Vitenskapskomiteen for mattrygghet
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

Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety

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Preface

The Norwegian Food Safety Authority has requested the Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) to make a risk and benefit assessment of marine oils. Marine oils (fish oils, cod liver oils, krill oils and seal oils) represent the product group accounting for the largest sales volume of food supplements, and fortification of regular foods with these oils is increasing.

The task from the Norwegian Safety Authority is divided in three parts:

Part 1: Risk assessment of decomposition substances and oxidation products in fish oils.

Part 2: Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods.

Part 3: Risk and benefit assessment of marine oils.

This report answers the terms of reference in the assessment of Marine oils - Part 2: Evaluation of negative and positive health effects of n-3 fatty acids as constituents of food supplements and fortified foods.

Summary

The Norwegian Food Safety Authority has requested the Norwegian Scientific Committee for Food Safety, VKM) to evaluate negative and positive human health effects from intake of n-3 fatty acids from food supplements and fortified foods.

N-3 fatty acids can be derived from marine or plant sources and are present in supplements and fortified foods as triacylglycerol, phospholipids or synthetic ethyl esters. The most important sources of marine n-3 fatty acids are fish oils produced either from fatty fish or from livers of lean fish and food supplements. Other sources are oils from marine mammals and krill, and oils rich in docosahexanoic acid (DHA) isolated from the micro algae *Schizochytrium sp*. and *Ulkenia sp*. are under certain conditions accepted as Novel Foods in the EU. Most studies concerning n-3 fatty acids in food supplements and fortified foods include eicosapentaenoic acid (EPA) and DHA either alone or in combinations. Plant oils rich in α-linolenic acid (ALA) are e.g. linseed oil and rapeseed oil.

What are the negative health effects of n-3 fatty acids?

The following negative health effects have been identified in studies with EPA and DHA; bleeding tendency, lipid peroxidation, impaired inflammation and other immune functions, impaired lipid and glucose metabolism and gastrointestinal disturbances.

An increased bleeding time has been found after intake of 6.9 g/day EPA and DHA in coronary heart disease patients on anti-coagulant medication. However, no negative health effects regarding bleeding complication in connection with EPA and DHA supplementations have been reported.

A limited number of studies have reported data on lipid peroxidation following n-3 fatty acid supplementation. Most of these did not show any increase in lipid peroxidation biomarkers. One large study with myocardial infarction patients taking 3.5 g EPA and DHA per day as ethyl ester showed increased thiobarbituric acid reactive substances (TBARS) in plasma. The relationship between *in vivo* lipid peroxidation and TBARS is uncertain. Moreover, none of the oxidative stress biomarkers are presently defined as risk factors of disease. The clinical relevance of lipid peroxidation is therefore unclear.

In several studies biomarkers of systemic inflammation in healthy subjects and different patient groups supplemented with n-3 fatty acids have been measured. No increase in C-reactive protein (CRP) after intake of marine n-3 fatty acids has been observed. EPA and DHA at doses of 5 g/day have been shown to activate endothelial cells (increased sVCAM-1 and sE-selectin) among individuals at high risk of cardiovascular diseases and in patients with coronary heart disease. Although low-grade systemic inflammation plays an important role in the pathology of some diseases, such as cardiovascular disease and type 2 diabetes, the clinical relevance of an increase of low-grade systemic inflammation is still uncertain.

The findings in the reviewed literature indicates no effects on glucose control in subjects with type 2 diabetes of supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). A minor increase in LDL-cholesterol (1-3%) in subjects with type 2 diabetes has been reported in meta-analyses following supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). No dose response relationship has been reported. However, the clinical relevance in subjects with type 2 diabetes of this minor increase in LDL-cholesterol is unclear because of a concomitant reduction in serum triacylglycerol and unchanged apolipoprotein B in the same subjects. No change in LDL-cholesterol was reported in the large coronary heart intervention trials including both subjects

with and without type 2 diabetes. Since the effect on LDL-cholesterol is minor, and of uncertain clinical significance, the Scientific Steering Committee will put less emphasis on this effect.

Negative health effects regarding gastrointestinal function, including abdominal cramps, flatulence, eructation, vomiting and diarrhea, have been reported, but seem to be associated with intake of an oily substance and not ascribed specifically to EPA and/or DHA.

Based on the reviewed literature, it is not possible to identify clear adverse effects from EPA and/or DHA, which can be used for setting tolerable upper intake levels.

In the studies investigating ALA, no negative health effects have been observed. Intake of ALA from linseed oil and margarine up to 8 g/day in addition to the contribution from a Western diet has not shown any negative health effects and it is therefore no rationale to set an upper tolerable intake level for ALA.

What are the positive health effects of n-3 fatty acids?

Positive health effects have been evaluated in the following domains; cardiovascular diseases, inflammation and immune function, CNS and mental health functioning. The studies have investigated EPA and DHA mainly as fish oils or as ethyl esters.

The strongest evidence for possible beneficial effects of n-3 fatty acid supplementation in humans is provided by large randomised controlled trials involving more than 43 000 study participants suffering from cardiovascular disease (secondary prevention). In patients given either 0.8 g EPA and DHA or 1.8 g of EPA as ethyl ester daily the risk of cardiovascular events and mortality was reduced.

Primary prevention from EPA and DHA supplementation has been less studied. However, EFSA has based its recommendation for adults on scientific evidence indicating that oily fish consumption (1-2 meals per week or dietary supplements containing EPA and DHA and equivalent to a range of 0.25 to 0.50 g of EPA and DHA daily) decrease the risk of mortality from coronary heart disease and sudden cardiac death.

Evidence suggests that intake of fish oil (containing from 1.6 to 7.1g/day EPA and DHA) might lessen symptoms or reduce the use of anti-inflammatory drugs in patients with rheumatoid arthritis.

EPA and DHA have been observed to have positive effects on early neurodevelopment, especially supplementation to preterm infants, and given to the pregnant women during the last half of pregnancy.

Positive effects in various CNS disorders are reported from EPA and DHA with doses ranging from 0.5 to 2.8 g/day.

This evaluation has shown that given a Western diet, the positive health effects are linked to EPA and DHA and not ALA. Therefore, the Scientific Steering Committee recommends that considerations on adequate intakes of n-3 fatty acids should be specific on ALA, on EPA and on DHA.

What are the health consequences of using n-3 fatty acids as ethyl esters?

Ethyl esters are synthetic compounds not naturally present in any foods. Ethyl esters of EPA and DHA were developed as a pharmaceutical to treat patients with cardiovascular diseases, and not for a healthy population. It is currently in use as food supplements.

The safety of EPA and DHA ethyl esters has only been evaluated as a drug in clinical settings. From the reviewed literature it has not been possible to distinguish the health effects from EPA and DHA as triacylglycerol from those of EPA and DHA as ethyl esters.

What is the intake of n-3 fatty acids in the Norwegian population and the status according to potential negative or positive health effects of n-3 fatty acids?

The main source of EPA and DHA for those who eat little or no fish is food supplements. The average intakes of EPA, docosapentaenoic acid (DPA) and DHA in different age groups range between 0.1-0.6 g/day without supplements and 0.3-0.9 g/day with supplements. The intake of EPA and DHA among children is low.

The intake of EPA and DHA does not exceed the doses associated with increased bleeding time, bleeding complications, or, although of uncertain significance as risk factors of disease, markers of lipid peroxidation (increased TBARS) and endothelial activation (increased sVCAM and sE-selectin) as reported in the reviewed studies.

The main dietary n-3 fatty acid in the Norwegian population is ALA and average intakes of ALA in the different age groups are 0.7-1.8 g/day and according to the Norwegian recommendation. The intake of ALA is well below an amount considered safe.

The Scientific Steering Committee notes that the intake of EPA and DHA is below the EFSA recommendation in a large fraction of children and adolescents. An intake below the EFSA recommendation may miss the opportunity of positive effects from EPA and DHA on neurodevelopment and prevention of coronary heart disease.

The evidence presented in this evaluation show that it is possible to obtain positive health effects in the Norwegian population from intake of EPA and DHA, including from food supplements, without any appreciable risk of negative or adverse health effects.

Sammendrag

Mattilsynet har bedt Vitenskapskomiteen for mattrygghet (VKM) om en vurdering av negative og positive helseeffekter av n-3 fettsyrer fra kosttilskudd og berikede matvarer.

N-3 fettsyrer kan utvinnes fra marine kilder eller planter, og forekommer i kosttilskudd og berikede matvarer i form av triacylglyseroler, fosfolipider eller som syntetiske etylestere. Den viktigste kilden til marine n-3 fettsyrer i kosttilskudd er fiskeolje fra fiskelever og fet fisk. Fiskeoljer fra sjøpattedyr og krill er eksempler på andre kilder. I EU er dokosaheksaenrike oljer isolert fra mikroalgene *Schizochytrium sp*. og *Ulkenia sp*. godkjent som såkalt ny mat. De fleste studiene med n-3 fettsyrer i tilskudd eller beriket mat er gjort med eikosapentaensyre (EPA) eller dokosaheksaensyre (DHA) enten hver for seg, eller sammen. Eksempler på planteoljer med høyt innhold av n-3 fettsyrer er linfrøolje og rapsolje, men da som α-linolensyre (ALA).

Hvilke potensielt negative helseeffekter av n-3 fettsyrer har vært studert?

Følgende negative helseeffekter av EPA og DHA har vært undersøkt i vitenskpelige studier; økt blødningstendens, lipidperoksidering, nedsatt betennelse- og immunfunksjoner, endret glukose- og fettmetabolisme og gastrointestinale plager.

Økt blødningstid er rapportert hos pasienter med hjerte- og karsykdom etter inntak av 6,9 g EPA og DHA per dag. Disse pasientene fikk i tillegg blodfortynnende medisin. Det er imidlertid ikke rapporter om noen tilfeller av blødningskomplikasjoner i forbindelse med tilskudd av EPA og DHA.

Enkelte studier har rapportert om lipidperoksidering i forbindelse med tilskudd av n-3 fettsyrer, men de fleste studiene viser ingen endring i biomarkører som skulle indikere lipidperoksidering. En økning i tiobarbitursyrereaktive substanser (TBARS) i plasma er vist i en stor studie hvor pasienter etter hjerteinfarkt fikk tilskudd av 3,5 g EPA og DHA per dag som etylester. Sammenhengen mellom *in vivo* lipidperoksidering og TBARS er usikker. Foreløpig er heller ingen biomarkører for såkalt oksidativt stress definert som risikofaktorer for utvikling av sykdom. Den kliniske betydningen av økt TBARS er derfor uklar.

