37.5)	3 (37.5)	2 (25.0)	1 (12.5)	4 (50.0)	3 (42.9)	
	≥2 times a week		18 (38.3)	3 (37.5)	3 (37.5)	2 (25.0)	0 (0)	0 (0)	3 (42.9)	
Fatty fish		47								
	≤ 3 times a month		33 (70.2)	5 (62.5)	5 (62.5)	6 (75.0)	7 (87.5)	4 (50.0)	6 (85.7)	0.704
	≥ once a week		14 (29.8)	3 (37.5)	3 (37.5)	2 (25.0)	1 (12.5)	4 (50.0)	1 (14.3)	
Lean fish		47								
	≤ 3 times a month		35 (74.5)	7 (87.5)	7 (87.5)	7 (87.5)	2 (25.0)	3 (37.5)	4 (57.1)	0.741
	≥ once a week		12 (25.5)	1 (12.5)	1 (12.5)	1 (12.5)	6 (75.0)	5 (62.5)	3 (42.9)	
Fruit		47								
	≤ 3 times a week		15 (31.9)	2 (25.0)	5 (62.5)	2 (25.0)	2 (25.0)	1 (12.5)	3 (42.9)	0.648
	≤ once a day		20 (42.6)	4 (50.0)	2 (25.0)	3 (37.5)	5 (62.5)	4 (50.0)	2 (28.6)	
	≥ twice a day		12 (25.5)	2 (25.0)	1 (12.5)	3 (37.5)	1 (12.5)	3 (37.5)	2 (28.6)	
Vegetables		47								
	≤ 3 times a week		8 (17.0)	3 (37.5)	4 (50.0)	0 (0)	1 (12.5)	0 (09	0 (0)	0.042*
	≤ once a day		32 (68.1)	4 (50.0)	4 (50.0)	5 (62.5)	7 (87.5)	7 (87.5)	5 (71.4)	
	≥ twice a day		7 (14.9)	1 (12.5)	0 (0)	3 (37.5)	0 (0)	1 (12.5)	2 (28.6)	
Dairy		40[3]								
	1 portion a day		18 (45.0)	2 (28.6)	4 (66.7)	4 (57.1)	3 (50.0)	3 (37.5)	2 (33.3)	0.717
	≥ 2 portions a day		22 (55.0)	5 (71.4)	2 (33.3)	3 (42.9)	3 (50.0)	5 (62.5)	4 (66.7)	

Egg		46[4]								
	≤ 1 egg a week		18 (17.4)	2 (28.6)	0 (0)	0 (0)	1 (12.5)	2 (25.0)	3 (42.9)	0.366
	≤ 3 eggs a week		23 (50.0)	4 (57.1)	5 (62.5)	6 (75.0)	4 (50.0)	3 (37.5)	1 (14.3)	
	≥4 eggs a week		15 (32.6)	1 (14.3)	3 (37.5)	2 (25.0)	3 (37.5)	3 (37.5)	3 (42.9)	
Liquid *n*-3 supplement		47								
	Yes		10 (20.8)	3 (37.5)	0 (0)	2 (25.0)	1 (12.5)	3 (37.5)	1 (14.3)	0.379
	No		37 (77.1)	5 (62.5)	8 (100.0)	6 (75.0)	7 (87.5)	5 (62.5)	6 (85.7)	
***n*-3 supplement**		47								
	Yes		14 (29.2)	2 (25.0)	2 (25.0)	2 (25.0)	0 (0)	6 (75.0)	2 (28.6)	0.043*
	No		33 (68.8)	6 (75.0)	6 (25.0)	6 (75.0)	8 (100.0)	2 (25.0)	5 (71.4)	
Supplements		47								
	Yes		15 (31.3)	5 (62.5)	1 (12.5)	1 (12.5)	3 (37.5)	3 (37.5)	2 (28.6)	0.262
	No		32 (66.7)	3 (37.5)	7 (87.5)	7 (87.5)	5 (62.5)	5 (62.5)	5 (71.4)	

* Significant at a level of $p<0.05$

[1] *p*-value given by Chi-square test differences between intervention groups.

[2] FFQ was missing for one participant in Low-OX, thus analysis is based on dietary data from 47 out of 48 subjects.

[3] Data missing for seven subjects.

[4] Data missing for one subject.

3.1.2 Diet- and lifestyle habits in the study population

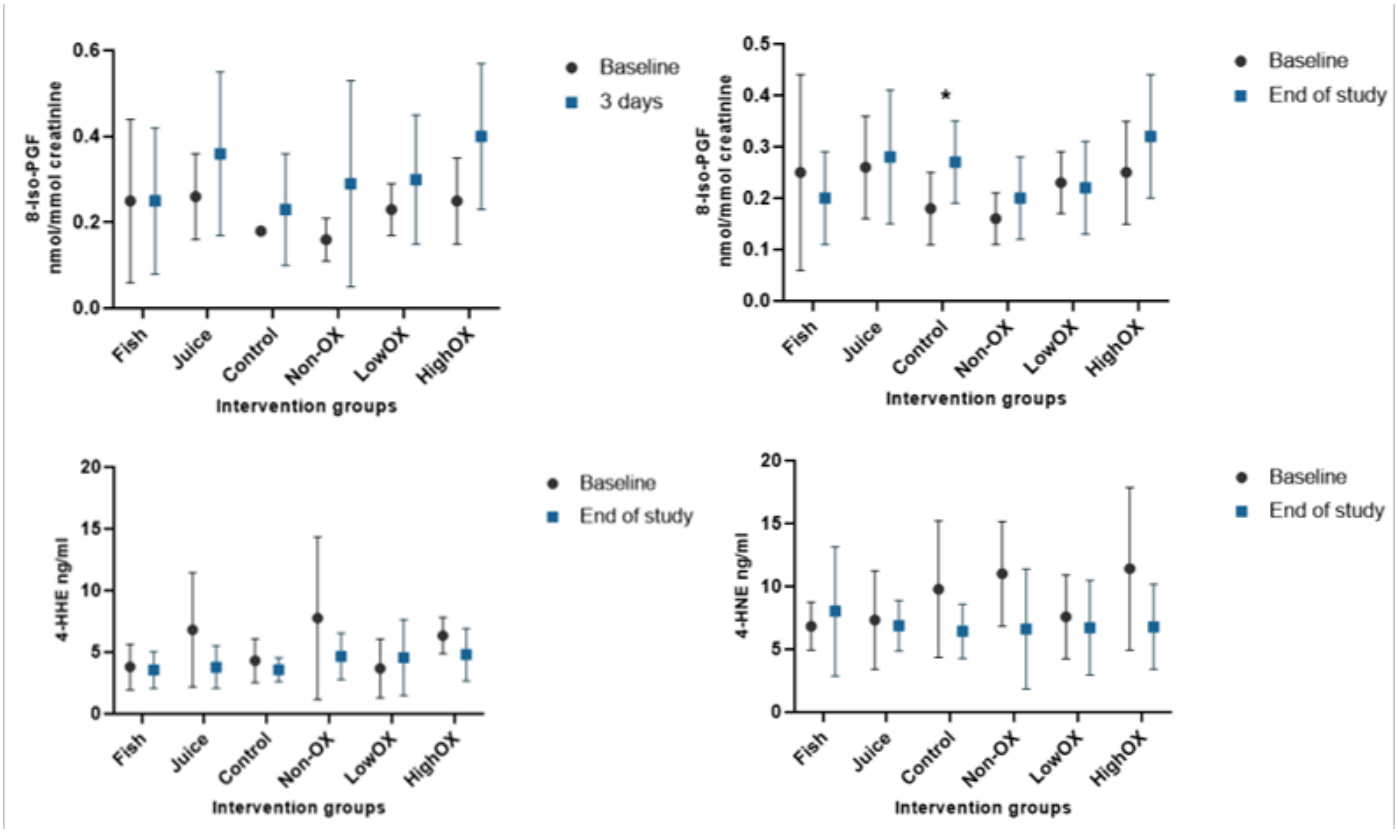
Dietary data assessed through FFQ was obtained from 47 out of 48 study subjects. Significant between-group differences in vegetable intake (p=0.042) and use of *n*-3 capsule supplement (p=0.043) were observed between groups at baseline. Otherwise, no significant differences in consumption of various food groups were detected between groups (Table 3). Based on the inclusion criteria, the study population comprised subjects with less favorable diet-and activity habits. Hence, most subjects had a diet low in fish, seafood, fruit and vegetables and a low physical activity level. Two out of five had an intake of fish in accordance with the recommended 2-3 portions a week, while about 60% reported consuming fish once a week or more seldom. Looking at fatty fish alone, approximately 30% ate fatty fish once a week or more. Combined about 20% used either liquid or capsule *n*-3 supplements. About one out of four had an intake of fruit in line with the recommended two out of five fruits and vegetables a day, while none reached the recommended three vegetables a day. Of the 74% who did not meet the recommendation, 6% reported eating fruit less than once a week. Further, 70% reported that they usually chose bread with a wholegrain content of 50% or more. The subjects were asked to subjectively report their own focus on having a healthy diet on scale from "very little" to "very large", where 43% described their emphasis as intermediate, while 57% reported having a "large" or "very large" focus on a healthy diet.

Most subjects had a physical activity level that did not meet the recommendations for physical activity. Approximately one third of the subjects reported walking for 30 minutes more than four times a week, which corresponds to the recommended weekly 150 minutes of moderate activity. One out of five reported that they never or less than once a month would take a walk for 30 minutes. The education level in the study population ranged from primary and lower secondary education to four years or more of higher education. About 70% of the subjects had completed higher education at the university level, of which about 37% had completed more than four years of higher education. Fifteen percent evaluated their income as low, here defined as e.g., not affording healthy foods and gym membership.

3.2 Primary outcomes

Plasma 4-HHE and 4-HNE were measured at baseline and after 21 days of intervention, while urinary 8-isoPGF were measured at baseline and after three and 21 days (Table 4). After both three and 21 days of intervention no significant differences in plasma 8-isoPGF was found between intervention groups. However, a significant increase in urine 8-isoPGF from mean (SD) 0.18 ± 0.07 nmol/mmol creatinine at baseline to 0.27 ± 0.08 nmol/mmol creatinine at end of study was observed within the Control group (p=0.008) (Figure 4). Delta variables suggested a tendency of 8-isoPGF increasing during the first three days of intervention and then decreasing towards end of study within all intervention groups receiving *n*-3 products, though insignificant in statistical analysis. The changes in plasma 4-HHE and 4-HNE after 21 days of intervention were not significantly different neither between nor within intervention groups.

Figure 4. Box plots presenting markers of lipid peroxidation and oxidative stress at baseline and after three and 21 days (n=48). Data are shown as mean values and standard deviations.



Abbreviations: 8-isoPGF, 8-iso-prostaglandin-F2α; 4-HHE, 4-hydoxy-2-hexenal; 4-HNE, 4-hydoxy-nonenal

* Paired-samples t-test for changes within groups from baseline to end of study. Level of significance set to p<0.05.

Table 4. Markers of lipid peroxidation and oxidative stress at baseline and after three and 21 days of intervention (*n*=48). Data are presented as mean values and standard deviations or mean values and 95% CI.

Variables	Fish *n*=8	Juice *n*=8	Control *n*=8	Non-OX *n*=8	LowOX *n*=9	HighOX *n*=7	*p*-value[1]
8-isoPGF (nmol/mmol creatinine)							
Baseline	0.25 ± 0.19	0.26 ± 0.10	0.18 ± 0.07	0.16 ± 0.05	0.23 ± 0.06	0.25 ± 0.10	
3 days	0.25 ± 0.17	0.36 ± 0.19	0.23 ± 0.13	0.29 ± 0.24	0.30 ± 0.15	0.40 ± 0.17	
End of study	0.20 ± 0.09	0.28 ± 0.13	0.27 ± 0.08	0.20 ± 0.08	0.22 ± 0.09	0.32 ± 0.12	
p-value[2]	0.932	0.070	0.335	0.157	0.265	0.086	
p-value[3]	0.412	0.420	0.008*	0.149	0.827	0.109	
Change[4] Mean ± (95% CI)	0.02 (-0.11, 0.14)	0.11 (-0.01, 0.23)	0.04 (-0.09, 0.16)	0.11 (0.02, 0.23)	0.08 (-0-04, 0.19)	0.16 (0.03, 0.29)	0.600
Change[5] Mean ± (95% CI)	-0.05 (-0.16, 0.06)	-0.07 (-0.18, 0.04)	0.03 (-0.08, 0.14)	-0.10 (-0.21, -0.01)	-0.08 (-0.18, 0.02)	-0.08 (-0.19, 0.04)	0.615
Plasma 4-HHE (ng/ml)							
Baseline	3.80 ± 1.84	6.81 ± 4.62	4.31 ± 1.77	7.75 ± 6.59	3.68 ± 2.38	6.34 ± 1.46	
End of study	3.55 ± 1.47	3.79 ± 1.71	3.57 ± 0.96	4.65 ± 1.88	4.55 ± 3.08	4.80 ± 2.11	
p-value[3]	0.679	0.191	0.375	0.310	0.186	0.081	
Change[5] Mean ± (95% CI)	-2.01 (-3.60,-0.43)	-1.44 (-3.02, 0.14)	-1.95 (-3.64, -0.25)	-0.97 (-2.71, 0.77)	0.11 (-1.60, 1.82)	-1.27 (-2.96, 0.42)	0.479
Plasma 4-HNE (ng/ml)							
Baseline	6.83 ± 1.89	7.32 ± 3.91	9.78 ± 5.43	11.0 ± 4.16	7.58 ± 3.32	11.4 ± 6.46	
End of study	8.02 ± 5.12	6.88 ± 2.00	6.42 ± 2.15	6.61 ± 4.76	6.71 ± 3.76	6.79 ± 3.38	
p-value[3]	0.310	0.452	0.364	0.105	0.888	0.194	
Change[5] Mean ± (95% CI)	0.28 (-2.73, 3.29)	-2.18 (-5.16, 0.80)	-2.09 (-5.29, 1.10)	-2.34 (-5.58, 0.91)	-1.10 (-4.30, 2.11)	-2.23 (-5.50, 1.05)	0.809

Abbreviations: 8-isoPGF, 8-iso-prostaglandin-F2α; 4-HHE, 4-hydoxy-2-hexenal; 4-HNE, 4-hydoxy-nonenal.