Biomarkører for systemisk inflammasjon har vært målt i flere studier på både friske og i ulike pasientgrupper etter tilskudd med n-3 fettsyrer. Det er ikke observert noen økning i C-reaktivt protein (CRP) etter inntak av n-3 fettsyrer. I èn studie er det vist at en dose på 5 g EPA og DHA per dag kan aktivere cellene i karveggene hos personer med høy risiko for kardiovaskulære sykdommer og hos pasienter med hjerte- og karsykdommer (bestemt ved økt sVCAM og sE-selctin). Selv om en svak systemisk inflammasjon er en vesentlig patologisk faktor i enkelte sykdommer, for eksempel kardiovaskulære sykdommer og type 2 diabetes, er den kliniske betydningen fremdeles uavklart.

Resultatene fra litteraturen som er vurdert indikerer at glukosekontroll forblir uendret i personer med type 2 diabetes etter tilskudd med EPA og DHA i doser fra 0,8 til 4,8 g per dag (gjennomsnittlig: 2,4 g/dag).

En mindre økning i LDL-kolesterol (1-3%) hos personer med type 2 diabetes etter tilskudd med EPA og DHA i doser fra 0,8 til 4,8 g per dag (gjennomsnittlig: 2,4 g/dag) er funnet i meta-analyser, men et dose- responsforhold er ikke funnet. Den kliniske betydningen av denne mindre økningen i LDL-kolesterol er imidlertid usikker fordi det samtidig ble funnet en reduksjon i serum triacylglyserol og uendret apolipoprotein B i de samme individene. Det er ikke rapportert om endring i LDL-kolesterol verken i individer med eller uten type 2 diabetes

i de store hjerte/kar-intervensjonsstudiene. Ettersom serum triacylglyserol reduseres, og økningen LDL-kolesterol er liten, er den kliniske betydningen av denne økningen usikker. VKM har derfor lagt mindre vekt på disse funnene.

Det er rapportert om gastrointestinale plager som magekrampe, flatulens, gulping, oppkast og diaré etter tilskudd med EPA og DHA, men disse plagene synes å være assosiert med inntak av oljer generelt, og kan ikke relateres til EPA og DHA som sådan.

Det er ikke beskrevet klart definerte negative helseeffekter av EPA og/eller DHA i den litteraturen som er gjennomgått som kan benyttes til å fastsette tolerable inntaksnivåer.

Det er ikke observert negative helseeffekter i de studiene som har studert ALA. Inntak av ALA fra linfrøolje og margarin i doser opp til 8 g per dag i tillegg til bidrag fra et typisk vestlig kosthold har ikke vist noen negative helseeffekter, og det er derfor ikke rasjonale for å fastsette et øvre tolerabelt inntaksnivå for ALA.

Hvilke positive effekter fra n-3 fettsyrer har vært studert?

Potensielt positive helseeffekter på hjerte- og karsykdommer, betennelses- og immun funksjoner, utvikling av sentralnervesystem og mental helse fra n-3 fettsyrer har vært undersøkt. De fleste studiene omhandler EPA og DHA, og hovedsakelig i form av triacylglyserol eller etylestere.

Det beste bevisgrunnlaget for positive helseeffekter av EPA og DHA finner vi i store randomiserte kontrollerte studier som inkluderer mer enn 43 000 pasienter med hjerte- og karsykdommer. Pasienter som enten fikk 0,8 g EPA og DHA per dag eller 1,8 g EPA i form av etylester fikk redusert risiko for nye kardiovaskulære hendelser og død (sekundærprevensjon).

Primærprevensjon av hjerte- og karsykdommer (altså forebygging hos friske) med tilskudd av EPA og DHA er ikke undersøkt i like stor grad som ved sekundærprevensjon. European Food Safety Authority (EFSA) anbefaler imidlertid alle å spise 1-2 fiskemåltider i uken eller å ta kosttilskudd med EPA og DHA tilsvarende 0,25-0,50 g per dag. Anbefalingene er basert på vitenskaplig grunnlag for at EPA og DHA kan redusere dødelighet ved hjerte- og karsykdommer og redusere risiko for plutselig hjertestans.

Det finnes studier som viser at fiskeolje med 1,6-7,1 g EPA og DHA per dag lindrer symptomer for pasienter med reumatoid artritt, og kan lede til redusert bruk av anti-inflammatoriske medisiner.

EPA og DHA har vist å ha positive effekt på utvikling av det sentrale nervesystemet, og særlig har tilskudd til for tidlig fødte samt tilskudd til gravide i siste halvdel av svangerskapet vist seg å være positivt.

Det er rapportert om positive effekter på mental helse av EPA og DHA ved ulike doser mellom 0,5 og 2,8 g per dag.

Denne utredningen har vist at gitt et typisk vestlig kosthold, er de positive helseeffektene fra n-3 fettsyrer knyttet til EPA og DHA, og ikke ALA. VKM anbefaler derfor at det gjøres spesifikke vurderinger av tilfredsstillende inntak for hver enkelt n-3 fettsyre – altså ALA, EPA og DHA.

Har det noen betydning å ta n-3 tilskudd i form av etylestere?

Etylestere er syntetiske forbindelser som ikke forekommer naturlig i noen matvarer. I utgangspunktet ble etylestere av EPA og DHA utviklet som et legemiddel til behandling av pasienter med hjerte- og karsykdommer, og altså ikke til den friske befolkningen. Etylestere inngår nå i flere vanlige n-3 kosttilskudd.

Hvor trygt det er å bruke EPA og DHA i form av etylestere har kun vært vurdert for legemidler til pasienter med hjerte- og karsykdommer. Det har imidlertid ikke vært mulig å skille mellom helseeffekter fra EPA og DHA i form av triacylglyserol og helseeffekter fra EPA og DHA i form av etylestere.

Hva er inntaket av n-3 fettsyrer i den norske befolkningen vurdert opp mot potensielt negative og positive helseeffekter fra n-3 fettsyrer?

Den viktigste kilden til EPA og DHA for de som spiser lite eller ingen fisk er kosttilskudd. De gjennomsnittlige inntakene av EPA, dokosapentaensyre (DPA) og DHA i ulike aldersgrupper uten kosttilskudd er 0,1-0,6 g per dag, og med kosttilskudd 0,3-0,9 g per dag. Inntaket av EPA og DHA er lavt hos barn.

Inntaket av EPA og DHA overstiger ikke doser som har vært assosiert med økt blødningstid, blødningskomplikasjoner eller biomarkører for lipidperoksidering (økt TBARS) og aktivering av cellene i karveggen (økt sVCAM og sE-selectin) i enkelte studier. Det må poengteres at disse biomarkørenes rolle som indikatorer for sykdomsutvikling er usikre.

ALA er den n-3 fettsyren som vi har det høyeste inntaket av fra kosten i den norske befolkningen. Det gjennomsnittlige inntaket av ALA i de ulike aldersgruppene er 0,7-1,8 g/dag, og ligger rundt anbefalingen fra Helsedirektoratet. Inntaket av ALA vurderes som trygt.

VKM bemerker at inntaket av EPA og DHA er under EFSAs anbefalinger for en stor andel barn og unge. Et inntak som er lavere enn EFSAs anbefaling vil kunne medføre at disse går glipp av positive helseeffekter fra EPA og DHA på nevrologisk utvikling og forebygging av hjerte- og karsykdommer.

Litteraturen som er gjennomgått i denne vurderingen viser at det er mulig å oppnå positive helseeffekter i den norske befolkningen fra inntak av EPA og DHA, inkludert fra tilskudd, uten nevneverdig risiko for negative helseeffekter fra disse fettsyrene.

Definitions and terms

Acceptable macronutrient distribution ranges (AMDR)

AMDRs have been established for macronutrients as percentages of total energy intake. These are ranges of macronutrient intakes that are associated with reduced risk of chronic disease, while providing recommended intakes of other essential nutrients.

Adequate intake (AI)

The recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate—used when an RDA cannot be determined.

ALA (α-linolenic acid)

Essential polyunsaturated fatty acid in the n-3 series (18:3n-3) that must be supplied through the diet. Main source in the diet is terrestrial plants, nuts and particularly plant oils such as linseed oil and rapeseed oil. ALA is often referred to as plant n-3 fatty acid.

ARA (arachidonic acid)

Polyunsaturated fatty acid in the n-6 series (20:4 n-6) that can be synthesised in the human body from linoleic acid (LA). Main sources in the diet are animal meat, eggs and dairy products.

CABG

Coronary artery bypass graft surgery.

CHD

Coronary heart disease.

CNS

Central nervous system.

CRP

C-reactive protein. A commonly used inflammation marker.

CVD

Cardiovascular disease.

DRI

Dietary reference intake.

E%

Energy percent. Percent of total energy intake.

EPA (eicosapentaenoic acid)

Polyunsaturated fatty acid in the n-3 series (20:5n-3) that can be synthesised in the human body from ALA. Main sources are marine plants, fish and other seafood and food supplements. EPA, DPA and DHA are often referred to as marine n-3 fatty acids.

DPA (docosapentaenoic acid)

Polyunsaturated fatty acid in the n-3 series (22:5n-3) that can be synthesised in the human body from ALA. Main sources are marine plants, fish and other seafood and food supplements. EPA, DPA and DHA are often referred to as marine n-3 fatty acids. In the nomenclature, DPA may also refer to 22:5n-6. In the present evaluation DPA refers only to 22:5n-3.

DHA (docosahexaenoic acid)

Polyunsaturated fatty acid in the n-3 series (22:6n-3) that can be synthesised in the human body from ALA. Main sources are marine plants, fish and other seafood and food supplements. EPA, DPA and DHA are often referred to as marine n-3 fatty acids.

IBD

Inflammatory bowel disease.

INR

International normalised ratio related to bleeding. INR is a measure of the biological effect of vitamin K-dependent coagulation proteins. High INR level involves a higher risk of uncontrolled bleeding.

IOM

Institute of Medicine. IOM is an independent, nonprofit organization that works outside of government to provide unbiased and authoritative advice to decision makers and the public in USA.

LA (linoleic acid)

Essential polyunsaturated fatty acid in the n-6 series (18:2 n-6) that must be supplied through the diet. Main source in the diet is terrestrial plants and particularly plant oils such as soybean oil and sunflower oil.

LDL

Low density lipoprotein.

Lowest adverse effect level (LOAEL)

The lowest dose of a substance for which an adverse effect can be observed in a long-term toxicity animal study.

Marine n-3 fatty acids

EPA, DPA and DHA.

MDA

Malondialdehyd.

No observed adverse effect level (NOAEL)

The highest dose of a substance for which no adverse effect has been observed in long-term toxicity study.

n-3 fatty acids

ALA, EPA, DPA and DHA.

n-3 and n-6

The number of the expression indicates the position in an unsaturated fatty acid of the first carbon of the first double bond. The n indicates that the counting starts from the methyl end including the methyl carbon.

PCI

Percutaneous coronary intervention. Commonly known as coronary angioplasty.

Primary prevention

Prevention of diseases and conditions before their biological onset. In this evaluation this means positive health effects of n-3 fatty acids in healthy subjects.

PUFA

Polyunsaturated fatty acid.

Recommended dietary allowance (RDA)

The average daily dietary nutrient intake level sufficient to meet the nutrient requirement of nearly all (97-98 percent) healthy individuals in a particular life stage and gender group.

Secondary prevention

Action performed to take care of early symptoms of a disease and preclude the development of possible irreparable medical conditions. In this evaluation this means positive health effects of n-3 fatty acids in patients.

sVCAM

Soluble vascular cell adhesion molecule.

TAG

Triacylglycerol.

TBARS

Thiobarbituric acid reactive substances.

Tolerable upper intake level (UL)

The highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase. An UL is set taking into account the scientific uncertainties in the data by dividing the NOAEL by an uncertainty factor. This factor accounts for uncertainties in human inter-variability and extrapolation of data from animals to humans, as well as other uncertainties in the data.

Contents

1 Background

The consumption of n-3 fatty acids from fortified foods and food supplements is increasing due to claimed positive health effects. According to the Norwegian Food Safety Authority (Mattilsynet), the sale of n-3 fatty acid supplements including cod liver oil constituted 22% of the total market of food supplements in 2006.

N-3 fatty acids can be derived from marine or plant sources and can have different chemical structures (triacylglycerol, phospholipids or free fatty acids) and concentrations in the fortified foods and supplements. Ethyl esters of n-3 fatty acids can be produced synthetically.

The most important sources of marine n-3 fatty acids are fish oils produced from fatty fish or oils from livers of lean fish. Remaining material from processing of fish and other sea foods will probably become a more important source in the future. Other sources are oils from marine mammals and krill.

N-3 fatty acids can also be isolated from algae. Oils rich in docosahexanoic acid (DHA) isolated from the micro algae *Schizochytrium sp*. and *Ulkenia sp*. are under certain conditions accepted as Novel Foods for use in the EU.

The scientific literature regarding the different n-3 fatty acids, particularly eicosapentaenoic acid (EPA) and DHA and their positive impact on health is emerging. However, a prolonged high intake of EPA and DHA has been negatively associated with bleeding, lipid peroxidation, inflammation and immune function, impaired lipid and glucose metabolism.

In 2006, VKM published: A comprehensive assessment of fish and other seafood in the Norwegian diet (Alexander *et al.*, 2006). Similar assessments have been done by the authorities in Denmark (Fødevaredirektoratet, 2003), UK (SACN, 2004) and Sweden (Becker *et al.*, 2007). The European Food Safety Authority (EFSA) has in recent years assessed several health claims and nutritional claims related to n-3 fatty acids (EFSA, 2005; EFSA, 2008a; EFSA, 2008b; EFSA, 2008c; EFSA, 2008d; EFSA, 2008e; EFSA, 2008f; EFSA, 2008g; EFSA, 2009b) and recently published labelling reference intake values for n-3 and n-6 polyunsaturated fatty acids and dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol (EFSA, 2009a; EFSA, 2010b).

All these reports and opinions are valuable background documents for the present evaluation.

The several risk and benefit assessments from different countries (including Norway) are based mainly upon studies of health outcomes from consumption of fish and other seafood (Alexander *et al.*, 2006). Thus, the present evaluation addresses the intake of n-3 fatty acids as constituents of food supplements and fortified foods.

2 Terms of reference

The Norwegian Food Safety Authority has requested the Norwegian Scientific Committee for Food Safety to evaluate the positive and negative health effects of n-3 fatty acids added to food supplements and fortified foods. The various forms of n-3 fatty acid and various ratios of the n-3 fatty acids should be evaluated.

- What are the negative health effects of n-3 fatty acids?
  - Is it possible to set tolerable upper intake levels (UL) for the n-3 fatty acids?
  - What are the health consequences of using n-3 fatty acids as ethyl esters?
- What are the positive health effects of n-3 fatty acids?

- What is the intake of n-3 fatty acids in the Norwegian population and status according to potential positive or negative health effects of n-3 fatty acids?

3 Introduction

There are two main sources of the four dietary n-3 fatty acids, i.e. α-linolenic acid (ALA), EPA, docosapentaenoic acid (DPA) and DHA. Terrestrial plants are the main source of ALA which is present in edible oils such as rapeseed, walnut and linseed oil, whereas EPA, DPA and DHA are mainly derived from fish and other seafoods and to a smaller extent from other sources like algae and fungi. Hence, ALA is often referred to as *plant* n-3 and EPA, DPA and DHA as *marine* n-3. The n-3 fatty acids are present in foods as free fatty acids, triacylglycerols (TAGs) and phosphoplipids. In concentrated supplements synthetically produced ethyl esters are often used.

Fish oils have traditionally been used as the main source of marine n-3 fatty acids in supplements. Particularly cod liver oil has been widely used in Norway, traditionally as a source of the fat soluble vitamins A and D, but later also with a focus on EPA and DHA. The n-3 fatty acids used in food supplements and for fortification of regular foods vary, but the marine n-3 fatty acids seem to be most commonly used. The ratios of EPA to DPA and DHA differ between the various sources of marine n-3 fatty acids, but little attention has been given to the individual n-3 fatty acids.

No specific recommendation related to the intake of EPA, DPA or DHA is given to the Norwegian population (Sosial- og helsedirektoratet, 2005). The Nordic countries recommend a total intake of n-3 fatty acids of approximately 0.5 E%. In general, such a recommendation indicate that all n-3 fatty acids are of the same biological activity, and the interpretation and implementation of such recommendations can be made in many ways, i.e. the requirement can be covered by mixtures of fatty acids with varying proportions of ALA, EPA, DPA and DHA or by one single n-3 fatty acid. However, the individual n-3 fatty acids have shown different physiological effects. Moreover, the documented positive health effects have mostly been observed in studies with EPA and DHA. EFSA recommends an intake of EPA and DHA at 0.25 g/day, which is equivalent to 0.125 E% at an intake of 1800 kcal (7.5MJ).

Thus, during the last decade several official agencies as well as different organisations have issued specific recommendations for EPA and DHA for health promotion and decreased risk of cardiovascular diseases, and the consumption of EPA and DHA from food supplements or fortified foods have increased.

Currently many questions regarding potential negative health effects as a consequence of rancid fish oils and/or high doses of n-3 fatty acids, particularly EPA and DHA, have been raised. It is therefore important to assess the total intake of EPA and DHA in the Norwegian population related to potential risks and benefits as well as to recommendations for the n-3 fatty acids.

EFSA has developed a guidance for performing human risk-benefit assessments of food. The guidance recommends a stepwise method and that the risk- and benefit assessment should be comprised of three elements, i.e. risk assessment, benefit assessment and risk-benefit comparison (EFSA, 2010a). As for the risk assessment paradigm which is well established, the benefit assessment should also include the following steps: positive health effect identification, positive health effect characterisation (dose-response assessment), exposure assessment and benefit characterisation. The present evaluation addresses to some extent the first steps in the EFSA guidance i.e. an initial assessment, addressing the question whether the

health risks outweigh the health benefits or vice versa and a refined assessment aiming at providing semi-quantitative or quantitative estimates of risks and benefits at relevant exposure by using common metrics.

The conclusions in the present evaluation regarding the negative and postitive health effects of n-3 fatty acids are based on systematic literature searches, previous assessments from official organisations e.g. US Institute of Medicine (IOM), EFSA, Food and Agricultural Organization of the United Nations) FAO, US Food and Drug Administration (FDA) and World Health Organization (WHO) and intake assessments of n-3 fatty acids from nationally representative dietary surveys in Norway. In several of the included studies, the background diet is not known, but is assumed to be a typical Western diet low in ALA. The present evaluation is based on human studies although results of animal and cell studies are mentioned in some cases.

Rancid fish oils or lipid peroxidation that may occur during processing and storage of food supplements and fortified foods are adressed in a separate opinion from the Steering Committee of the Norwegian Scientific Committee for Food Safety (VKM) – Marine oils part 1 and is therefore not addressed in this evaluation.

4 Fatty acids

4.1 Classification and nomenclature of fatty acids

Fatty acids are chemical molecules consisting of a hydrocarbon chain (-CH_2-) varying in length from 2 to 22 or more carbons with a carboxyl group (-COOH) at one end and a methyl group (-CH_3) at the other end (Figure 4.1). The carbon atoms in the fatty acid are numbered (in red above the molecule) from the carboxyl group (COOH) and the last carbon atom has the designation n or omega (ω), see Figure 4.1. For example, EPA, which has 20 carbon atoms and 5 double bonds, with the first one located at n-3, can also be written as 20:5 n-3. The Greek nomenclature is also often used (as ω, see blue below). Note that according to the former nomenclature, it is carbon atom number 2 that is α. The two designations (n-3 and ω-3 fatty acids) are the same and mean that the first double bond counted from the n or ω–end is positioned between carbon atoms three and four. If the first double bond is between carbon atoms six and seven or nine and ten counted from the ω–end, it would be an n-6 or n-9 fatty acid, respectively.