[1] General linear model was used for changes between groups from baseline to the 3-days visit adjusted for baseline and for changes between groups from baseline to the 21-days visit adjusted for baseline. *Post hoc* Bonferroni test was used when significant changes were detected.

[2] Paired-samples t-test changes within groups from baseline to the 3-days visit.

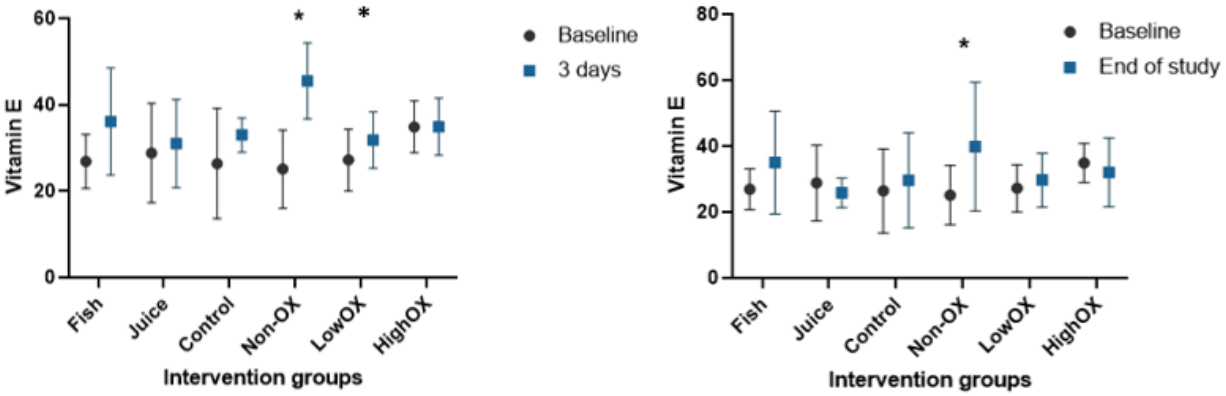
[3] Paired-samples t-test for changes within groups from baseline to end of study.

[4]: V_3-V_2 [5]: V_4-V_2

3.3 Secondary outcomes

The secondary outcomes in the study were serum vitamin E, TAG, cholesterol, LDL-cholesterol and HDL-cholesterol, presented by mean values and standard deviations in Table 5. From baseline to the three days follow-up a significant increase in serum vitamin E was observed between intervention groups (p=0.009). Furthermore, pairwise comparison in the *post hoc* analysis showed that changes were significant in the Juice-group compared with the Non-OX group (p=0.010) and in the Non-OX group compared with the LowOX group (p=0.022). When looking at individual changes serum vitamin E increased significantly within the Non-OX group from mean (SD) 27.2 ± 9.7 µmol/L at baseline to 44.0 ± 8.84 µmol/L at the three days follow-up (p=0.001) and from 27.2 ± 9.74 µmol/L at baseline to 44.0 ± 8.84 µmol/L at end of study (p=0.023). Also, a significant increase was found from baseline to the three days visit in the LowOX group (p=0.023) (Figure 5). Changes in other secondary outcomes during follow-up were not significant neither between nor within intervention groups.

Figure 5. Box plot presenting serum vitamin E at baseline and after three and 21 days of intervention (n=48) given in µmol/L. Data are shown as mean values and standard deviations.



* Paired-samples t-test for changes within groups from baseline to end of study. Significant on a level of p<0.05

Table 5. Serum secondary outcomes (*n*=48). Data presented as mean values and standard deviations or mean values (95% CI).

Variables		Fish *n*=8	Juice *n*=8	Control *n*=8	Non-OX *n*=8	LowOX *n*=9	HighOX n=7	*p*-value[1]
Vitamin E (µmol/L)								
	Baseline	26.9 ± 6.20	28.8 ± 11.54	26.4 ± 12.79	25.1 ± 9.01	27.2 ± 7.15	34.9 ± 5.96	
	3 days	36.1 ± 12.4	31.0 ± 10.2	33.0 ± 3.93	45.5 ± 8.80	31.8 ± 6.52	34.9 ± 6.64	
	End of study	35.0 ± 15.6	25.8 ± 4.50	29.6 ± 14.4	39.8 ± 19.5	29.7 ± 8.16	32.0 ± 10.4	
	p-value[2]	0.140	0.545	0.112	0.001*	0.023*	1.000	
	p-value[3]	0.207	0.376	0.365	0.023*	0.547	0.563	
	Change[4] Mean± (95% CI)	8.37 (2.41, 14.3)	2.81 (-3.21, 8.88)	5.38 (-0.60, 11.4)	18.2 (12.2, 24.2)	3.90 (-2.06, 9.86)	5.01 (-1.65., 11.7)	0.009*[6]
	Change[5] Mean± (95% CI)	-0.75 (-11.9, 10.4)	-5.47 (-16.6, 5.66)	-2.84 (-14.0, 8.32)	-4.81 (-16.1, 6.42)	-1.00 (-12.1, 10.1)	-5.02 (-17.4,7.40)	0.982
TAG (mmol/L)								
	Baseline	1.06 ± 0.65	0.96 ± 0.37	0.97 ±0.36	2.53 ± 1.75	1.32 ± 0.96	1.28 ± 0.35	
	End of study	1.10 ± 0.45	1.06 ± 0.37	0.92 ± 0.30	1.84 ± 0.82	1.23 ± 0.58	1.20 ± 0.64	
	p-value[3]	0.782	0.320	0.659	0.207	0.715	0.638	
	Change[5] Mean± (95% CI)	-0.13 (-0.44, 0.18)	-0.13 (-0.44, 0.17)	-0.28 (-0.59, 0.03)	0.01 (-0.34, 0.36)	-0.10 (-0.42, 0.23)	-0.12 (-0.44,0.21)	0.907
Cholesterol (mmol/L)								
	Baseline	5.36 ± 1.15	5.13 ± 0.96	5.08 ± 0.54	6.26 ± 1.79	6.09 ± 1.61	6.31 ± 0.92	
	End of study	5.25 ± 0.79	5.59 ± 0.84	4.97 ± 0.50	6.36 ± 2.47	6.09 ± 1.31	6.11 ± 1.42	
	p-value[3]	0.667	0.178	0.380	0.786	1.000	0.383	
	Change[5] Mean± (95% CI)	-0.14 (-0.70, 0.42)	0.41 (-0.16, 0.98)	-0.17 (-0.74, 0.40)	0.15 (-0.42, 0.72)	0.36 (-0.50, 0.57)	-0.14 (-0.75,0.47)	0.652
HDL (mmol/L)								
	Baseline	1.48 ± 0.30	1.78 ± 0.34	1.63 ± 0.33	1.33 ± 0.34	1.90 ± 0.42	1.56 ± 0.35	
	End of study	1.43 ± 0.28	1.85 ± 0.33	1.69 ± 0.36	1.29 ± 0.32	1.92 ± 0.45	1.56 ± 0.22	
	p-value[3]	0.573	0.402	0.329	0.644	0.782	1.000	
	Change[5] Mean± (95% CI)	-0.08 (-0.23, 0.07)	0.11 (-0.05, 0.26)	0.06 (-0.08, 0.21)	-0.10 (-0.25, 0.05)	0.08 (-0.60, 0.23)	-0.01 (-0.17, 0.15)	0.305
LDL								

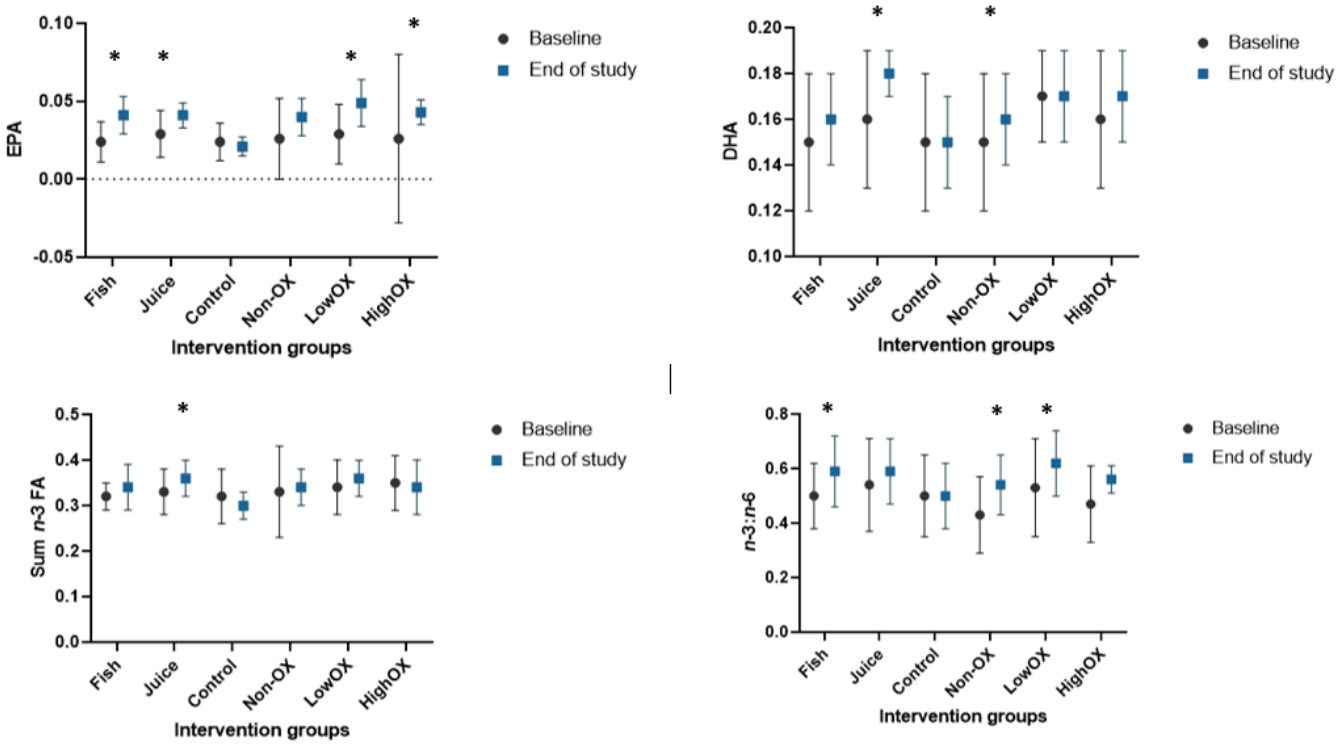
(mmol/L)								
	Baseline	3.56 ± 1.00	2.88 ± 0.74	3.15 ± 0.60	3.93 ± 1.05	3.62 ± 1.25	4.29 ± 1.13	
	End of study	3.38 ± 0.79	3.20 ± 0.72	3.00 ± 0.56	4.00 ± 1.80	3.63 ± 1.13	4.07 ± 1.42	
	p-value[3]	0.163	0.318	0.270	0.862	0.960	0.178	
	Change[5] Mean± (95% CI)	1.93 (1.37, 2.49)	2.00 (1.42, 2.59)	1.70 (1.14, 2.23)	2.36 (1.79, 2.92)	1.65 (1.12, 2.18)	1.82 (1.20, 2.44)	0.505

Abbreviations: HDL, high-density lipoprotein in plasma; LDL, low-density lipoprotein in plasma; TAG, triacylglycerol.
* Significant at a level of $p<0.05$
[1] General linear model was used for changes between groups from baseline to the 3-days visit adjusted for the pre-variable and for changes between groups from baseline to the 21-days visit adjusted for the pre-variable. *Post hoc* Bonferroni test was used when significant changes were detected.
[2] Paired-samples t-test for changes within groups from baseline to the 3-days visit.
[3] Paired-samples t-test for changes within groups from baseline to end of study.
[4]: V_3-V_2
[5]: V_4-V_2
[6] Pairwise comparisons show a significant difference between Non-OX and Juice ($p=0.010$) and between Non-OX and LowOX ($p=0.022$).