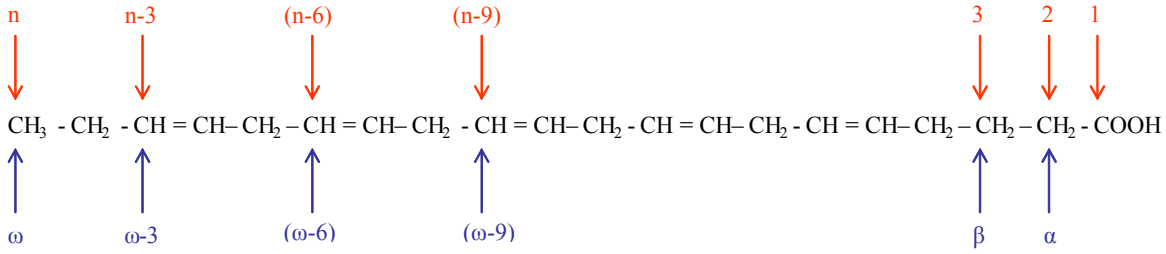


Figure 4.1: A schematic outline of the structure and nomenclature of the polyunsaturated n-3 fatty acid eicosapentaenoic acid with 20 carbon atoms and 5 double bonds, often abbreviated EPA (20:5 n-3).

The most common fatty acids in foods have an even number of carbon atoms ranging from 12-18 carbons. Fatty acids are mainly categorised according to chain length, number of double bonds and the configuration of the double bond.

Fatty acids carrying the maximum number of hydrogen atoms are termed saturated. A fatty acid becomes unsaturated when a pair of hydrogen atoms is removed, thereby creating a double bond between the adjacent carbon atoms where the hydrogen atoms disappeared. In the human body, enzymes with high specificity regarding position catalyze desaturation at certain positions, and therefore the location of double bonds is tightly controlled.

Humans and animals can synthesize most of the fatty acids they need, except for linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3). As a result, these two fatty acids are called essential and must be supplied through the diet.

The different types of fatty acids are present in many types of fat, but the ratios may vary considerably. It is common to divide fatty acids into saturated, monounsaturated (one double bond) and polyunsaturated (two or more double bonds). Examples are given in Table 4.1 and Figure 4.1 which illustrates the structure and nomenclature of fatty acids.

Table 4.1: Examples of common fatty acids, abbreviations and typical sources.

Trivial name	Short name	Abbrev ation	Typical sources
Saturated			
Palmitic acid	16:0	PA	Animal meat, eggs, plant oils, dairy products
Stearic acid	18:0	SA	Animal meat, eggs, plant oils, dairy products
Monounsaturated			
Oleic acid	18:1 n-9	OA	Plant oils (particularly olive oil), animal meat, eggs, dairy products
Polyunsaturated			
N-6			
Linoleic acid	18:2 n-6	LA	Plant oils (e.g. soybean oil, sunflower oil and corn oil)
Arachidonic acid	20:4 n-6	ARA	Animal meat, eggs, dairy products
N-3			
α-linolenic acid	18:3 n-3	ALA	Plant oils (e.g. linseed oil, rapeseed oil and walnut oil)
Eicosapentaenoic acid	20:5 n-3	EPA	Fish and seafood, food supplements, fortified foods
Docosapentaenoic acid	22:5 n-3	DPA	Fish and seafood, food supplements, fortified foods
Docosahexaenoic acid	22:6 n-3	DHA	Fish and seafood, food supplements, fortified foods

4.2 Dietary sources of n-3 fatty acids

Plant oils, such as linseed oil, rapeseed oil and walnut oil, contain significant amounts of ALA. But in the Western diet the plant oils used for food production are quite low in ALA and high in linoleic acid, like soybean oil, sunflower oil and corn oil. Fish and other seafoods, especially fatty fish and phospholipids from lean fish fillet, cod liver oil and other n-3 fatty acid supplements are our main sources of EPA, DPA and DHA. DPA is a minor fatty acid compared to EPA and DHA in fish and fish oils, and relevant scientific data regarding DPA is

limited. During the last decades, supplements and fortified foods with various fish oils as well as oils extracted from the blubber of sea mammals and krill have become important sources. Also plant oils rich in ALA are used in fortification of foods and supplements. Table 4.2 shows the content of n-3 fatty acids in some examples of regular foods, fortified foods and food supplements.

Table 4.2: Content of n-3 fatty acids in some examples of regular foods, fortified foods and food supplements in g/100g.

Food	ALA	EPA	DPA	DHA	EPA+DPA+DHA
Farmed salmon, fillet[1]	0.6	0.7	0.3	1.0	2.0
Farmed trout, smoked fillet[1]	0.2	0.8	0.3	1.3	2.4
Cod, fillet[1]		0.09	0.01	0.2	0.3
Mackrel (autumn), fillet[2]		1.0	0.2	2.5	3.7
Shrimps[2]		0.1	0.01	0.1	0.21
Spreadable caviar, regular[2]		0.1	-	0.2	0.3
Spreadable caviar, fortified[3]	2.4	1.4	-	1.3	2.7
Margarine, soy[2]	3.1	-	-	-	
Bread, fortified	0.2	0.05	-	0.06	0.11
Sunflower oil[2]	0.3				
Corn oil[2]	1.24				
Soybean oil[4]	5.2				
Linseed oil[3]	50.6				
Rapeseed oil[3]	8.4				
Cod liver oil[2]		8.8		10.4	20.6
N-3 supplement with ALA[3]	31.3				

[1]www.nifes.no/sjomatdata/.
[2]From KBS (Dietary Assessment System), IE96 mainly based on data from Norwegian Food Composition Table 1995 (Matvaretabellen, 1995) including fatty acids in 400 food items.
[3]Average content in products used in the intake assessment. Information provided by the manufacturers.
[4]Araujo *et al.*, 2010.

In high quality oils of plant and marine origin fatty acids are present mainly in the form of TAGs, and only small amounts are free fatty acids or bound in phospholipids. Oils made from fish and sea mammals are used as raw materials for the production of highly concentrated marine n-3 ethyl esters. Recently, phospholipids extracted from krill have become an additional source for marine n-3 fatty acids.

4.3 Interconvertion of C18, C20 and C22 fatty acids

Dietary ALA and linoleic acid are utilized in many pathways including the elongations and further desaturation to the n-3 fatty acids EPA, DPA and DHA and the n-6 arachidonic acid (ARA), respectively. Desaturases remove two hydrogen atoms from a fatty acid, creating a carbon-carbon double bond. Delta (Δ) denotes that the double bond is created at a fixed position from the carboxyl group of a fatty acid, i.e. Δ-5 and Δ-6 desaturase introduces a double bond at the 5^{th} and 6^{th} position from the carboxyl end, respectively. ALA is metabolised to DHA by Δ-6 desaturation, elongation, and Δ-5 desaturation to yield EPA. EPA will be further elongated, Δ-6 desaturated, and chain-shortened by partial peroxisomal β-oxidation to DHA (Voss *et al.*, 1991; Moore *et al.*, 1995; Sprecher *et al.*, 1999). Linoleic acid is desaturated and elongated to arachidonic acid, which can be further elongated to 22:4 n-6 and 22:5 n-6 using the same pathway as in synthesis of DHA from ALA (Voss *et al.*, 1991; Moore *et al.*, 1995; Sprecher *et al.*, 1999). Thus, they are competing for the same enzyme systems and tracer studies as well as dietary supplementation studies have revealed that the conversion of ALA to DHA is in the range of 0.5% (Plourde & Cunnane, 2007). The fractional conversion of ALA to EPA and DHA appears to be greater in women, possibly mediated through estrogen (Graham, 2004; Giltay *et al.*, 2004). Also, a greater proportion of ALA appears to be β-oxidized in men and used as an energy source, leading to lower plasma levels at similar intakes (Graham, 2004). Consistent with these sex differences in metabolism, it has been reported that women achieved higher plasma levels of DHA and ALA in comparison with men on the same diet (Giltay *et al.*, 2004). In a typical Western diet with high intake of linoleic acid, the conversion of ALA to EPA, DPA and DHA is reduced.

4.4 Molecular biological actions of n-3 PUFA

Recent research on the metabolic pathways of fatty acids has altered the view of fat metabolism and the importance of fatty acids in tissue function. Any positive and/or negative health effects of n-3 fatty acids are mediated by underlying molecular mechanisms of action of these fatty acids. The effects can be mediated by one specific mechanism of action or combinations of mechanisms, and it may be difficult to distinguish between these mechanisms when looking at physiological effects in humans. In general PUFAs mechanism of action is believed to be due to its capability to alter the membrane lipid composition, its impact on cellular metabolism, signal transduction, and regulation of gene expression. The use of *in vitro* cell culture systems and animal models has revealed unique mechanisms of action of n-3 fatty acids in gene regulation, metabolic and signal transduction pathways. Most of the studies, however, have not examined the effects of the different single n-3 fatty acids, but rather compared the effects of mixtures of n-3 fatty acids. The exact molecular and cellular effects of each of the n-3 fatty acids are therefore not known.

4.4.1 Fatty acids as structural components

Fatty acids are incorporated into phospholipids of cell membranes. DHA and arachidonic acid are by far the most prevalent n-3 and n-6 fatty acids in membranes wheras the concentration of membrane bound EPA is much lower. Because of their flexible structures they contribute to the fluidity of membranes. This fluidity is important for proper functioning of proteins embedded in the membrane lipid bilayer, such as receptors, ion channels, transporters and enzymes. Any changes in the fatty acid composition of the membranes affect their activity, leading to changes in cellular metabolism and signal transduction. No studies have been identified that has addressed possible functional differences between EPA and DHA in

membranes, but since biological membranes are dependent on acyl chain length and degree of saturations it is expected that alterations in content of these fatty acids will differently affect membrane structure and function. The membranes contain heterogenous domains composed of different structures and physicochemical properties called lipid rafts. *In vitro* studies have shown that treatment with EPA can increase the amount of EPA and DHA in lipids isolated from rafts and thus influences the movement of proteins in the membrane (Stulnig *et al.*, 2001).