3.4 Fatty acids in erythrocyte plasma membrane

Erythrocyte levels of *n*-3 and *n*-6 FAs were measured at baseline and after the intervention. Mean erythrocyte EPA, DPA, DHA and total *n*-3 FAs increased in all groups except the Control group. After 21 days of intervention changes in EPA were significant between intervention groups ($p<0.001$). The Control group were significantly different from all other intervention groups in groupwise comparisons in the *Post hoc* analysis. Analysis of within-group changes showed that erythrocyte EPA increased significantly within the Fish-group ($p=0.007$), Juice-group ($p=0.028$), LowOX group ($p=0.002$) and HighOX ($p=0.003$). Changes in DPA during follow-up was found insignificant between groups but increased significantly within the Fish-group ($p=0.030$), Non-OX group ($p=0.048$) and LowOX group ($p<0.001$). Group differences were observed for erythrocyte DHA ($p=0.001$). Furthermore, *post hoc* analysis revealed that this was reflected by a significantly greater increase in the Juice-group compared with the Control group ($p<0.001$). The individual changes were significant within the Juice-group ($p=0.044$) and Non-OX group ($p=0.045$). Total *n*-3 FAs tended to increase in all intervention groups receiving *n*-3 products but were only significant for the Juice-group ($p=0.022$). However, a significant between-group difference was observed ($p=0.034$). Changes in the *n*-3 FAs/*n*-6 FAs ratio from baseline to end of study was significant between groups ($p=0.034$). Also, the *n*-3 *FAs/n*-6 FAs ratio shows a tendency of increasing during intervention in all groups given *n*-3 products, though only significantly in the Fish-group ($p=0.001$) and the Non-OX group ($p=0.015$). Changes in LA, AA and total *n*-6 FAs remained insignificant both within and between intervention groups.

Figure 6: Box plots presenting fatty acids in erythrocyte plasma membrane given in mg fatty acid/g erythrocyte (*n*=48). Data are shown as mean values and standard deviations.



* Paired-samples t-test for changes within groups from baseline to end of study. Significant on a level of $p<0.05$

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; sum *n*-3 FA, sum omega-3 fatty acids; *n*-6/*n*-3, ratio omega-6 fatty acids to omega-3 fatty acids

Table 6. Fatty acids in erythrocyte plasma membrane given as mg fatty acid/g erythrocyte (*n*=48). Data presented as mean values and standard deviations or mean values (95 % CI).

Variables	Fish *n*=8	Juice *n*=8	Control *n*=8	Non-OX *n*=8	LowOX *n*=9	HighOX *n*=7	*p*-value[1]
20:5*n*-3 (EPA)							
Baseline	0.024 ± 0.013	0.029± 0.015	0.024 ± 0.012	0.026 ± 0.026	0.029 ± 0.019	0.026 ± 0.054	
End of study	0.041 ± 0.012	0.041 ± 0.008	0.021 ± 0.006	0.040 ± 0.012	0.049 ± 0.015	0.043 ± 0.008	
p-value[2]	0.007*	0.028*	0.563	0.102	0.002*	0.003*	
Change[3] Mean ± (95% CI)	0.016 (0.009, 0.023)	0.014 (0.008, 0.020)	-0.004 (-0.010, 0.002)	0.014 (0.007, 0.020)	0.022 (0.015, 0.028)	0.017 (0.010, 0.024)	<0.0001**[4]
22:5*n*-3 (DPA)							
Baseline	0.043 ± 0.009	0.044 ± 0.011	0.040 ± 0.008	0.039 ± 0.011	0.041 ± 0.008	0.044 ± 0.005	
End of study	0.050 ± 0.006	0.050 ± 0.006	0.005 ± 0.009	0.046 ± 0.009	0.049 ± 0.009	0.049 ± 0.007	
p-value[2]	0.030*	0.095	0.140	0.048*	<0.0001**	0.289	
Change[3] Mean ± (95% CI)	0.007 (0.002, 0.012)	0.007 (0.003, 0.012)	0.005 (0.000, 0.10)	0.006 (0.001, 0.010)	0.008 (0.004, 0.013)	0.006 (0.001, 0.011)	0.905
22:6*n-3* (DHA)							
Baseline	0.15 ± 0.03	0.16 ± 0.03	0.15 ±0.03	0.15 ± 0.03	0.17 ± 0.02	0.16 ± 0.03	
End of study	0.16 ± 0.02	0.18 ± 0.01	0.15 ± 0.02	0.16 ± 0.02	0.17 ± 0.02	0.17 ± 0.02	
p-value[2]	0.172	0.044*	1.000	0.045*	0.316	0.604	
Change[3] Mean ± (95% CI)	0.008 (-0.001, 0.016)	0.022 (0.014, 0.030)	-0.005 (-0.013, 0.003)	0.010 (0.002, 0.018)	0.011 (0.002, 0.018)	0.009 (0.000, 0.017)	0.001*[5]
Sum *n*-3 FA							
Baseline	0.32 ± 0.03	0.33 ± 0.05	0.32 ± 0.06	0.33 ± 0.10	0.34 ± 0.06	0.35 ± 0.06	
End of study	0.34 ± 0.05	0.36 ± 0.04	0.30 ± 0.03	0.34 ± 0.04	0.36 ± 0.04	0.34 ± 0.06	
p-value[2]	0.237	0.022*	0.093	0.772	0.359	0.904	
Change[3] Mean ± (95% CI)	0.011 (-0.022, 0.043)	0.031 (0.001, 0.062)	-0.034(-0.064,-0.003)	0.008 (-0.022, 0.039)	0.036 (0.006, 0.066)	0.010 (-0.023, 0.042)	0.034*[6]

18:2*n*-6 (LA)							
Baseline	0.31 ± 0.17	0.32 ± 0.16	0.34 ± 0.17	0.43 ± 0.24	0.34 ± 0.25	0.39 ± 0.23	
End of study	0.24 ± 0.03	0.27 ± 0.07	0.27 ± 0.05	0.30 ± 0.07	0.26 ± 0.06	0.28 ± 0.07	
p-value[2]	0.234	0.483	0.213	0.111	0.338	0.281	
Change[3] Mean ± (95% CI)	-0.12 (-0.16, -0.07)	-0.08 (-0.12, -0.04)	-0.08 (-0.12, -0.04)	-0.07 (-0.11, -0.03)	-0.11 (-0.15, -0.07)	-0.07 (-0.12, -0.03)	0.509
20:4*n*-6 (AA)							
Baseline	0.28 ± 0.03	0.27 ± 0.02	0.25 ± 0.02	0.30 ± 0.03	0.28 ± 0.06	0.29 ± 0.04	
End of study	0.27 ± 0.02	0.28 ± 0.03	0.26 ± 0.03	0.30 ± 0.07	0.26 ± 0.06	0.27 ± 0.02	
p-value[2]	0.084	0.277	0.180	0.329	0.209	0.097	
Change[3] Mean ± (95% CI)	-0.100 (-0.026,0.007)	0.005 (-0.010, 0.021)	-0.004 (-0.020, 0.012)	-0.001 (-0.017, 0.015)	-0.019 (-0.034, -0.003)	-0.020 (-0.036,-0.003)	0.188
Sum *n*-6 FA							
Baseline	0.66 ± 0.19	0.66 ± 0.17	0.68 ± 0.17	0.81 ± 0.23	0.70 ± 0.33	0.76 ± 0.26	
End of study	0.58 ± 0.05	0.62 ± 0.07	0.62 ± 0.07	0.66 ± 0.09	0.59 ± 0.08	0.62 ± 08	
p-value[2]	0.187	0.536	0.259	0.087	0.274	0.190	
Change[3] Mean ± (95% CI)	-0.13 (-0.18, -0.08)	-0.08 (-0.13, -0.04)	-0.09 (-0.14, -0.05)	-0.07 (-0.12, -0.02)	-0.14 (-0.18, -0.10)	-0.10 (-0.15, -0.05)	0.283
n-6/*n*-3							
Baseline	0.50 ± 0.12	0.54 ± 0.17	0.50 ±0.15	0.43 ± 0.14	0.53 ± 0.18	0.47 ± 0.14	
End of study	0.59 ± 0.13	0.59 ± 0.12	0.50 ± 0.12	0.54 ± 0.11	0.62 ± 0.12	0.56 ± 0.05	
p-value[2]	0.001*	0.170	1.000	0.015*	0.038*	0.200	
Change[3] Mean ± (95% CI)	0.09 (0.03, 0.15)	0.07 (0.02, 0.13)	0.01 (-0.05, 0.06)	0.08 (0.03, 0.13)	0.14 (0.09, 0.19)	0.08 (0.02, 0.13)	0.034*[7]

Abbreviations: EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; sum *n*-3 FA, sum omega-3 fatty acids; LA, linolenic acid; AA, arachidonic acid; sum *n*-6 FA, sum omega-6 fatty acids; *n*-6/*n*-3, ratio omega-6 fatty acids to omega-3 fatty acids.

*Significant on a level of $p<0.05$

** Significant on a level of $p<0.001$

[1] General linear model using *post hoc* Bonferroni test when significant for changes between groups from baseline to end of study adjusted for the pre-variable.

[2] Paired-samples t-test for changes within groups from baseline to end of study.

[3] V_4-V_2

[4] Pairwise comparison shows that there is a significant difference between Control and all other intervention groups; Fish (*p=0.002)*, Juice (*p=0.004)*, Non-OX (*p=0.004)*, LowOX (*p <0.0001)* and HighOX (*p=0.001*).

[5] Pairwise comparison shows a significant difference between Juice and Control (p <0.0001).

[6] Pairwise comparison shows a significant difference between Control and LowOX (p=0.032).

[7] Pairwise comparison shows a significant difference between Control and LowOX ($p=0.012$.

3.5 Compliance and side-effects of study products

Compliance schema was obtained from 43 out of 48 subjects at end of study. At total of eight subjects in the capsule groups reported to have to consumed less than the daily dose of 18 capsules on one occasion during the intervention period, whereof four subjects in the Non-OX group, two subjects in the LowOX group and two subjects in the HighOX group. In the Fish group all subjects reported to have consumed all portions of their designated study product. Compliance schema was obtained from seven out of eight subjects in the Juice groups, whereof all subjects reported consuming their designated study product. Three subjects in LowOX group reported occurrence of adverse effects of the intervention (flatulence, stomach pain, diarrhea, nausea and belching), while two subjects in HighOX group reported incidents of flatulence and belching.

4 Discussion

The aim of the present thesis was to investigate the short-term effect of various *n*-3 products on markers of lipid peroxidation and oxidative stress in a healthy population after three and 21 days of intervention. Neither non-oxidized nor oxidized *n*-3 products were associated with significant changes in markers of lipid peroxidation and oxidative stress of interest. However, 21 days of intervention with various *n*-3 products significantly altered the composition of *n*-3 LC PUFAs in erythrocyte membranes. Also, a significant increment in vitamin E after three days of intervention was observed between groups.

4.1 Outcome variables

4.1.1 Markers of lipid peroxidation and *in vivo* oxidative stress

A major concern regarding high intake of *n*-3 LC PUFAs is the potential increased *in vivo* lipid peroxidation (64). However, few studies have investigated the effect of oxidized fish oils in human subjects. Most available evidence is based on animal studies, which have illustrated adverse effects of oxidized FAs on physiological systems. That said, dosages are typically higher compared to human consumption and the routes of administering the FAs are often unphysiological (e.g., intravenous injections). Feeding mice with oxidized *n*-3 LC PUFAs in both phospholipids or TAGs increased plasma 4-HHE and inflammatory markers. Other feeding trials have reported delayed growth and increased production of inflammatory markers (34). Also, studies investigating secondary *n*-3 PUFA oxidation products have shown that intravenous injections of 4-HHE induces severe liver damage and necrotizing peritonitis when administered intraperitoneally (65). Our results are in accordance with the study by Ottestad et al. where seven weeks of supplementation with oxidized fish oil did not significant changes in plasma 4-HHE and 4-HNE (66).