4.4.2 Fatty acids as precursors of bioactive metabolites

Fatty acids are enzymatically oxidised to generate a range of signalling molecules called eicosanoids, which are bioactive metabolites involved in a great number of regulatory mechanisms. Worth mentioning in this context are the roles of the prostaglandins, leukotrienes, and lipoxins in inflammation and the action of the thromboxanes and prostacyclins in haemostasis. In general, eicosanoids derived from arachidonic acid have a pro-inflammatory effect, whereas eicosanoids (e.g. thromoxanes, prostaglandines and leukotrienes) derived from EPA, as well as docosanoids (e.g. resolvines) derived from DHA, have a less pro-inflammatory effect. Other metabolites of EPA and DHA, called resolvins and protectins, reduce and help resolving the inflammatory response (Serhan *et al.*, 2008).

4.4.3 Fatty acids as modulators of enzyme activity

The extent to which fatty acids inhibit or stimulate enzymes depends on the type and the concentration of the fatty acid as well as if the fatty acids are esterified to TAGs or phospholipids. PUFA may alter cell membrane composition of microdomains and thereby modulating the relay of extracellular signals from surface receptors to downstream signalling networks. By altering cell membrane composition, PUFAs affect several enzymes essential for functioning of cells involved in signal transmission (neurons, cardiac cells, endocrine cells). EPA and DHA have shown to inhibit protein kinases, which activate other important enzymes (Mirnikjoo *et al.*, 2001; Seung Kim *et al.*, 2001). Differences in action and potency between EPA and DHA have been reported (Vreugdenhil *et al.*, 1996). PUFAs also interact directly with calcium regulatory enzymes, preventing a rise in intracellular calcium. N-3 fatty acids and particularly DHA have been shown to inhibit the Na^+-K^+ ATPase pump, preventing a rise in intracellular potassium. Changes in ion permeability seem to be directly dependent upon the degree of unsaturation of fatty acids and DHA has been shown to have a more pronounced effect on membrane ion permeability in comparison with ALA (Ehringer *et al.*, 1990).

4.4.4 Fatty acids as regulators of gene expression

Fatty acids affect gene expression at the nuclear level either directly or through one of their metabolites (Castrillo & Tontonoz, 2004; Jump *et al.*, 2005). They can also alter various signalling cascades within the cell, thereby raising second messenger concentrations and thus affecting gene expression. Well-characterized transcription factors involved in fatty acid-induced gene expression includes the peroxisome proliferator-activated receptors (PPARs), hepatic nuclear factor-4α (HNF-4α), the liver X receptors (LXRs) and the sterol regulator element binding protein (SREBPs) (Sampath & Ntambi, 2005). PPARs and LXRs can inhibit the activity of the transcription factor nuclear factor kappaB (NF-κB), which controls genes involved in inflammation, cell proliferation and apoptosis (DeBosscher *et al.*, 2006). Fatty

acids can also interfere with the Toll-like receptors involved in oxidative stress and inflammation (Lee *et al.*, 2003; Wong *et al.*, 2009). The G protein-coupled receptor 120 (GPR120) has recently been characterised as an n-3 fatty acid receptor which mediates the anti-inflammatory effects of EPA and DHA (Oh *et al.*, 2010). Jump has reviewed the effect of n-3 fatty acid regulation of hepatic gene transcription based on *in vitro* studies and animal experiments (Jump, 2008). ALA is a weak regulator of hepatic gene expression, while EPA and DHA are strong regulators. Furterhmore, EPA, but not DHA, is a potent activator of PPARα in the liver. DHA, but not EPA, seems to play a more important role in the regulation of SREBP acitivity. This shows that the different n-3 fatty acids can specifically regulate gene expression, but to which extend this occurs in all cell types is still unknown.

4.5 Lipid peroxidation in humans

Oxidative damage to lipids (lipid peroxidation) occurs to PUFAs as they have a greater number of double bonds in the hydrocarbon chain which are susceptible to oxidation. The first phase of lipid peroxidation *in vivo* is the initation when abstraction of $H^{\cdot}$ radical from the hydrocarbon chain gives a lipid radical. The second phase is propagation when the lipid radical react with oxygen to give a lipoperoxyl radical ($LOO^{\cdot}$) which in turn reacts with a second lipid to yield a lipid radical and a lipid hydroperoxyde (LOOH) which can generate secondary oxidation products such as aldehydes (Porter *et al.*, 1995). Lipid peroxidation can affect membrane fluidity, permeability and function and thereby change the functionality of the cells (Berlett & Stadtman, 1997). The common methods used today to measure lipid peroxidation in human samples are all indirect and includes malondialdehyde (MDA), lipid hydroperoxides, conjugated dienes, oxLDL and F2-isoprostanes. The evidence that the various methods actually reflect lipid peroxidation *in vivo* is limited. In order to combat lipid peroxidation a complex antioxidant defense system has evolved. This defense system can prevent or repair oxidative damage such as lipid peroxidation and includes a wide and diverse group of both endogenous antioxidants and exogenous antioxidants from the diet. If there is an imbalance between reactive oxygen species and the organisms capacity to neutralize or eliminate them oxidative stress may occur. Oxidative stress is involved in various pathological states including inflammation, atherosclerosis, neurogenerative diseases and cancer. Oxidation of LDL to oxLDL is linked to the initiation and progression of atherosclerosis (Steinberg *et al.*, 1989).

5 Existing recommendations for n-3 fatty acids

ALA is as described in chapter 4 an essential fatty acid which cannot be synthesized by humans. The recommendations for ALA and other n-3 fatty acids (EPA and DHA) vary in different countries due to a number of factors such e.g. total fat intake, type of fat, different background diets, etc. In addition, during the last decades it has become evident that the *de novo* production of EPA and DHA from ALA in humans is limited, especially with a high n-6 fatty acid intake, and that EPA and DHA possess important physiological effects. Therefore many official organisations have established specific recommendations for EPA and DHA.

In a recent expert consultation report from the Food and Agriculture Organization of The Unites Nations/The World Health Organization (FAO/WHO, 2010) it was concluded that the total n-3 fatty acid intake can range between 0.5-2E%[1], whereas the minimum dietary requirement for ALA (>0.5 E%) prevents deficiency symptoms in adults. For adult males and non-pregnant/non-lactating adult females 0.25 g EPA and DHA per day is recommended. For adult pregnant and lactating females, the minimum intake for optimal adult health and fetal and infant development is 0.3 g EPA and DHA per day, of which at least 0.2 g per day should be DHA (FAO/WHO, 2010).

The Nordic Nutrition Recommendations (NNR Project Group, 2004) have no specific recommendations for EPA, DPA or DHA, but recommend that the intake of n-3 fatty acids are at least 0.5 E% for children from 2 years of age and adults, and at least 1 E% for infants 6-11 months and pregnant and lactating women. For an adult person 1 E% will correspond to 2.0-2.6 g of n-3 fatty acids per day, if the total energy intake is 7.5-10 MJ. The Nordic recommendations are adopted in the the Norwegian recommendations (Sosial- og helsedirektoratet, 2005).

In USA there are no official recommendations for EPA, DPA or DHA, and the current Adequate Intake (AI) for ALA is 1.6 g/day for men 19- >70 years, and 1.1 g/day for women 19- >70 years. The US Institute of Medicine (IOM) has suggested an Acceptable Macronutrient Distribution Range (AMDR) for ALA at 0.6 to 1.2 E% (IOM, 2005).

Sweden follows the Nordic Nutrition Recommendations (NNR Project Group, 2004) on n-3 fatty acids, however, the National Food Administration in Sweden has recommended a dietary intake of 100-300 mg DHA per day, preferably from fatty fish during pregnancy and lactation (Becker *et al.*, 2007).

EFSA has recently proposed an AI of 0.10 g/day DHA for infants (>6 months of age) and small children below 24 months based on visual function.

Taking into account that available data are insufficient to derive an Average Requirement and that an intake of 0.25g/day of EPA and DHA appears to be sufficient for primary prevention in healthy subjects, EFSA has set an AI of 0.25 g/day for EPA and DHA for adults. EFSA has based its recommendation for adults on scientific evidence indicating that oily fish consumption (1-2 meals per week or dietary supplements containing EPA and DHA equivalent to a range of 0.25 to 0.50 g of EPA and DHA daily) decreases the risk of mortality from coronary heart disease and sudden cardiac death.

For children aged 2 to 18 years the dietary advice is consistent with advice for the adult population (EFSA, 2010b).

The main source of EPA and DHA for Norwegian consumers is fatty fish and cod liver oil. Fish, fish oils and cod liver oil, is considered to be a part of a healthy diet and an increased intake of fish in Norway is considered to be beneficial for health (http://www.helsedirektoratet.no/vp/multimedia/archive/00015/IS-0210_pdf_15034a.pdf). Cod liver oil is recommended to children from 4 weeks of age in Norway as vitamin D supplementation (Sosial- og helsedirektoratet, 2005). An overview of the different recommendations for n-3 fatty acids is given in Table 5.1.

[1]Energy percent; percent of total energy intake (e.g. at energy intake at 2000 kcal (8.4 MJ), 0.5 E% is equivalent to 1.3 g n-3 fatty acids per day).

Table 5.1: Recommendations for n-3 fatty acids.

Organisation, source	Recommended intake
Norwegian Directorate of Health, Sosial- og helsedirektoratet, 2005.	At least 0.5 E% n-3 fatty acids for children from 2 years of age and adults, and at least 1 E% for infants 6-11 months and pregnant and lactating women.
Nordic Nutrition Recommendations, NNR Project Group, 2004.	1 E% n-3 fatty acids from 6 months of age.
The National Food Administration in Sweden, Becker *et al.*, 2007.	As the Norwegian recommendations. 100-300 mg DHA per day, preferably from fatty fish in pregnancy and during lactation.
EFSA, 2010b.	ALA 0.5 E%. Children from 2 to 18 years and adults; an intake of 0.25 g EPA and DHA per day. Pregnant and lactating women; 0.25 g EPA and DHA per day plus additional 0.10-0.20 g DHA per day. Older infants (>6 months of age) and young children below the age of 24 months of age; 0.10 g DHA per day.

In addition to the general n-3 recommendations, epidemiologic studies and randomised controlled trials of coronary heart disease (CHD) events in patiens, have led to specific recommendations for EPA and DHA independent of source, although many official organisations emphasize regular fish consumption, i.e. a food based approach (Table 5.2).