A hypothesis was that consumption of oxidized fish oil would activate an oxidative stress-related response within the body. 8-isoPGF is established as the most reliable marker of *in vivo* oxidative stress (54), thus we speculated that urinary levels of 8-isoPGF would increase upon intervention with oxidized products. In the beforementioned study by Ottestad et al. consumption of either oxidized or non-oxidized did not induce any changes in urinary 8-isoPGF in healthy subjects. Similarly, seven weeks of daily consumption of capsules containing either krill oil or fish oil did not produce significant changes in 8-isoPGF and other markers of oxidative stress, though the oxidative status of these oils were not given (67). We observed a tendency of urinary 8-isoPGF increasing during the first three days of the intervention, followed by a decrease towards end of study, though insignificant in the groups receiving *n*-3 products. Surprisingly, a significant increment in urinary 8-isoPGF was detected in the control group receiving sunflower oil in our study. Sunflower oil comprises about 60 % *n*-6 PUFAs (68), and with 8-isoPGF being an oxidation

product of AA in mind, one might speculate if the elevated urinary levels in the control group could be a reflection of increased lipid peroxidation of *n*-6 PUFAs.

4.1.2 Serum vitamin E, triacylglycerol and lipoproteins

A significant increment in serum vitamin E after three days was observed between intervention groups, a finding that potentially indicates some sort of immediate immune response upon start of study. Interestingly, though statistically insignificant, a pattern of an immediate increment in serum vitamin E after three days and a subsequent decrease towards end of study was apparent within all intervention groups. Though insignificant, this observation could reflect an immediate activation of the antioxidant defense followed by coping as serum vitamin E decreases. Following this thought, we may speculate if the increment in vitamin E could be set in context with the observed increment in 8-isoPGF during the first three days. Vitamin E is an important constituent in the human antioxidant defense; thus it was hypothesized that an increment in vitamin E in the oxidized fish oil-groups could reflect an activation of the immune system. This was not validated, rather we observed a surprising increase in vitamin E after both three and 21 days in the non-oxidized fish oil group. To our knowledge, only one study has investigated the effect of oxidized fish oil on serum vitamin E, where neither oxidized nor non-oxidized fish oil induced significant alterations in serum levels (66).

Elevated fasting and postprandial TAG-levels are recognized as markers of cardiovascular risk. Consumption of EPA and DHA may improve cardiovascular health through decrease in TAG-levels, and a dose-dependent relation has been reported across studies. Also, effect on TAG-levels seems to depend on baseline levels, with highest therapeutic potential in hypertriacylglycerolaemic subjects and CVD patients (69, 70). Evidence on the effect of EPA and DHA on lipoprotein profiles is inconsistent and metanalyses has concluded with little or neutral effect on circulating HDL and LDL. Effect seems to be dependent on dose, form and population (69, 70). Little evidence exists on the effect of oxidized *n*-3 PUFAs on serum levels of TAGs, LDL and HDL. It has been suggested that fish oil with different quality elicits differential effects on blood lipids, posed as a potential explanator for the discrepancy in the literature (55, 69). One study has investigated the effect of capsules of high-quality fish oil, oxidized fish oil and high-oleic sunflower oil on circulating lipoproteins in healthy subjects. Here, seven weeks of intervention demonstrated that high quality fish oil reduced LDL-levels when compared to oxidized fish oil and high-oleic sunflower oil (71). Hernandez et al. reported that 30 days of intervention with less oxidized oil and highly oxidized fish oil capsule both decreased circulating TAG-levels (67). Similarly, the postprandial incorporation of *n*-3 LC PUFAs in TAG-rich lipoprotein after consumption of cod liver oil emulsified in yoghurt and juice was not affected by oxidative status (72).

The majority of studies have operated with a relatively high doses of EPA+DHA, typically 3.0 to 4.0 g of EPA+DHA (70). In the present study, average daily intake of EPA+DHA during the intervention period were approximately 1.6 g/d, hence, one might question if the dose of EPA+DHA was high enough to detect changes in TAG-levels. However, some studies have reported changes in TAG-levels at low dosages. Visoli et al. observed an 19% decrease in TAG-levels in healthy subjects after six weeks of intervention with bovine milk fortified with 300 mg EPA+DHA (73). Also, the intervention period in the present study was three weeks, which combined with insufficient dosage may not induce a substantial reduction in TAG-levels in normotriacylglyceroleamic subjects. Yet, reduction in TAG-levels has been reported in short-term study by Rambjør et al. where healthy subjects received capsules of 3.0 g/d of EPA+DHA over an intervention period of three weeks (74). That said, as the scope of the present study was oxidized fish oil, supplementing high dosages of oxidized EPA+DHA would probably introduce an ethical question.

4.1.3 *n*-3 polyunsaturated fatty acids in erythrocyte membranes

The various products investigated led to increments in *n*-3 LC PUFAs in erythrocyte membranes. Interestingly, both non-oxidized and oxidized exerted effects on the FA composition. Though measured in another material, Ottestad et al. also showed that circulating EPA and DHA increased whether consumed from oxidized or non-oxidized oil (66). To date, the understanding of how oxidized lipids are absorbed in human subjects is scare. Seemingly, oils with oxidative status exceeding acceptable limits also increases both plasma and erythrocyte levels. Following this thought, one may speculate which implications incorporation of oxidized PUFAs have for the physiological properties of the membrane.

As hitherto described, there is strong evidence for a dose-dependent relation between intake of *n*-3 LC PUFAs and composition of *n*-3 LC PUFAs in membranes, substantiated by several human studies showing that consumption of large amount of *n*-3 PUFAs induces a subsequent higher incorporation into phospholipids membranes (35). This may be of relevance for cardiovascular health, as an increased amount of *n*-3 PUFAs at expense of *n*-6 PUFAs potentially can reduce the negative effects of *n*-6 metabolites. Consumption of EPA and DHA reduces the synthesis of AA-derived PGs, TXAs and LTs. These compounds are known to be proinflammatory and prothrombotic, thus the lowering effect by *n*-3 PUFAs is considered an important health benefit. It is speculated if proinflammatory metabolites of *n*-6 PUFAs may increase the chronic inflammatory status in the body, which predisposes for chronic disease. That said, eicosanoid biochemistry is immensely complex and recent evidence argues that *n*-6 metabolites may exert both proinflammatory and anti-inflammatory effects in CVD. Seemingly, it is not as easy as *n*-6 PUFAs being deemed bad and *n*-3 PUFAs being deemed good (29, 34, 35). The present study showed that increasing the consumption of *n*-3 LC PUFAs in the form of fish and fish oil capsules efficiently

altered the ratio between *n*-6 and *n*-3 PUFAs. Due to the beforementioned implicated mechanisms, the importance of an ideal ratio between *n*-6 PUFAs and *n*-3 PUFAs in the diet is disputed. Some argues that the problem is a too low intake of seafood, rather than a too high consumption of *n*-3 PUFAs. Health authorities has suggested that a better strategy may be increasing the intake of n-3 PUFAs from seafood and plant sources without decreasing the intake of n-6 PUFAs. In this manner, the ratio is improved and the possible cardioprotective effects of both n-3 and n-6 PUFAs are maximized (16).

4.1.4 Chemical form of *n*-3 polyunsaturated fatty acids and bioavailability

Whether the *n*-3 LC PUFAs are more bioavailable when consumed from seafood as opposed to supplemental sources is under scrutiny. In the present study *n*-3 LC PUFAs was investigated as TAGs and ethyl esters. At end of study *n*-3 LC PUFAs had increased in all groups receiving *n*-3 products, without any apparent pattern dependent on chemical form. These findings are in concordance with other studies (75, 76). Harris et al. showed that *n*-3 LC PUFAs in erythrocytes and plasma phospholipids increased significantly after 16 weeks of daily consumption of 485 mg EPA+DHA from either fatty fish or fish oil capsules. Whether *n*-3 LC PUFAs was consumed from fatty fish or from fish oil capsules did not produce any differences in increment in FAs (76). Similarly, studies of changes in serum and plasma levels have shown inconsistent findings. In a randomized trial by Elvevoll et al. *n*-3 LC PUFAs from salmon were compared with cod liver oil, and after eight week of intervention serum EPA and DHA had increased in all intervention groups. The daily intake of EPA+DHA from cod liver oil was about three times higher than from salmon. Interestingly, at end of study, the increase in serum levels of EPA and DHA was nearly as high in the fish-eating groups compared with the cod liver group. These findings suggested that fish more efficiently enhances the levels of *n*-3 LC PUFAs compared with concentrated EPA and DHA in ethyl esters in capsules (75). Conversely, in a study by Kirkhus et al. similar intakes of *n*-3 PUFAs from fish pâté, fruit juice with fish oil and fish oil capsules did not produce any significant between-group differences. After seven weeks of intervention plasma EPA and DHA increased significantly in all intervention groups, which might indicate that fish pâte, enriched juice and fish oil capsules represents equivalent dietary sources of EPA and DHA (77).

Numerous factors appear to affect the bioavailability of *n*-3 PUFAs. Evidence suggests *n*-3 PUFAs are more bioavailable when they are bound to TAG and re-esterified TAGs as opposed to bound in ethyl esters. Schuchardt et al. postulates that this may be explained by digestion of these compounds differ in efficiency. The carboxyl ester lipases who cleave the FA-ethanol bond in EE appears to be far less efficient than the lipases cleaving FA-glycerol bond in TAGs. After absorption in the enterocytes, a prerequisite for further transport in the blood is re-esterification of free *n*-3 PUFAs to TAGs. For *n*-3 PUFA-TAG the necessary glycerol, as well as 2-MAG, are already provided as substrate. By contrast, the

necessary glycerol is not present for EE, thus further delivery of re-esterified TAGs is delayed (32). Also, concomitant food intake and interactions with other components affects the uptake of *n*-3 PUFAs. Simultaneous intake of fat appears to act synergistically on uptake *n*-3 PUFAs, seemingly through stimulating release of pancreatic lipases (32, 33, 78). Lawson et al. reported that the absorption of *n*-3 LC PUFA-EE from capsules increased threefold when consumed together with a high-fat meal as opposed to a low-fat meal (78). Thus, when utilizing *n*-3 LC PUFAs capsules, consumption together with a meal may be of importance.

Another aspect worth mentioning is how oxidative degeneration is detected dependent on *n*-3 LC PUFAs being administered through seafood, liquid supplements, or encapsulated supplements. Non-oxidized fish oils have little or no odor and the taste is difficult to differentiate from other cooking oils. Formation of secondary oxidation products introduces an unpleasant odor and taste (60). Oxidative degeneration in fish and liquid fish oil supplements is identified by rancid and "fishy" odor and taste and most consumers intuitively avoid consumption. Encapsulation material in fish oil capsules can mask odor of oxidation products and identifying early stages of oxidation requires opening the capsule, which most consumers probably do not do routinely.

4.2 Dietary data

Based on the inclusion criteria, the study population comprised subjects with less favourable diet- and lifestyle habits. Thus, most subjects had an intake of seafood, fruit and vegetables and a physical activity level that were not in line with the Norwegian dietary- and physical activity recommendations (10). Findings in the present study population do not differ remarkably from findings in a representative Norwegian population in Norkost 3, which showed that a scarce proportion had an intake of seafood, fruit and vegetables in line with the dietary recommendations. About 25 % had a sufficient intake of fruit and vegetables, while one out of three met the recommendation of seafood intake (11). Most plants contain high concentrations of redox-active secondary metabolites such as antioxidants and reductants, which are produced by plants in response to oxidative stress and activate antioxidant defense in plant cells. These plant-derived redox active metabolites are important constituents of the human antioxidant defense and are obtained through dietary intake of fruit and vegetables (79). Also, physical activity seems to be of importance for the antioxidant defense in human. The mild stress produced by physical activity triggers defense responses without causing significant oxidative damage (80). Altogether, a healthy lifestyle with a diet rich in fruit, vegetables and seafood combined with physical activity may reduce incidence of major chronic disease by creating a mild, unharmful stress that activates protective and beneficial mechanisms. We wanted to investigate the effect of oxidized *n*-3 LC PUFAs in a population with a baseline low intake

of fruit, vegetables and seafood and low physical activity level, and speculated that such lifestyle habits could reflect a weaker defense line against oxidative species.

Though less favourable diet-and lifestyle pattern, the study population present subjects at general good health based on the biochemical and anthropometrical assessments conducted at baseline. Average BMI was just above 25 kg/m^2, biochemical markers were within reference levels, and none used medications like lipid-lowering agents and blood thinners. The physiological systems in healthy subjects might be able to handle oxidized *n*-3 LC PUFAs. Oxidative stress is characterized by excess of oxidative species in physiological systems and oxidized *n*-3 LC PUFAs may possibly act differently in subjects at imbalance. Following this thought, consumption of exogenous oxidized FAs may be an accelerator of endogenous lipid peroxidation, however, which implications this have on cardiovascular health remains unclear. A general rule of thumb in chronic disease goes "the greater sum of risk factors, the greater the total risk". Several risk factors have been associated with CVD, with the most prominent being obesity, unhealthy diet, physical inactivity, diabetes, dyslipidemia and hypertension (1). With the concurrence of other risk factors, one might speculate if the presence of oxidized *n*-3 LC PUFAs can be a factor weighing the balance towards morbidity.