In summary, this evaluation refers to the Norwegian recommendation (based on the Nordic recommendation) for total n-3 fatty acids at 0.5 E%, as well as the recent recommendation from EFSA on 0.25 g/day EPA and DHA for children and adolescents above 2 years and adults and 0.10 g/day DHA for infants and small children (6-24 months).

Table 5.2: Recommendations of EPA and DHA for primary prevention of coronary heart diseases.

Organisation, source[1]	Recommendations
FAO/WHO, 2003.	Regular fish consumption, 1-2 servings per week; each serving should provide the equivalent of 200-500 mg of EPA and DHA.
The American Dietetic Association/Dietitians in Canada, (Kris-Etherton *et al.*, 2007).	500 mg/day of EPA and DHA provided by two servings of fatty fish/week.
American Heart Association, (Lichtenstein *et al.*, 2006).	Two servings of fish (preferably fatty) per week.
American Diabetes Association, (Bantle *et al.*, 2008).	Two or more servings of fish per week (with the exception of commercially fried fish fillets) providing omega-3 polyunsaturated fatty acids are recommended.
Australia and New Zealand National Health and Medical Research Council., 2006.	A suggested dietary target for women and men 19- >70 years of age is 430 and 610 mg/day of DHA/EPA/DPA, respectively.
EFSA, 2010b.	1-2 fish meals of fatty fish per week or 250 mg EPA and DHA per day.
European Society for Cardiology, (DeBacker *et al.*, 2003).	Fatty fish and n-3 fatty acids have particular protective properties for primary cardiovascular disease prevention.
The National Food Administration in Sweden, (Becker *et al.*, 2007).	2-3 servings of fish per week of which one serving with fatty fish covers the requirement for EPA and DHA.
AFFSA, CNERNA & CNRS in France, (A.Martin (Ed.), 2001).	500 mg per day of EPA and DHA; minimum 120 mg DHA per day.
Health Council of the Netherlands, 2010.	Fish twice per week, one of which should be fatty to achieve the dietary reference intake of 450 mg per day of n-3 fatty acids from fish.
Superior Health Council of Belgium, 2004.	Minimum 0.3E% from EPA and DHA for adults (approximately 667 mg per day).
International Society for the Study of Fatty Acids and Lipids, (ISSFAL, 2004).	A minimum intake of EPA and DHA (500 mg per day) is recommended for cardiovascular health.
United Kingdom Scientific Advisory Committee on Nutrition, (SACN, 2004).	Consume at least two portions of fish per week, of which one should be fatty, and provide 450 mg of EPA and DHA per day.
Report from the National Council of Nutrition, (Nasjonalt råd for ernæring, 2011).	A daily supplement of cod liver oil or other n-3 supplement may be an alternative to ensure a sufficient intake of long-chain n-3 fatty acids (EPA, DHA) for those who do not eat fatty fish. The primary advice is to eat fatty fish.

[1]Most references found in Kris-Etherton *et al.*, 2009.

6 Negative health effects related to n-3 fatty acids in humans

In this chapter, the possible negative health effects associated with high intake of n-3 fatty acids, mostly from food supplements, are evaluated. The evaluation of negative health effects is based on literature from a systematic search in MEDLINE and EMBASE, predominantly studies with EPA and DHA as supplements, previous safety assessments from Food and Drug Administration and US Institute of Medicine and also documentation from a safety assessment of an n-3 formulation as a registered drug.

"Negative health effect" and "adverse health effect" are broad terms and EFSA has recently established the following definition for "adverse effect": a change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (EFSA, 2010a). A wide range of negative effects related to the intake of n-3 fatty acids have been reported. These vary from mild and transient, such as a "fishy taste", to unpleasant gastrointestinal symptoms, i.a. erucation, abdominal discomfort, and frequent defaecations, to more serious effects such as increased bleeding time, negative impact on lipid and glucose metabolism and lipid peroxidation. Although unpleasant, some of these effects are clearly mild whereas others can be considered adverse effects as defined by EFSA. In the present evaluation the Norwegian Scientific Committee for Food Safety chose to use the term negative health effects in a more broad sense and describe and evaluate the whole range of effects reported, including also unpleasant symptoms usually termed side-effects.

6.1 Previous safety assessments of fish oils

There has been much emphasis on the positive health effects of n-3 fatty acids, but less on negative health effects, and no tolerable upper intake level (UL) has been set for fish oil, DHA, EPA or ALA. However, in 1997 the US Food and Drug Administration (FDA) performed a comprehensive review (more than 2600 articles) of the safety of fish oils and concluded that total intakes up to 3 g/day of EPA and DHA from the diet and supplements are generally recognized as safe (GRAS[2])[3]. This total daily intake of EPA and DHA was regarded as a safeguard against an increased bleeding time (the time taken for bleeding from a standardised skin wound to cease), impaired glycemic control in non-insulin-dependent diabetes, and increased levels of LDL-cholesterol. In the FDA document it was referred to some studies on type 2 diabetes reporting increased glucose levels when 4.5 to 8 g/day of fish oils were used in the diet. Many studies on hypertriglyceridemic or hypercholesterolemic subjects, and some studies on normolipemic subjects, reported an increase in LDL-cholesterol

[2] The definition of GRAS is available on the homepage of FDA (http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/default.htm.) GRAS is a FDA designation that a chemical or substance added to food is considered safe by experts, and so is exempted from the usual Federal Food, Drug, and Cosmetic Act food additive tolerance requirements.

[3] Department of Health and Human Services, US Food and Drug Administration. Substances affirmed as generally recognized as safe: menhaden oil. Federal Register. June 5, 1997. Vol. 62, No. 108: pp 30751–30757. 21 CFR Part 184 [Docket No. 86G-0289]. Available at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1997_register&docid=fr05jn97-5. Accessed October 3, 2002.

or apolipoprotein B following fish oil supplementation at a dose level of 5 g/day or more of EPA and DHA.

In the IOM 2005 report on dietary reference intakes (DRI) of macronutrients, the potential hazards of EPA and DHA were identified to be impaired immune response and excessively prolonged bleeding times. In addition, IOM described two subpopulations that should take supplements containing EPA and DHA with caution: those individuals who already exhibit glucose intolerance or diabetic conditions that require increased doses of hypoglycemic agents and individuals with familial hypercholesterolemia using anticoagulants. However, the IOM considered the scientific data insufficient to establish an UL (IOM, 2005).

Effect on bleeding time, immune response and glucose and lipid metabolism is further evaluated in section 6.4, 6.6 and 6.7, respectively.

6.2 Safety assessment of a registered drug consisting of EPA and DHA

One n-3 fatty acid formulation has been approved as a drug which implies a rigorous documentation on effect and safety. In the following description of studies on the safety information was retrieved from: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor.cfm.

The registered drug is a mixture of EPA and DHA in the form of ethyl esters. The n-3 fatty acids in this preparation are isolated from fish oil. The drug is supplied as liquid-filled capsules for oral administration. Each one gram capsule contains at least 900 mg of n-3 fatty acids (approximately 465 mg of EPA and 375 mg of DHA) and 4 mg α-tocopherol. The drug is mainly indicated as an adjunct to diet to reduce very high TAG concentrations in serum (hypertriglyceridemia) in adult patients. It is recommended as monotherapy at a dose of 4 g/day or adjuvant to dietary intervention. The efficacy of 4 g/day, which is regarded to be the optimal medical dose, has been demonstrated in a series of 8 clinical trials (Medical Reviews, see http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor.cfm). In several European countries the drug has been approved for both treatment of hypertriglyceridemia and for post-myocardial infarction. Several of the studies from the literature search described in chapter 6 and 7 include this n-3 formulation as test compound.

A search in the WHO Adverse Drug Events Database (April 1, 2010) identified 397 reports including 971 different adverse events related to DHA and EPA given as a drug. All adverse events reported 10 times or more are listed in Table 6.1 ranked after their frequency.

The following categories of events; medication error (38), drug dispensing error (24), oral administration complication (18), therapeutic response unexpected (17), medical condition aggravated (12), treatment non-compliance (10) were omitted from the table.

Pharmacokinetics

Pharmacokinetic studies have shown that there is a complete hydrolysis of EPA and DHA ethyl esters accompanied by absorption and incorporation of EPA and DHA into TAG, cholesterol esters and phospholipids. Information taken from the preclinical Pharmacology Reviews part of the drug approval package submitted for the registered drug (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor.cfm),

Table 6.1: Reported adverse events related to EPA and DHA as drugs.

Adverse events	Frequency
Nausea	23
Blood triglyceride increase	21
Eructation	20
Abdominal distension	17
Rash	17
Chest pain	16
Pruritus	15
Diarrhea	13
Dizziness	13
Blood glucose increase	12
Constipation	12
Abdominal discomfort	10
Flatulence	10
LDL-cholesterol increase	10
SUM	209

Chronic toxicity

In 1 year toxicity studies, 2000 mg/kg bw/day and 1000 mg/kg bw/day produced clinical signs such as fur staining in rats and dogs, respectively. In rats, the main target organ of toxicity was the liver, and in the dogs the main target organs were adrenals, kidneys and testes. The NOAEL in rats (both sexes) was identified to be 600 mg/kg bw/day. This dose corresponds to about 8 g/day in humans with bw of 60 kg, when the comparison is made on the basis of body surface area. Such a comparison takes into account that the metabolism and surface/volume ratio is progressively higher in smaller animals. The NOAEL (adrenal, kidney and testis) in male dogs was reported to be 50 mg/kg bw/day, which corresponds to 1.6 g/day in humans, based on body surface area comparison. In female dogs the NOAEL (adrenals) was reported to be 300 mg/kg bw/day, corresponding to 8 g/day in humans, based on body surface area.

Dermal symptoms were consistently observed in several studies in rats, mice and dogs and were mainly and more severely observed in males.

Carcinogenicity

In a 2-year rat carcinogenicity study with oral gavage doses of 100, 600, 2000 mg/kg bw/day, males were treated with n-3 fatty acid as ethyl esters for 101 weeks and females for 89 weeks without an increased incidence of tumors. The highest dose corresponds to 20 g/day in humans (5-fold the human dose for a standard body weight of 60 kg), when a comparison due to body surface area is done. Standard lifetime carcinogenicity bioassays were not conducted in mice.