To our knowledge, no studies have investigated whether oxidized fish oils is as efficacious as non-oxidized fish oils. Though the mechanisms are not fully understood, it may appear likely that lipid peroxides are less effective than their parent PUFA. Lipid peroxides have different shape, polarity and reactivity than their originating PUFA, thus they might be ineffective or act differentially in the implicated mechanisms of *n*-3 PUFAs (3). With the potential inefficacy and harm in mind, oxidation has been suggested as a major explanator for discrepancies in the literature. Despite epidemiological studies coupling high dietary or plasma *n*-3 LC PUFAs to lower risk of CVD, primary and secondary prevention studies have presented inconsistent results. Oxidative status in trial oils is rarely reported and the potential effects on study outcome remain unknown. Thus, oxidation may potentially be overlooked as an explanation for conflicting or disappointing results (3, 34, 55).

4.3 Methodological considerations

"The health effects of oxidized fish oil"-study was conducted in 2013 and raw data material of analytes in blood and urine samples, as well as dietary data from FFQs, has been systematized and statistically handled and forms the basis for this master thesis. The fact that the study was conducted some years back has introduced methodological challenges. It has been challenging to retrieve all necessary information of study procedure; thus, some methodological material is lacking. Ideally, data on analyses of fatty acid composition of the fish oil in the various products should have been included in the thesis, as well as more information about how the fish oils were oxidated. Unfortunately, this information was not possible to

retrieve. A major limitation in the present study was the small study population. It was estimated that each intervention group should comprise 20 subjects, giving a total of 120 subjects in the study population. However, the window of recruitment was narrow and resulted in only 48 subjects being enrolled in the study. Based on the low number of subjects, the present study can be considered a pilot study. Given the evident difference in appearance of intervention products, it was not possible to perform a blinded study. However, the master student who performed the statistical analysis were blinded for the randomization code, which can be highlighted as a strength. Another major strength was that study products was generally well accepted by study subjects. Few subjects reported adverse side-effects and subjects were generally pleased with palatability with regards to smell, taste and appearance of study products. Ultimately, this probably contributed to good compliance in study.

A strength of the FFQ used in the present study was that it included both general questions of seafood consumption followed by more detailed question about consumption of specific seafood species/products. Also, the FFQ included many possible answer alternatives for frequency of consumption, thus contributing to more accurate assessment of dietary intake. That said, gathering dietary data is a notorious problem in nutrition epidemiology. Registration of dietary data has inherent errors and may be the most prominent weakness in nutrition research. Collecting dietary data is a comprehensive process and relies on great accuracy by study participants. This is possible in short-term studies with few participants, whereas studies with longer duration and larger population often requires utilization of less accurate methods. Thus, FFQs are often used to estimate dietary intake in larger-scale studies. As a retrospective method, FFQs introduces recall bias because they are dependent on the participants ability to correctly remember what they have eaten over a period of time. Estimation of nutrient content based on FFQs is not errorless and must be based on mean values, even though the nutrient content in foods may vary considerably due to geographic area, harvesting, storage, preparation etc. Another major challenge is the tendency of misreporting dietary intake, consciously or unconsciously. A classic example is the tendency of overweight subjects tending to under-report food intake, while underweight tend to over-report food intake (81). To exemplify from the present study, almost 60 % reported having “a large focus” or “a very large focus” on a healthy diet when they were asked to subjectively report their own focus on having a healthy diet. Here, one could argue that the subjects’ perception of a healthy diet contradicts with our interpretation of the dietary data.

4.4 Conclusions

Observations in the present study contributes to a field of research that to a lesser degree has been explored. Findings do not indicate any negative short-term effects of intake of oxidized fish oils on markers of *in vivo* oxidative stress and lipid peroxidation, thus our hypothesis of the most oxidized *n*-3 products leading to a larger increment in these markers was not validated. As of today, most studies on oxidized fish oil have been of short duration and there is little evidence on the effects of long-term intake. Several studies have shown that many *n*-3 products available on the commercial market have oxidation levels exceeding the limits defined by the industry. Knowing that many do not consume enough seafood and rely on supplemental sources, further investigation of possible long-term effects of consumption of oxidized products could be of interest. As of today, most studies have included a low number of participants and larger studies with longer duration could clarify the discrepancies around oxidized fish oil. Also, oxidized *n*-3 PUFAs may exert differential effects in different populations (e.g., healthy vs patient), thus a matter that needs to be further investigated. However, with the suspected adverse biological effects of oxidized fish oil in mind, it might be difficult to conduct long-span studies in an ethical order.

References

1. FAO. Cardiovascular diseases (CVDs): FAO; 2017 [Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
2. Norwegian Scientific Committee for Food Safety. Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods. 2011.
3. Albert BB, Cameron-Smith D, Hofman PL, Cutfield WS. Oxidation of marine omega-3 supplements and human health. Biomed Res Int. 2013;2013:464921.
4. Lund EK. Health benefits of seafood; Is it just the fatty acids? Food Chem. 2013;140(3):413-20.
5. EFSA Panel on Dietic Products NaAN. Scientific Opinion on health benefits of seafood (fish and shellfish) consumption in relation to health risk associated with exposure to methylmercury. Parma: European Food Safety Authority; 2014.
6. FAO. Fats and fatty acids in human nutrition. Report of an expert consultation. Genova, Italy: Food and Agriculture Organization of the United Nations; 2008.
7. EFSA Panel on Dietic Products NaAN. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA Journal. 2010;8(3):107.
8. Rimm EB, Appel LJ, Chiuve SE, Djoussé L, Engler MB, Kris-Etherton PM, et al. Seafood Long-Chain n-3 Polyunsaturated Fatty Acids and Cardiovascular Disease: A Science Advisory From the American Heart Association. Circulation. 2018;138(1):e35-e47.
9. Helsedirektoratet. Kostrådene og næringsstoffer Oslo: Helsedirektoratet; 2016 [Available from: https://www.helsedirektoratet.no/faglige-rad/kostradene-og-naeringsstoffer#referere.
10. Totland T, Melnes B, Lundberg-Hallén N, Helland-Kigen K, Lund-Blix N, Myhre J, et al. Norkost 3. En landsomfattende kostholdundersøkelse blant menn og kvinner i Norge i alderen 18-70 år, 2010-11. . Oslo: Helsedirektoratet; 2012.
11. Bang HO, Dyerberg J, Hjøorne N. The composition of food consumed by Greenland Eskimos. Acta Med Scand. 1976;200(1-2):69-73.
12. Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L, et al. Associations of Omega-3 Fatty Acid Supplement Use With Cardiovascular Disease Risks: Meta-analysis of 10 Trials Involving 77 917 Individuals. JAMA Cardiol. 2018;3(3):225-34.
13. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Marine n-3 Fatty Acids and Prevention of Cardiovascular Disease and Cancer. N Engl J Med. 2019;380(1):23-32.
14. Bowman L, Mafham M, Wallendszus K, Stevens W, Buck G, Barton J, et al. Effects of n-3 Fatty Acid Supplements in Diabetes Mellitus. N Engl J Med. 2018;379(16):1540-50.
15. Rizos EC, Ntzani EE, Bika E, Kostapanos MS, Elisaf MS. Association between omega-3 fatty acid supplementation and risk of major cardiovascular disease events: a systematic review and meta-analysis. Jama. 2012;308(10):1024-33.
16. Abdelhamid AS, Brown TJ, Brainard JS, Biswas P, Thorpe GC, Moore HJ, et al. Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. Cochrane Database Syst Rev. 2020;3(2):Cd003177.
17. Rustan A, Drevon A. Fatty Acids: Structures and Properties eLS. 2005.
18. Cottin SC, Sanders TA, Hall WL. The differential effects of EPA and DHA on cardiovascular risk factors. Proc Nutr Soc. 2011;70(2):215-31.
19. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet. 2010;376(9740):540-50.
20. Massaro M, Scoditti E, Carluccio MA, De Caterina R. Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. Prostaglandins Leukot Essent Fatty Acids. 2008;79(3-5):109-15.
21. Harris WS, Poston WC, Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis. 2007;193(1):1-10.
22. Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. Atherosclerosis. 2008;197(1):12-24.
23. Harris WS. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J Lipid Res. 1989;30(6):785-807.
24. Berg J, Tymoczko J, Gatto G. Biochemistry. 8th ed: W.H. Freeman & Co Ltd; 2015.

25. Thompson T. Lipids: Encyclopedia Britannica; 1999 [Available from: https://www.britannica.com/science/lipid/additional-info#history.
26. Gropper S, Smith J. Advanced Nutrition and Human Metabolism. 6 ed: Wadsworth Publishing Co Inc; 2012.
27. Nordic Council of Ministers. Nordic Nutrition Recommendations 2012. Copenhagen; 2014.
28. Catalá A. A synopsis of the process of lipid peroxidation since the discovery of the essential fatty acids. Biochem Biophys Res Commun. 2010;399(3):318-23.
29. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol. 2011;58(20):2047-67.
30. Zhang H, Zhao H, Zhang Y, Shen Y, Su H, Jin J, et al. Characterization of Positional Distribution of Fatty Acids and Triacylglycerol Molecular Compositions of Marine Fish Oils Rich in Omega-3 Polyunsaturated Fatty Acids. Biomed Res Int. 2018;2018:3529682.
31. Schuchardt JP, Hahn A. Bioavailability of long-chain omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2013;89(1):1-8.
32. Dyerberg J, Madsen P, Møller JM, Aardestrup I, Schmidt EB. Bioavailability of marine n-3 fatty acid formulations. Prostaglandins Leukot Essent Fatty Acids. 2010;83(3):137-41.
33. Visioli F, Risé P, Barassi MC, Marangoni F, Galli C. Dietary intake of fish vs. formulations leads to higher plasma concentrations of n-3 fatty acids. Lipids. 2003;38(4):415-8.
34. Maehre HK, Jensen IJ, Elvevoll EO, Eilertsen KE. ω-3 Fatty Acids and Cardiovascular Diseases: Effects, Mechanisms and Dietary Relevance. Int J Mol Sci. 2015;16(9):22636-61.
35. Harris WS. The Omega-6:Omega-3 ratio: A critical appraisal and possible successor. Prostaglandins Leukot Essent Fatty Acids. 2018;132:34-40.
36. Calder PC. Fatty acids and inflammation: the cutting edge between food and pharma. Eur J Pharmacol. 2011;668 Suppl 1:S50-8.
37. Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem Phys Lipids. 2009;157(1):1-11.
38. Rundblad A, Holven KB, Bruheim I, Myhrstad MC, Ulven SM. Effects of krill oil and lean and fatty fish on cardiovascular risk markers: a randomised controlled trial. J Nutr Sci. 2018;7:e3.
39. Yashodhara BM, Umakanth S, Pappachan JM, Bhat SK, Kamath R, Choo BH. Omega-3 fatty acids: a comprehensive review of their role in health and disease. Postgrad Med J. 2009;85(1000):84-90.
40. Das UN. Essential fatty acids: biochemistry, physiology and pathology. Biotechnol J. 2006;1(4):420-39.
41. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014;2014:360438.
42. Girotti AW. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J Lipid Res. 1998;39(8):1529-42.
43. van 't Erve TJ, Lih FB, Kadiiska MB, Deterding LJ, Eling TE, Mason RP. Reinterpreting the best biomarker of oxidative stress: The 8-iso-PGF(2α)/PGF(2α) ratio distinguishes chemical from enzymatic lipid peroxidation. Free Radic Biol Med. 2015;83:245-51.
44. van 't Erve TJ, Kadiiska MB, London SJ, Mason RP. Classifying oxidative stress by F(2)-isoprostane levels across human diseases: A meta-analysis. Redox Biol. 2017;12:582-99.
45. Vasilaki AT, McMillan DC. Lipid Peroxidation: SpringerLink; 2017 [Available from: https://link.springer.com/referenceworkentry/10.1007%2F978-3-642-16483-5_3373.
46. Catalá A. An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. Int J Biochem Cell Biol. 2006;38(9):1482-95.
47. Massey KA, Nicolaou A. Lipidomics of polyunsaturated-fatty-acid-derived oxygenated metabolites. Biochem Soc Trans. 2011;39(5):1240-6.
48. Spickett CM. The lipid peroxidation product 4-hydroxy-2-nonenal: Advances in chemistry and analysis. Redox Biol. 2013;1(1):145-52.
49. Roy J, Le Guennec JY, Galano JM, Thireau J, Bultel-Poncé V, Demion M, et al. Non-enzymatic cyclic oxygenated metabolites of omega-3 polyunsaturated fatty acid: Bioactive drugs? Biochimie. 2016;120:56-61.
50. Staff A, Halvorsen B. Isoprostaner – nye markører for oksidativt stress. Tidsskriftet for den norske legeforeningen. 2003.

51. Galli C, Calder PC. Effects of fat and fatty acid intake on inflammatory and immune responses: a critical review. Ann Nutr Metab. 2009;55(1-3):123-39.
52. Morrow JD, Roberts LJ, 2nd. The isoprostanes. Current knowledge and directions for future research. Biochem Pharmacol. 1996;51(1):1-9.
53. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med. 2000;28(4):505-13.
54. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ, 2nd. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A. 1990;87(23):9383-7.
55. Turner R, McLean CH, Silvers KM. Are the health benefits of fish oils limited by products of oxidation? Nutr Res Rev. 2006;19(1):53-62.
56. Sand O, Sjaastad Ø, Haug E. Menneskets fysiologi. 2 ed. Oslo: Gyldendal; 2016.
57. Ytrehus K. Aterosklerose: Store norske leksikon; 2020 [
58. Yamada S, Funada T, Shibata N, Kobayashi M, Kawai Y, Tatsuda E, et al. Protein-bound 4-hydroxy-2-hexenal as a marker of oxidized n-3 polyunsaturated fatty acids. J Lipid Res. 2004;45(4):626-34.
59. Ahotupa M, Suomela JP, Vuorimaa T, Vasankari T. Lipoprotein-specific transport of circulating lipid peroxides. Ann Med. 2010;42(7):521-9.
60. Nofima. Lite oksiderte omega-3 oljer og potensielle helsefordeler. En screening av omega-3 oljer med hensyn til variasjon i oksidasjonsgrad, innhold av oksidasjonsprodukter og effekt på markørsystemer. . Trondheim: Nofima; 2012.
61. Council of Europe. European Pharmacopeia. 6th ed. Strasbourg: Council of Europe; 2008.
62. Haefeli M, Elfering A. Pain assessment. Eur Spine J. 2006;15 Suppl 1(Suppl 1):S17-24.
63. Dahl L, Mæland CA, Bjørkkjær T. A short food frequency questionnaire to assess intake of seafood and n-3 supplements: validation with biomarkers. Nutr J. 2011;10:127.
64. Bays HE. Safety considerations with omega-3 fatty acid therapy. Am J Cardiol. 2007;99(6a):35c-43c.
65. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):81-128.
66. Ottestad I, Vogt G, Retterstøl K, Myhrstad MC, Haugen JE, Nilsson A, et al. Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. Br J Nutr. 2012;108(2):315-26.
67. Higdon JV, Liu J, Du SH, Morrow JD, Ames BN, Wander RC. Supplementation of postmenopausal women with fish oil rich in eicosapentaenoic acid and docosahexaenoic acid is not associated with greater in vivo lipid peroxidation compared with oils rich in oleate and linoleate as assessed by plasma malondialdehyde and F(2)-isoprostanes. Am J Clin Nutr. 2000;72(3):714-22.
68. Mattilsynet. Matvaretabellen 2020: Mattilsynet; 2020 [Available from: https://www.matvaretabellen.no/solsikkeolje-08.106.
69. Balk EM, Lichtenstein AH, Chung M, Kupelnick B, Chew P, Lau J. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. Atherosclerosis. 2006;189(1):19-30.
70. Harris WS. n-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr. 1997;65(5 Suppl):1645s-54s.
71. Davidson MH, Benes LB. The future of n-3 polyunsaturated fatty acid therapy. Curr Opin Lipidol. 2016;27(6):570-8.
72. Rundblad A, Holven KB, Ottestad I, Myhrstad MC, Ulven SM. High-quality fish oil has a more favourable effect than oxidised fish oil on intermediate-density lipoprotein and LDL subclasses: a randomised controlled trial. Br J Nutr. 2017;117(9):1291-8.
73. Ottestad I, Nordvi B, Vogt G, Holck M, Halvorsen B, Brønner KW, et al. Bioavailability of n-3 fatty acids from n-3-enriched foods and fish oil with different oxidative quality in healthy human subjects: a randomised single-meal cross-over study. J Nutr Sci. 2016;5:e43.
74. Visioli F, Risè P, Plasmati E, Pazzucconi F, Sirtori CR, Galli C. Very low intakes of N-3 fatty acids incorporated into bovine milk reduce plasma triacylglycerol and increase HDL-cholesterol concentrations in healthy subjects. Pharmacol Res. 2000;41(5):571-6.

75. Rambjør GS, Wålen AI, Windsor SL, Harris WS. Eicosapentaenoic acid is primarily responsible for hypotriglyceridemic effect of fish oil in humans. Lipids. 1996;31 Suppl:S45-9.
76. Elvevoll EO, Barstad H, Breimo ES, Brox J, Eilertsen KE, Lund T, et al. Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. Lipids. 2006;41(12):1109-14.
77. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. Am J Clin Nutr. 2007;86(6):1621-5.
78. Kirkhus B, Lamglait A, Eilertsen KE, Falch E, Haider T, Vik H, et al. Effects of similar intakes of marine n-3 fatty acids from enriched food products and fish oil on cardiovascular risk markers in healthy human subjects. Br J Nutr. 2012;107(9):1339-49.
79. Lawson LD, Hughes BG. Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. Biochem Biophys Res Commun. 1988;156(2):960-3.
80. Blomhoff R. Dietary antioxidants and cardiovascular disease. Curr Opin Lipidol. 2005;16(1):47-54.
81. Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, et al. Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget. 2018;9(24):17181-98.
82. Maki KC, Slavin JL, Rains TM, Kris-Etherton PM. Limitations of observational evidence: implications for evidence-based dietary recommendations. Adv Nutr. 2014;5(1):7-15.

Appendices

Appendix I: Invitation to participate in the “Health effects of oxidized fish oil study”.

Appendix II: Food frequency questionnaire.

Forespørsel om deltakelse i forskningsprosjektet

" Omega-3 inntak og helseeffekter"

Bakgrunn og hensikt

Dette er et spørsmål til deg som er over 18 år om å delta i forskningsstudien" Omega-3 inntak og helseeffekter". Før du bestemmer deg for om du vil delta, er det viktig at du forstår hvorfor studien gjennomføres, hva det innebærer og hvilke fordeler og eventuelle ulemper som kan være forbundet med å delta. Du bør derfor lese denne informasjonen nøye.

Forskning viser at omega-3 fettsyrer fra fisk og fiskeoljer kan virke positivt inn på ulike sykdomstilstander. Den gunstige effekten er særlig knyttet til redusert risiko for hjerte og karsykdom. Kilder for omega-3 fettsyrer i kosten er først og fremst fet fisk og tran, men også kosttilskudd med omega-3 eller matvarer som berikes med omega-3 fra fiskeoljer. Kvaliteten på fiskeoljer på markedet kan variere. Det er også kjent at omega-3 kan oksideres og føre til endring på smak og lukt av produktet. Det er derimot ikke vist noen ugunstige helseeffekter av inntak av oksiderte omega-3 fettsyrer hos friske personer.

Hensikten med studien er å sammenligne om kvalitet på utvalgte omega-3 produkter påvirker betennelses- og oksidasjon markører i blod og urin sammenlignet med kontrollgruppen. Dette ønsker vi å undersøke ved at alle deltakerne fordeles tilfeldig til enten å spise laks, drikke juice tilsatt lakseolje, ta kapsler med omega-3 hvor den ene har god kvalitet og to andre som har noe dårligere kvalitet eller få kapsler med vegetabilsk olje. Kapsler har kvalitet i likhet med andre kapsler som i dag finnes på det norske marked.

Prosjektansvarlig er Nasjonalt institutt for ernærings- og sjømatforskning (NIFES), ett offentlig forskningsinstitutt med forvaltningsansvar på sjømatområdet.

Hva innebærer studien?

Som deltaker i studien er det svært viktig at du kun inntar omega-3 i form av det produktet du får utdelt, og ikke i tillegg inntar sjømat, tran eller kosttilskudd med omega-3 fra fiskeoljer. Du skal etter nærmere avtale møte oss 4 ganger i løpet av studieperioden som varer i 3 uker.

- Ved første visitt er det legeundersøkelse og utfylling av et spørreskjema om kostholdet ditt og et skjema med bakgrunnsinformasjon om deg som deltager.

- Ved andre visitt må du møte fastende til blodprøvetaking og ha med en morgenurinprøve.
  Deretter fordeles du tilfeldig til en av en av de seks forsøksgruppene som i 3 uker skal innta enten; 1) Laks 3 dager i uka 2) Juice tilsatt lakseolje 6 dager i uka 3) Omega-3 kapsler daglig (god kvalitet) 4) og 5) Omega-3 kapsler daglig (dårligere kvalitet) eller 6) kapsler med vegetabilsk olje. Den første uken av studien skal deltaker innta sitt omega-3 produkt i løpet av de tre første dagene. Du vil også bli bedt om

å føre dagbok hvor du krysser av når du inntar ditt utleverte omega-3 produkt samt registrerer medisinbruk og hvis du er i fysisk aktivitet sammenhengende mer enn 30 minutter.

- Tredje visitt er på dag 4 i studien og da skal det tas fastende blodprøve og du skal ha med en morgenurinprøve.

- Fjerde visitt er tre uker etter studiestart. Det skal tas fastende blodprøve og du skal ha med en morgenurinprøve. Det vil også være en avsluttende samtale hvor du skal svare på spørreskjema (ett om din gjennomføring av studien og ett om eventuelle bivirkninger) og levere inn dagboken din.

Mulige fordeler og ulemper

Det er ingen direkte fordeler for deltakerne å bli med i studien, annet enn at du får utlevert gratis et omega-3 produkt (deltagere i kontroll-gruppen kan i etterkant av studien få utdelt et omega-3 produkt). Deltakere i lakse-gruppen vil kunne få med laks til partner hvis ønskelig. Alle deltakerne som får omega-3 produkt utdelt vil i studieperioden få dekket sitt omega-3 behov. Alle deltagerne vil kunne få tilbakemelding etter at studien er avsluttet på egne blodprøvesvar. Din deltakelse vil være viktig for å bedre forstå betydningen omega-3 fra fisk og fiskeoljer har for helsen vår. I forhold til mulig ulemper er det kjent at noen opplever ubehag ved blodprøvetaking. Ved å bruke erfarne bioingeniører sørger vi for at dette ubehaget skal bli minst mulig. Enkelte kan finne det utfordrende å spise et kosthold som utelater inntak av sjømat i studieperioden på 3 uker.

Hva skjer med prøvene og informasjonen om deg?

Prøvene tatt av deg og informasjonen som registreres om deg skal kun brukes slik som beskrevet i hensikten med studien. Alle opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger, men unntak av blodprøver som sendes til Fürst laboratorium for analyse. Prøvesvarene vi får tilsendt fra Fürst vil vi behandle uten bruk av navn og fødselsnummer. En kode knytter deg til dine opplysninger og prøver gjennom en navneliste. Det er kun autorisert personell knyttet til prosjektet som har adgang til navnelisten og som kan finne tilbake til deg. Kodenøkkelen vil bli slettet og prøvematerialet vil bli destruert når prosjektet er avsluttet i 2022. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

Frivillig deltakelse

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i studien. Dette vil ikke få konsekvenser for din videre behandling. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side. Selv om du nå sier ja til å delta, kan du senere trekke tilbake ditt samtykke uten at det påvirker din øvrige behandling. Dersom du har spørsmål til studien eller dersom du senere ønsker å trekke deg fra studien, kan du kontakte prosjektmedarbeider Lisbeth Dahl (41450308) eller forskningslege Snorre Øfjord (55277955).

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Kriterier for deltagelse

Vi ønsker å forsikre oss om at de endringene vi eventuelt måler i blod- og/eller urinprøver bare skyldes egenskaper knyttet til inntatt omega-3 produkt. Derfor må følgende forhold være oppfylt for å delta i studien:

Inklusjonskriterier:

- Friske menn og kvinner i fra 18 år og eldre.
- Svarer ja på tre eller flere av følgende kost- og aktivitetsvaner:
  1) spiser mindre enn 5 porsjoner frukt, bær eller grønnsaker per dag
  2) spiser fet fisk tilsvarende en middagsporsjon 1 gang per uke eller sjeldnere
  3) har mindre enn 30 minutter sammenhengende fysisk aktivitet daglig
  4) røyker
  5) har BMI minst 25, men mindre enn 40.
- BMI 18.5 – 40.

Eksklusjonskriterier:

- Kjent smitte av HIV eller hepatitt
- Bløder eller bruker medisiner som kan ha betydning for studien
- Spiser fet fisk til middag og eller som pålegg mer enn to ganger per uke eller tilsvarende mengde omega-3 tilskudd.
- Allergi/intoleranse for omega-3 produkt i studien (laktose i juicen, fisk)
- Gravide og ammende
- Planlegger vektreduksjon

Tidsskjema – hva skjer og når skjer det?

Et detaljert tidsskjema vil bli utarbeidet for hver deltaker i studien etter avtale med den enkelte.

Eventuell kompensasjon til og dekning av utgifter for deltagere

Deltagerne får dekket reisekostnad og eventuelt parkering etter gjeldende sats. Senter for klinisk studier har noen parkeringsplasser for de som har behov for å kjøre bil. Omega-3 produkter i studien er gratis.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern

Opplysninger som registreres om deg er alder, kjønn, midje- og hofteomkrets, høyde, vekt, blodtrykk, puls, livstilsstilsvaner (soling, medisiner, kosthold, trening og røyking), samt resultater fra analyser av blodprøver og urinprøver.