Genotoxicity

N-3 fatty acid as ethyl esters were not mutagenic or clastogenic with or without metabolic activation in the bacterial mutagenesis (Ames) test with *Salmonella typhimurium* and *Escherichia coli* or in the chromosomal aberration assay in Chinese hamster V79 lung cells or human lymphocytes. N-3-acid ethyl esters were negative in the *in vivo* mouse micronucleus assay.

Reproductive and developmental toxicity

In a rat fertility study with oral gavage doses of 100, 600, 2000 mg/kg bw/day, males were treated for 10 weeks prior to mating and females were treated for 2 weeks prior to and throughout mating, gestation and lactation. No adverse effect on fertility was observed even at the highest dose 2000 mg/kg bw/day, which corresponds to 20 g/day in humans (5-fold the human dose for standard body weight of 60 kg), when a comparison due to body surface area is done. In rabbits, the maternal NOAEL for reproductive toxicity was 375 mg/kg bw/day and embryo-fetal NOAEL was 750 mg/kg bw/day, representing 8-16 g/day in humans based on body surface area comparison. Studies in pregnant rats have reported conflicting results. In one study, no adverse reproductive effects were observed at doses up to 6000 mg/kg bw/day. Two studies reported embryotoxicity and/or decreased survival to postnatal day 4 (40% reduction) at doses resulting in exposures 7 times the recommended human dose of 4 g/day based on a body surface area comparison.

Adverse effects in humans

Controlled safety data are available from 226 patients treated with 4 g/day of EPA and DHA as ethyl ester (Medical Reviews part of the drug approval package for the registered drug http://www.accessdata.fda.gov/drugsatfda_docs/nda/2004/21-654_Omacor.cfm). A total of 665 patients received EPA and DHA therapy (any dose) in studies for which case report forms were available. More patients treated with EPA and DHA as ethyl ester experienced an adverse effect compared to placebo (35% vs 28%). However, the reported adverse effects were mild and only 8 patients on drug treatment discontinued as a result of adverse effects. The incidence of serious adverse effects was similar between EPA and DHA-group (3.1%) and placebo (2.6%). The most common adverse effects in the treatment group were eructation (4.9%) followed by infection (4.4%), flu syndrome (3.5%), and diarrhea (3.5%). Adverse effects in the digestive system such as eructation, dyspepsia, nausea and diarrhea accounted for 15% of the reported effects. The differences in incidences of adverse effects were not statistically significant between the treatment and the placebo group. The only side effect that occurred at a significantly higher rate in the 4 g/day EPA and DHA group in comparison with placebo was taste perversion (primarily “fishy taste”), with an incidence of 2.7% in the treatment group versus none in the placebo group (p=0.015).

In summary: In the preclinical animal studies with this registered n-3 fatty acid drug it was indicated NOAELs in the range of 50-600 mg/kg bw/day for various toxic effects (8-20 g/day equivalent to human exposure by body surface using a standard body weight of 60 kg). In humans, the only side effect that occurred at a significantly higher rate in the 4 g/day EPA and DHA group compared to placebo was taste perversion, primarily "fishy taste".

6.3 Literature search

Relevant publications for the negative health effects in humans reported in section 6.4-6.8 were identified by literature searches. Because of the extensive literature existing and its particular relevance only human studies were included. The searches were perfomed as follows: the MEDLINE database (March 2009) were searches using the following map terms: alpha-linolenic acid, eicosapentaenoic acid/or docosahexaenoic acid, docosapentaenoic acid, fatty acids, n-3, fish oils, cod liver oils in combination with one of the following two groups of map terms:

- adverse events, dose-response relationship, drugs, risk assessment/or risk factors, toxicity tests/or drug toxicity
- hemorrhage, bleeding time, bleeding tendency, restenosis, endothelium, oxidative stress, lipid peroxides, lipid peroxidation, peroxides, malondialdehyde, thiobarbiturates, thiobarbituric acid reactive substances

The MEDLINE search was limited to year 2000–2009 and human studies. Reviews and original papers with the word "safety" in the title or abstract and furthermore relevant publications with one of the following words in title or abstract "high-dose/bleeding/hypergly/gastro/dose-response/oxidative stress/peroxidation/malondialdehyde/thiobarbituratic" were selected.

Similar procedure was followed in a search in the EMBASE database. Instead of using map terms for the fatty acids (alpha-linolenic acid, eicosapentaenoic acid/or docosahexaenoic acid, docosapentaenoic acid, fatty acids, n-3, fish oils, cod liver oils), only articles with one of these keywords in the title were included in the EMBASE search.

An additional MEDLINE search was conducted using the map terms for the fatty acids, but selecting only those articles with the words "safety" or "high-dose" in the title, and no other limitations.

Publications in section 6.6 "(Negative health effects related to) inflammation and modulated immune function" were found by a PubMed search using the keywords eicosapentaenoic acid OR docosapentaenoic acid OR docosahexaenoic acid OR alpha-linolenic acid OR omega-3 OR fish oil OR cod liver oil AND inflammation. With the limitation added 2000-2009, Humans, Clinical Trial, English. Studies reporting effect of n-3 fatty acids on circulating inflammation markers on healthy subjects, individuals at high risk for heart disease and subjects with CHD were selected. In addition publications from reference lists in published articles, including systematic reviews, were included.

Meta-analyses and systematic reviews were given priority if recent meta-analyses were available. Particularly relevant trials have been included some places as examples in addition to these meta-analyses or systematic reviews. In areas with no available meta-analyses or systematic reviews, all relevant human studies from the literature searches have been included. In addition, the members of the *ad hoc* group have included a few articles relevant to their expert areas that were not identified in the search.

The systematic search revealed five areas where negative health effects in humans have been discussed:

- Bleeding
- Lipid peroxidation
- Inflammation and modulated immune function
- Impaired lipid and glucose metabolism
- Gastrointestinal disturbances

6.4 Bleeding

Although excessively prolonged bleeding times and increased incidence of bleeding have been observed in Inuits, whose diets are rich in EPA and DHA (mean 6.5 g/day) (Dyerberg & Bang, 1979), information is lacking to conclude that EPA and DHA were the sole basis for these observations

It has been known for many years that EPA and DHA reduce platelet aggregation, increase tissue plasminogen activator and decrease tissue plasminogen inactivator (n-3 polyunsaturated fatty acids and cytokine production) in health and disease (Calder, 1997). What consequences this might have for bleeding time is not known. Some studies have suggested an increased risk of bleeding, for example, a higher frequency of nasal bleeding was observed after intake of 1.5 g fish oil in young individuals with hypercholesterolemia more than 20 years ago (Clarke *et al.*, 1990). However, small studies must be interpreted with causion due to the risk of confounding factors, such as other dietary changes. A Cochrane review from 2005 including 48 intervention studies with 0.7-7 g EPA and DHA found no change in bleeding (Hooper *et al.*, 2004). Further, a prospective US study showed no association between the intake of DHA and EPA and hemorragic stroke (Iso *et al.*, 2001), and a recent Korean study reported that a low level of n-3 fatty acids in the erythrocytes was associated with increased risk of hemorragic stroke (Park *et al.*, 2009).

Evidence from controlled studies on healthy subjects is scarce. The report from IOM discusses that 11 short-term studies with EPA and DHA doses ranging from 2 to 15 g/day have shown a significant increase in bleeding time, while there was no change in 3 studies using similar doses (IOM, 2005). IOM report that analysis of these studies collectively indicates no dose-response for EPA and DHA intake and the percent increase in bleeeding time and none of the studies reported any increase in bleeding episodes.

Hereditary or acquired defects of von Willebrand Factor (vWF – a glycoprotein required for platelet adhesion) lead to von Willebrand disease. This hereditary condition involves increased bleeding risk such as increased risk for nosebleeds, menorrhagia, and gastrointestinal bleeding. Patients with von Willebrand disease normally require no treatment.

There are also other factors that influence on increased bleeding tendency, such as use of aspirin or warfarin. The effect of n-3 fatty acids in high doses has been investigated in relation to the use of warfarin and aspirin, but not in relation to von Willebrand disease.

A number of intervention studies with n-3 fatty acid supplementation (3-6 g/day of EPA and DHA) have been reported after cardiovascular by-pass surgery or percutaneous coronary intervention (PCI). The participants in such studies are usually on various anticoagulant drugs and thus at increased risk for bleeding in addition to the risk of bleeding associated with the procedure consisting of puncture of arteries or open heart operation. Nineteen secondary

prevention studies were identified and reviewed (Harris, 2007) regarding the safety of long-chain n-3 fatty acid supplementation. The studies included a total of 4397 participants and EPA and DHA were supplemented by various sources in the different studies and with duration between 4-28 months. Five studies used an n-3 fatty acid supplement registered as a drug (EPA and DHA as ethyl esters), while the others used various n-3 ethyl esters (4 studies), n-3 as fish oil supplementation or capsules with n-3 as TAG (11 studies). The main topic of these studies was to evaluate any effects of n-3 fatty acid supplementation on cardiovascular outcome, not to evaluate the safety of the products as such. Thus, any potential negative health effects such as increased bleeding tendency were discussed only shortly, and phrased "no difference in clinically significant bleeding was noted". Harris concluded that EPA and DHA do not increase risk for adverse bleeding episodes and considered the evidence to be at "A" (well designed randomised controlled clinical trials) (Harris, 2007).

Importantly, none of the studies reported any increase in bleeding complications. In four of the studies the effects of EPA and DHA on bleeding time were assessed more specifically, and in one study the increase was statistically significant (Leaf *et al.*, 1994). The four studies are described in more detail below.

In a 12 month study with 610 patients undergoing coronary bypass operation, 317 patients were assigned to receive 1.8 g EPA and 1.5 g DHA per day as ethyl esters and 293 to constitute the control group (Eritsland *et al.*, 1996). The bleeding time (sec) increased from 243 ± 76 before intervention to 282 ± 93 after intervention. In the control group, who also underwent bypass operation, bleeding time (sec) increased from 249 ± 79 to 283 ± 84. The differences between the fish oil and control groups in bleeding time were however not significant. Fifty seven and 59% of the prothrombin time measurements were within the target level for the treatment, INR (international normalised ratio) 2.5-4.2. (INR is a measure of the biological effect of vitamin K-dependent coagulation proteins and a high INR level (such as INR=5) means a high chance of bleeding). Hence, there was no statistical difference in bleeding complications between the fish oil group (3.4 g/day EPA and DHA as ethyl esters) and the control group. Approximately half of the patients were given warfarin and the other half received aspirin. Measurement of INR was only relevant for those patients who used warfarin.