NIFES ved administrerende direktør er databehandlingsansvarlig.

Biobank

Blod- og urinprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en generell forskningsbiobank ved NIFES. Hvis du sier ja til å delta i studien, gir du også samtykke til at det biologiske materialet og analyseresultater fra studien inngår i biobanken. Livar Frøyland er ansvarshavende for forskningsbiobanken. Biobanken planlegges å vare ut år 2022. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer.

Utlevering av materiale og opplysninger til andre

Hvis du sier ja til å delta i studien, gir du også ditt samtykke til at prøver og avidentifiserte opplysninger utleveres til prosjektansvarlig NIFES, samt samarbeidspartnere Universitetet i Oslo og Senter for kliniske studier Bergen AS, Høyskolen i Akershus, NOFIMA, alle i Norge, og Uppsala Universitet i Sverige/Universitetet i Debrecen, Ungarn.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

Økonomi og finansiørers rolle

Studien og biobanken er forskerinitiert, finansiering fra Forskningsrådet, Marine Harvest Ingredients AS, Smartfish AS, NIFES, Prosjektet er forskerstyrt, og alle resultater vil bli publisert. Kommersielle aktører bidrar med produkter og generiske forskningsmidler. Involverte forskere eller institusjoner har ingen økonomiske interesser i studien.

Forsikring

NIFES, som prosjektansvarlig, er kontaktpunkt på vegne av leverandørene av de ulike omega-3 produktene som undersøkes i studien. Produktene er dekket av produktansvarsforsikring til leverandør for omega-3 produkt.

Informasjon om utfallet av studien

Som deltaker har du rett til å få informasjon om utfallet av studien.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien "***Omega-3 inntak og helseeffekter***"

Dato:

Navn:

(Signert av prosjektdeltaker, dato)

(Prosjektdeltagers navn med BLOKKBOKSTAVER)

Jeg bekrefter å ha gitt informasjon om studien
"***Omega-3 inntak og helseeffekter***"

(Signert, rolle i studien, dato)

(Informatør sitt navn med BLOKKBOKSTAVER)

Spørreskjema om livsstil og hva du spiser☺

Ha de ***3 siste månedene*** i bakhodet når du fyller ut skjemaet. Med sjømat mener vi fisk, fiskeprodukter og andre sjømatprodukter som for eksempel skjell og skalldyr. Vi er klar over at kostholdet varierer fra dag til dag. Prøv likevel så godt du kan å gi et "gjennomsnitt" av ditt sjømatinntak spist til middag, som pålegg, i salat og eller spist som mellommåltid. Du skal bare sette ETT kryss på hvert spørsmål med mindre noe annet er spesifisert, og krysset skal være inne i en boks, ikke mellom boksene.

1. Hvor ofte har du spist fisk, fiskeprodukter eller annen sjømat som middagsmat de siste 3 mnd?

Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
☐	☐	☐	☐	☐	☐

2. Hvis du spiser fisk, fiskeprodukter eller annen sjømat til middag, hvor mye spiser du vanligvis?
(1 porsjon = 150 gram, tilsvarer for eksempel 1 laksekotelett eller 3 fiskekaker eller 2 dl reker u/skall)

½ porsjon	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner eller mindre
☐	☐	☐	☐	☐

3. Hvor ofte har du spist sjømat som pålegg, i salat, mellommåltid, snacks eller lignende de siste 3 mnd?

Aldri	Sjelden	1-3 ganger/ måned	1-2 ganger / uke	3-5 ganger / uke	Mer enn 5 ganger / uke
☐	☐	☐	☐	☐	☐

4. Hvis du bruker sjømat som pålegg, i salat, mellommåltid, snacks eller lignende, beskriv hvor mye du vanligvis spiser?
(for eksempel boks makrell i tomat, antall fiskekaker, dl reker til antall brødskiver/knekkebrød)

_____

_____

_____

_____

5. Hvor ofte har du spist følgende sjømat <u>som middag</u> siste 3 mnd?

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger /måned	1-2 ganger/uke	3 ganger eller mer/uke
Fet fisk (Laks, ørret , makrell, sild)	☐	☐	☐	☐	☐
Halvfet fisk (Kveite, uer, steinbit, flyndre, rødspette)	☐	☐	☐	☐	☐
Mager fisk (Torsk, sei, hyse)	☐	☐	☐	☐	☐
Sushi	☐	☐	☐	☐	☐
Ferskvannsfisk (Abbor, gjedde, røye, sik)	☐	☐	☐	☐	☐
Skalldyr og skjell (Reker, krabbe, hummer, blåskjell, kamskjell)	☐	☐	☐	☐	☐
Fiskeprodukter (Fiskekaker/boller/pudding/ grateng/pinner/suppe)	☐	☐	☐	☐	☐

6. Hvor ofte har du spist følgende sjømat <u>som pålegg</u> siste 3 mnd?

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Makrell på boks	☐	☐	☐	☐	☐
Sardin på boks	☐	☐	☐	☐	☐
Laks på boks/røkt laks el ørret/ gravet laks el ørret	☐	☐	☐	☐	☐
Brisling	☐	☐	☐	☐	☐

Ansjos	☐	☐	☐	☐	☐

Forts. spm. 6. Pålegg

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/måned	1-2 ganger/uke	3 ganger eller mer/uke
Reker	☐	☐	☐	☐	☐
Tunfisk på boks	☐	☐	☐	☐	☐
Sild (sursild, rømmesild, kryddersild el.lign.)	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐
Crabsticks	☐	☐	☐	☐	☐
Svolværpostei	☐	☐	☐	☐	☐
Lofotpostei	☐	☐	☐	☐	☐
Annet sjømat (spesifiser):	☐	☐	☐	☐	☐

_____

_____

7. Spiser du fiskerogn eller fiskelever?

☐ Nei ☐ Ja

Hvis ja, hvor mange ganger per år spiser du fiskeinnmat?

	1-3 ganger/år	4-6 ganger/år	7-9 ganger/år	≥ 10 ganger/år
Fiskerogn	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐

8. Andre generelle spørsmål om kostholdet ditt A. Hvor ofte spiser du frukt og grønnsaker?

	Sjeldnere enn 1 gang/uke	1-3 ganger/uke	4-6 ganger/uke	Hver dag	2 ganger/dag	3-4 ganger/dag	5 ganger eller mer/dag
Frukt og bær (inkludert juice og smoothie)	☐	☐	☐	☐	☐	☐	☐
Grønnsaker	☐	☐	☐	☐	☐	☐	☐

B. Spiser du meieriprodukter (melk, yoghurt, ost) daglig?

☐ Nei ☐ Ja

Hvis ja, hvor mange ganger per dag spiser du meieriprodukter?
(en gang er for eksempel ett glass melk eller en yoghurt eller ost til en skive brød)

1 gang/dag	2-3 ganger/dag	4-6 ganger/dag	7-9 ganger/dag	≥ 10 ganger/dag
☐	☐	☐	☐	☐

C. Hvor mange egg spiser du per uke? (stekt, kokt, eggerøre, omlett)

Mindre enn 1 egg/uke	1 egg/uke	2-3 egg/uke	4-5 egg /uke	6-7 egg/uke	8 eller flere egg/uke
☐	☐	☐	☐	☐	☐

6. Har du siste 3 måneder før studiedeltagelse spist særlig av matvarer tilsatt marine omega-3 fettsyrer (EPA og/eller DHA), eks. enkelte typer juice/yoghurt/brød (spesifiser evnt. hvilke merker)?

☐ ☐ Vet ikke ☐ Nei ☐ Ja

_____

_____

D. Bruker du smør eller margarin på skiven/knekkebrødet?

☐ Nei ☐ Ja

Hvis ja, fyll inn til hvor mange brødskiver/knekkebrød/rundstykker du vanligvis bruker smør/margarin per uke

____ Margarin ____ Lettmargarin ____ Smør

Hvor mye smører du pr. brødskive/knekkebrød/rundstykke?
En porsjonspakning på 10-12 gram rekker til antall skiver/knekkebrød/rundstykke:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

E. Hvilken brødtype bruker du vanligvis?

Fint (0 -25% sammalt/hele korn) ☐

Halvgrovt (25-50% sammalt/hele korn)

☐ Grovt (50-75% sammalt/hele korn)

☐

Ekstra grovt (75-100% sammalt/hele korn) ☐

F. Angi hvilken type fett du vanligvis bruker til matlaging (kun ett kryss per linje)?

	Aldri	Sjelden	Månedlig	Ukentlig	Daglig
Margarin	☐	☐	☐	☐	☐
Lettmargarin	☐	☐	☐	☐	☐
Smør	☐	☐	☐	☐	☐
Olivenolje	☐	☐	☐	☐	☐
Soyaolje	☐	☐	☐	☐	☐
Rapsolje	☐	☐	☐	☐	☐
Solsikkeolje	☐	☐	☐	☐	☐
Maisolje	☐	☐	☐	☐	☐
Annen olje	☐	☐	☐	☐	☐

G. Hvor mye kaffe og te drikker du?

	Aldri/sjelden	1 kopp/dag	2-3 kopper/dag	4-6 kopper/dag	7 + kopper/dag
Kaffe	☐	☐	☐	☐	☐
Kaffe med melk	☐	☐	☐	☐	☐
Te (vanlig svart)	☐	☐	☐	☐	☐
Grønn te/urtete	☐	☐	☐	☐	☐

H. Hvor ofte drikker du vin?

	Aldri/sjelden	2-4 glass/måned	1-2 glass/uke	3-6 glass/uke	7+ glass/uke
Rødvin	☐	☐	☐	☐	☐
Hvitvin	☐	☐	☐	☐	☐

9. Kosttilskudd

A. Har du tatt tran, fiskeolje- eller omega-3 tilskudd (flytende eller som kapsler) de siste 3 mnd?

	Nei	Ja
Flytende	☐	☐
Kapsler	☐	☐

B. Hvor mye tran, fiskeolje eller omega-3 (flytende) tar du per gang?

1 teskje	1 barneskje	1 spiseskje
☐	☐	☐

C. Hvor mange tran-, fiskeolje- eller omega-3 kapsler tar du per gang?

1-2 kapsler	3-4 kapsler	5 eller flere kapsler
☐	☐	☐

D. Hvilken type tran- eller fiskeolje/omega-3 tilskudd pleier du å bruke?

☐ Møllers tran
☐ Møllers dobbel
☐ Krillolje
☐ Selolje
☐ Møllers omega-3 + folat
☐ Trippel Omega-3
☐ Nycopluss omega-3
☐ Høykonsentrert omega-3
☐ Annen (spesifiser):

_____

_____

_____

E. Bruker du annet kosttilskudd (vitaminer og mineraler)?

☐ Nei ☐ Ja

(spørsmål forsetter på neste side)

Hvis ja, hvilke type kosttilskudd bruker du og hvor ofte?

	1-3 ganger/måned	1-3 ganger/uke	4-6 ganger/uke	Daglig

Multivitamin og mineral	☐	☐	☐	☐
Multivitamin	☐	☐	☐	☐
Multimineral	☐	☐	☐	☐
Antioksidanter	☐	☐	☐	☐
B-vitaminer	☐	☐	☐	☐
C-vitaminer	☐	☐	☐	☐
Kalsium og Vitamin D	☐	☐	☐	☐
Annet	☐	☐	☐	☐

Spesifiser hvilke(t) merke på kosttilskudd og hvor mye du tar hver gang:

_____

_____

_____

10. Solvaner

A. Hvor ofte de siste 3 månedene har du brukt solarium?

☐ Aldri ☐ Sjeldnere enn 1gang/måned ☐ 1 gang/måned ☐ 2-3 ganger/måned ☐ 1-2 ganger/uke

B. Hvor mange uker de siste tre månedene har du vært på badeferie (Norge eller Syden)? ☐ Har ikke vært på badeferie ☐ 1 uke ☐ 2-3 uker ☐ 4-6 uker ☐ 7 uker eller mer

C. Hvor mange dager/uker de siste tre månedene har du vært på fjellet i snø?

☐ Har ikke vært på fjellet i snø ☐ 1-6 dager ☐ 7-13 dager ☐ 2-3 uker ☐ 4 uker eller mer

D. Hvor mye utendørsaktivitet har du om sommeren (turer, hagearbeid, jobb)?

☐ Lite ☐ Middels ☐ Ganske mye ☐ Ute nesten hele tiden

11. Hvor ofte mosjonerer du sammenhengende i minst 30 minutter?

Aldri	Sjeldnere enn 1 gang/uke	1 gang/ uke	2-3 ganger/uke	4-6 ganger /uke	Hver dag

Går	☐	☐	☐	☐	☐	☐
Jogger/Sykler	☐	☐	☐	☐	☐	☐
Svømmer	☐	☐	☐	☐	☐	☐
Styrketrening	☐	☐	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐

---

12. Kryss av for feltene under som ev. gjelder for deg

☐ Har diabetes (sukkersyke)

☐ Har matvareallergi/intoleranse

☐ Spiser ikke melprodukter

☐ Spiser ikke melkeprodukter

☐ Spiser ikke kjøttprodukter

☐ Spiser ikke grønnsaker

13. Hvor stor vekt legger du på å ha et sunt kosthold?

Svært liten	Liten	Middels	Stor	Svært stor
☐	☐	☐	☐	☐

14. Vil du si at du har litt dårlig råd

(for eksempel liten mulighet for å kjøpe sunn mat eller trene på treningssenter eller lignende)?

☐ Nei ☐ Ja

15. Hva er din høyest fullførte utdannelse?

☐ Grunnskole

☐ Videregående skole

☐ Høyskole (3år)

☐ Universitet (1-3 år)

☐ Universitet (4 år eller mer)

TAKK FOR INNSATSEN!!! Ha de ***3 siste månedene*** i bakhodet når du fyller ut skjemaet. Med sjømat mener vi fisk, fiskeprodukter og andre sjømatprodukter som for eksempel skjell og skalldyr. Vi er klar

over at kostholdet varierer fra dag til dag. Prøv likevel så godt du kan å gi et "gjennomsnitt" av ditt sjømatinntak spist til middag, som pålegg, i salat og eller spist som mellommåltid. Du skal bare sette ETT kryss på hvert spørsmål med mindre noe annet er spesifisert, og krysset skal være inne i en boks, ikke mellom boksene.

1. Hvor ofte har du spist fisk, fiskeprodukter eller annen sjømat som middagsmat de siste 3 mnd?

Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1 gang/uke	2-3 ganger/ uke	4 ganger eller mer/uke
☐	☐	☐	☐	☐	☐

4. Hvis du spiser fisk, fiskeprodukter eller annen sjømat til middag, hvor mye spiser du vanligvis?
(1 porsjon = 150 gram, tilsvarer for eksempel 1 laksekotelett eller 3 fiskekaker eller 2 dl reker u/skall)

½ porsjon	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner eller mindre
☐	☐	☐	☐	☐

5. Hvor ofte har du spist sjømat som pålegg, i salat, mellommåltid, snacks eller lignende de siste 3 mnd?

Aldri	Sjelden	1-3 ganger/ måned	1-2 ganger / uke	3-5 ganger / uke	Mer enn 5 ganger / uke
☐	☐	☐	☐	☐	☐

6. Hvis du bruker sjømat som pålegg, i salat, mellommåltid, snacks eller lignende, beskriv hvor mye du vanligvis spiser?
(for eksempel boks makrell i tomat, antall fiskekaker, dl reker til antall brødskiver/knekkebrød)

_____

_____

_____

_____

7. Hvor ofte har du spist følgende sjømat <u>som middag</u> siste 3 mnd?

	Sjeldnere enn 1 gang/måned	1-3 ganger /måned	1-2 ganger/uke	3 ganger eller mer/uke	Aldri

Fet fisk (Laks, ørret , makrell, sild)	☐	☐	☐	☐	☐
Halvfet fisk (Kveite, uer, steinbit, flyndre, rødspette)	☐	☐	☐	☐	☐
Mager fisk (Torsk, sei, hyse)	☐	☐	☐	☐	☐
Sushi	☐	☐	☐	☐	☐
Ferskvannsfisk (Abbor, gjedde, røye, sik)	☐	☐	☐	☐	☐
Skalldyr og skjell (Reker, krabbe, hummer, blåskjell, kamskjell)	☐	☐	☐	☐	☐
Fiskeprodukter (Fiskekaker/boller/pudding/ grateng/pinner/suppe)	☐	☐	☐	☐	☐

6. Hvor ofte har du spist følgende sjømat <u>som pålegg</u> siste 3 mnd?

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/ måned	1-2 ganger/uke	3 ganger eller mer/uke
Makrell på boks	☐	☐	☐	☐	☐
Sardin på boks	☐	☐	☐	☐	☐
Laks på boks/røkt laks el ørret/ gravet laks el ørret	☐	☐	☐	☐	☐
Brisling	☐	☐	☐	☐	☐
Ansjos	☐	☐	☐	☐	☐

Forts. spm. 6. Pålegg

	Aldri	Sjeldnere enn 1 gang/måned	1-3 ganger/måned	1-2 ganger/uke	3 ganger eller mer/uke
Reker	☐	☐	☐	☐	☐
Tunfisk på boks	☐	☐	☐	☐	☐
Sild (sursild, rømmesild, kryddersild el.lign.)	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐
Crabsticks	☐	☐	☐	☐	☐
Svolværpostei	☐	☐	☐	☐	☐
Lofotpostei	☐	☐	☐	☐	☐
Annet sjømat (spesifiser):	☐	☐	☐	☐	☐

_____

_____

9. Spiser du fiskerogn eller fiskelever?

☐ Nei ☐ Ja

Hvis ja, hvor mange ganger per år spiser du fiskeinnmat?

	1-3 ganger/år	4-6 ganger/år	7-9 ganger/år	≥ 10 ganger/år
Fiskerogn	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐

10. Andre generelle spørsmål om kostholdet ditt A. Hvor ofte spiser du frukt og grønnsaker?

	Sjeldnere enn 1 gang/uke	1-3 ganger/uke	4-6 ganger/uke	Hver dag	2 ganger/dag	3-4 ganger/dag	5 ganger eller mer/dag
Frukt og bær (inkludert juice og smoothie)	☐	☐	☐	☐	☐	☐	☐
Grønnsaker	☐	☐	☐	☐	☐	☐	☐

B. Spiser du meieriprodukter (melk, yoghurt, ost) daglig?

☐ Nei ☐ Ja

Hvis ja, hvor mange ganger per dag spiser du meieriprodukter?
(en gang er for eksempel ett glass melk eller en yoghurt eller ost til en skive brød)

1 gang/dag	2-3 ganger/dag	4-6 ganger/dag	7-9 ganger/dag	≥ 10 ganger/dag
☐	☐	☐	☐	☐

C. Hvor mange egg spiser du per uke? (stekt, kokt, eggerøre, omlett)

Mindre enn 1 egg/uke	1 egg/uke	2-3 egg/uke	4-5 egg /uke	6-7 egg/uke	8 eller flere egg/uke
☐	☐	☐	☐	☐	☐

6. Har du siste 3 måneder før studiedeltagelse spist særlig av matvarer tilsatt marine omega-3 fettsyrer (EPA og/eller DHA), eks. enkelte typer juice/yoghurt/brød (spesifiser evnt. hvilke merker)?

☐ ☐ Vet ikke ☐ ☐ Nei ☐ ☐ Ja

_____

_____

D. Bruker du smør eller margarin på skiven/knekkebrødet?

☐ Nei ☐ Ja

Hvis ja, fyll inn til hvor mange brødskiver/knekkebrød/rundstykker du vanligvis bruker smør/margarin per uke

____ Margarin ____ Lettmargarin ____ Smør

Hvor mye smører du pr. brødskive/knekkebrød/rundstykke?
En porsjonspakning på 10-12 gram rekker til antall skiver/knekkebrød/rundstykke:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

H. Hvilken brødtype bruker du vanligvis?

Fint (0 -25% sammalt/hele korn) ☐

Halvgrovt (25-50% sammalt/hele korn)

☐ Grovt (50-75% sammalt/hele korn)

☐

Ekstra grovt (75-100% sammalt/hele korn) ☐

I. Angi hvilken type fett du vanligvis bruker til matlaging (kun ett kryss per linje)?

	Aldri	Sjelden	Månedlig	Ukentlig	Daglig
Margarin	☐	☐	☐	☐	☐
Lettmargarin	☐	☐	☐	☐	☐
Smør	☐	☐	☐	☐	☐
Olivenolje	☐	☐	☐	☐	☐
Soyaolje	☐	☐	☐	☐	☐
Rapsolje	☐	☐	☐	☐	☐
Solsikkeolje	☐	☐	☐	☐	☐
Maisolje	☐	☐	☐	☐	☐
Annen olje	☐	☐	☐	☐	☐

J. Hvor mye kaffe og te drikker du?

	Aldri/sjelden	1 kopp/dag	2-3 kopper/dag	4-6 kopper/dag	7 + kopper/dag
Kaffe	☐	☐	☐	☐	☐
Kaffe med melk	☐	☐	☐	☐	☐
Te (vanlig svart)	☐	☐	☐	☐	☐
Grønn te/urtete	☐	☐	☐	☐	☐

H. Hvor ofte drikker du vin?

	Aldri/sjelden	2-4 glass/måned	1-2 glass/uke	3-6 glass/uke	7+ glass/uke
Rødvin	☐	☐	☐	☐	☐
Hvitvin	☐	☐	☐	☐	☐

9. Kosttilskudd

C. Har du tatt tran, fiskeolje- eller omega-3 tilskudd (flytende eller som kapsler) de siste 3 mnd?

	Nei	Ja
Flytende	☐	☐
Kapsler	☐	☐

D. Hvor mye tran, fiskeolje eller omega-3 (<u>flytende</u>) tar du per gang?

1 teskje	1 barneskje	1 spiseskje
☐	☐	☐

C. Hvor mange tran-, fiskeolje- eller omega-3 <u>kapsler</u> tar du per gang?

1-2 kapsler	3-4 kapsler	5 eller flere kapsler
☐	☐	☐

F. Hvilken type tran- eller fiskeolje/omega-3 tilskudd pleier du å bruke?

☐ Møllers tran
☐ Møllers dobbel
☐ Krillolje
☐ Selolje
☐ Møllers omega-3 + folat
☐ Trippel Omega-3
☐ Nycopluss omega-3
☐ Høykonsentrert omega-3
☐ Annen (spesifiser):

_____

_____

_____

_____

G. Bruker du annet kosttilskudd (vitaminer og mineraler)?

☐ Nei ☐ Ja

Hvis ja, hvilke type kosttilskudd bruker du og hvor ofte?

	1-3 ganger/måned	1-3 ganger/uke	4-6 ganger/uke	Daglig
Multivitamin og mineral	☐	☐	☐	☐
Multivitamin	☐	☐	☐	☐
Multimineral	☐	☐	☐	☐
Antioksidanter	☐	☐	☐	☐
B-vitaminer	☐	☐	☐	☐
C-vitaminer	☐	☐	☐	☐
Kalsium og Vitamin D	☐	☐	☐	☐
Annet	☐	☐	☐	☐

Spesifiser hvilke(t) merke på kosttilskudd og hvor mye du tar hver gang:

_____

_____

_____

_____

_____

_____

10. Solvaner

E. Hvor ofte de siste 3 månedene har du brukt solarium?

☐ Aldri ☐ Sjeldnere enn 1gang/måned ☐ 1 gang/måned ☐ 2-3 ganger/måned ☐ 1-2 ganger/uke

F. Hvor mange uker de siste tre månedene har du vært på badeferie (Norge eller Syden)? ☐ Har ikke vært på badeferie ☐ 1 uke ☐ 2-3 uker ☐ 4-6 uker ☐ 7 uker eller mer

G. Hvor mange dager/uker de siste tre månedene har du vært på fjellet i snø?

☐ Har ikke vært på fjellet i snø ☐ 1-6 dager ☐ 7-13 dager ☐ 2-3 uker ☐ 4 uker eller mer

H. Hvor mye utendørsaktivitet har du om sommeren (turer, hagearbeid, jobb)?

☐ Lite ☐ Middels ☐ Ganske mye ☐ Ute nesten hele tiden

14. Hvor ofte mosjonerer du sammenhengende i minst 30 minutter?

	Aldri	Sjeldnere enn 1 gang/uke	1 gang/ uke	2-3 ganger/uke	4-6 ganger /uke	Hver dag
Går	☐	☐	☐	☐	☐	☐
Jogger/Sykler	☐	☐	☐	☐	☐	☐
Svømmer	☐	☐	☐	☐	☐	☐
Styrketrening	☐	☐	☐	☐	☐	☐
Annet	☐	☐	☐	☐	☐	☐

_____

_____

15. Kryss av for feltene under som ev. gjelder for deg

☐ Har diabetes (sukkersyke)

☐ Har matvareallergi/intoleranse

☐ Spiser ikke melprodukter

☐ Spiser ikke melkeprodukter

☐ Spiser ikke kjøttprodukter

☐ Spiser ikke grønnsaker

16. Hvor stor vekt legger du på å ha et sunt kosthold?

Svært liten	Liten	Middels	Stor	Svært stor
☐	☐	☐	☐	☐

16. Vil du si at du har litt dårlig råd

(for eksempel liten mulighet for å kjøpe sunn mat eller trene på treningssenter eller lignende)?

☐ Nei ☐ Ja

17. Hva er din høyest fullførte utdannelse?

☐ Grunnskole ☐ Videregående skole ☐ Høyskole (3år)

☐ Universitet (1-3 år) ☐ Universitet (4 år eller mer)

TAKK FOR INNSATSEN!!!