In another placebo-controlled 6 month study by Leaf *et al*. 275 patients using aspirin were randomised to ten 1.0 g capsules containing 80.6% ethyl esters of n-3 fatty acids providing 4.1 g EPA + 2.8 g DHA and 276 matched subjects received placebo corn oil (Leaf *et al.*, 1994). No significant differences between the study groups for negative health effects were oberved. There were 3% bleeding episodes in each treatment group. After 3 months of intervention with a total of 6.9 g EPA and DHA per day bleeding time (min) increased from 6.22 ± 0.2 (SEM) to 7.02 ± 0.2. This change was statistically significant ($p<0.01$), but still within the reference values. In the placebo group, the bleeding time did not change (6.43 ± 0.2 versus 6.47 ± 0.2 NS). No difference between the groups in clinical significant bleeding events was noted. Although the increased bleeding time was within the normal range, this indicates an effect on bleeding time of EPA and DHA as ethyl esters at 6.9 g/day.

In a randomised unblinded study Dehmer *et al*. investigated the rate of PCI. Eighty-two patients largely using aspirin and dipyradimole were supplemented with 5.4 g/day EPA and DHA (3.2 + 2.2 g/day) as TAG for 6 months. The control group did not receive another oil as placebo. In both groups the bleeding time was not significantly changed after 3 month therapy, but there was a trend towards increased bleeding time in the n-3 treated group showing a numerical increase from 6.8 ± 3.0 (SD) to 9.0 ± 3.1 min after one month ($p<0.1$) (Dehmer *et al.*, 1988).

In a study by Bender *et al*. 16 patients with cardiovascular disease on stable warfarin treatment were randomised to a 4-week treatment period of either placebo capsules (n=6), 0.9 g/day of EPA and DHA (3 g/day fish oil, n=5) or 1.8 g/day of EPA and DHA (6 g/day fish oil, n=5) for 4 weeks (Bender *et al.*, 1998). The results show that INRs remained the same during fish oil supplementation, and there were no incidences of an increase in bleeding episodes.

Table 6.2: Human studies in CHD patient undergoing various treatments reporting effects on bleeding time (min) with high doses EPA and DHA.

Reference	Patients (n)	Measure of effect	PUFA (TAG/Ethyl ester)	No effect (g/d)	Effect (g/d)
Eritsland et al., 1996	CABG[1] (610)	Bleeding time	EPA + DHA (Ethyl ester)	3.4	
Leaf et al., 1994	PCI (447)	Bleeding time	EPA + DHA (Ethyl ester)		6.9
Dehmer et al., 1988	PCI (82)	Bleeding time	EPA + DHA (TAG)	5.4	
Bender et al., 1998	Chronic warfarin (16)	INR[2]	EPA + DHA (TAG)	1.8	

[1]Cardiovascular by-pass surgery.
2International normalised ratio.

Other relevant studies report no changes in bleeding complications. One study with 108 CHD patients using aspirin and 3 g/day EPA and DHA reported "No patient suffered from bleeding complications" (Grigg *et al.*, 1989), another reported "Bleeding less frequent in fish oil group" after 4.5 months on 5.4 g/day EPA and DHA where all the CHD patients (n= 814) used aspirin and 50% of them were also taking low-molecular-weight heparin (Cairns *et al.*, 1996).

In addition 20 controlled studies including a total of 4659 patients did not report any excess bleeding tendency despite the fact that many patients used anticoagulant. Both TAG and ethyl ester formulations were used in the studies.

The question regarding bleeding time is still not resolved since there are studies reporting no significant changes in bleeding time (EPA and DHA 1.8 – 5.4 g/day), but also one study reporting a significant increase in bleeding time at 6.9 g/day EPA and DHA in CHD patients on anticoagulant medication. INR does not seem to be influenced by EPA and DHA according to the limited number of studies on INR. The effect on bleeding of EPA and DHA among vulnerable individuals, like persons with inherited or acquired hemorrhagic diathesis, has not been examined.

Randomised studies have not shown any adverse effects on clinical bleeding complication with EPA and DHA, but the studies were not designed for this purpose.

In summary, only a few controlled studies have assessed the effect of EPA and DHA (dose range 1.8 – 6.9 g/day) on bleeding time, bleeding tendency and international normalised ratio (INR). A significant increase in bleeding time has been observed at 6.9 g/day EPA and DHA in one study with coronary heart diseased patients on anticoagulant medication. No significant impact on bleeding time was observed in two other studies in patients on anticoagulation medication using 3.4 and 5.4 g EPA and DHA per day.

Comment: In Western societies thrombo-embolic vascular disorders are quantitatively a larger clinical problem in comparison with bleeding. Therefore, it has been assumed that for most adults somewhat reduced reactivity of the blood platelets would probably be advantageous.

6.5 Lipid peroxidation following intake of EPA and DHA

Oxidative stress and resulting lipid peroxidation is involved in various pathological states including inflammation, atherosclerosis, neurogenerative diseases and cancer. Evidence for the role of oxidative stress in the pathogenesis of CVD is primarily based on experimental cell culture studies and observational human studies. A recent review concluded that the ability of oxidative stress biomarkers to predict cardiovascular diseases in cell culture studies and observational human studies has yet to be established (Strobel *et al.*, 2010). It is also important to note that the methods used to assess lipid peroxidation in human samples including MDA, lipid hydroperoxides, conjugated dienes, oxLDL and F2-isoprostanes are indirect, and the evidence that the various methods actually reflects lipid peroxidation *in vivo* is limited.

In the IOM report from 2005 reviewing the dietary refence intakes it is stated that laboratory animals have shown increased lipid peroxidation and oxidative damage of erythrocytes, liver, and kidney membranes with consumption of DHA (IOM, 2005). The oxidative damage was reduced or prevented with a simultaneous consumption of supplementary vitamin E. In the present evaluation only data from human dietary intervention studies are included. In most of these studies the fish oil was stabilized with antioxidants. In some of the studies it is not specified whether the oils contained an antioxidant or not. The level of primary and secondary oxidation products in the oils measured as peroxide value (PV) and anisidine value (AV) are reported only in a few studies.

There various plasma markers of lipid peroxidation include malondialdehyde (MDA), lipid hydroperoxides, conjugated dienes, oxidation of LDL and F2-isoprostanes. The most common method has been quantification of MDA as the thiobarbituric acid reactive substances (TBARS). Oxidation of LDL to oxLDL is linked to the initiation and progression of atherosclerosis (Steinberg *et al.*, 1989). The susceptibility of LDL to oxidation can be measured *ex vivo* by isolating LDL directly from plasma and as oxidizing agent use the patients' own mononuclear cells, Cu^{2+} or 2,2'-azobis-(2-amidinopropane hydrochloride) (AAPH). In total, 14 studies[4] have assessed TBARS or MDA in plasma, urine or skin. In five studies[5] plasma-conjugated dienes or total lipid peroxides were determined. In ten studies[6] F_2-isoprostanes in urine or plasma were measured and in 11 remaining studies[7] the susceptibility of LDL to oxidative modification was determined. None of the studies found any significant increases in conjugated dienes, total lipid peroxidation or F_2-isoprostanes in plasma or urine following treatment with n-3 fatty acid supplements.

The concentration of TBARS in plasma was significantly increased in particants in two intervention studies where 3.5 g EPA and DHA per day were taken for either 5 or 8 weeks (Higdon *et al.*, 2000; Grundt *et al.*, 2003b). In the first study 15 post-menopausal women took

[4]Stalenhoef *et al.*, 2000; Wander & Du, 2000; Higdon *et al.*, 2000; Yaqoob *et al.*, 2000; Rhodes *et al.*, 2003; Engstrom *et al.*, 2003; Koletzko *et al.*, 2003; Shidfar *et al.*, 2003; Piolot *et al.*, 2003; Grundt *et al.*, 2003b; Grundt *et al.*, 2004; Shoji *et al.*, 2006; Siahanidou *et al.*, 2007; Shidfar *et al.*, 2008.

[5]Wander & Du, 2000; Jain *et al.*, 2002; Piolot *et al.*, 2003; Pedersen *et al.*, 2003; Takeuchi *et al.*, 2007.

[6]Higdon *et al.*, 2000; Mori *et al.*, 2000; Stier *et al.*, 2001; Mori *et al.*, 2003; Barden *et al.*, 2004; Engler *et al.*, 2004; Tholstrup *et al.*, 2004; Wu *et al.*, 2006; Himmelfarb *et al.*, 2007; Mostad *et al.*, 2009.

[7]Nenseter *et al.*, 1992; Suzukawa *et al.*, 1995; Wander *et al.*, 1996; Bonanome *et al.*, 1996; Brude *et al.*, 1997; Stalenhoef *et al.*, 2000; Turini *et al.*, 2001; Higgins *et al.*, 2001; Piolot *et al.*, 2003; Mesa *et al.*, 2004; Bloomer *et al.*, 2009.

fish oil, safflower oil or sunflower oil for 5 weeks in a cross-over designed study (Higdon *et al.*, 2000). The authors observed an increase in TBARS in plasma after intake of fish oil and safflower oil, but not with sunflower oil. In the study by Grundt *et al*. 300 acute myocardial infarction (MI) patients were taking EPA and DHA as ethyl ester for one year in a randomised placebo controlled trial. Despite an increase in TBARS in plasma among those taking EPA and DHA, the incidence of recurrent myocardial infarction was the same in the two groups.

In contrast, no significant changes in plasma TBARS were reported in 3 randomised controlled trials using doses 3.2-4.3 g/day of EPA and DHA (Stalenhoef *et al.*, 2000; Wander & Du, 2000; Yaqoob *et al.*, 2000) and one intervention trial with only fish oil (6 g/day)(Piolot *et al.*, 2003) all with duration from 5 to 12 weeks. In these studies the numbers of study subjects were 28 patients with primary hypertriglyceridemia, 46 post-menopausal women, 40 and 16 healthy men and women. Interestingly, in the study by Wander and Du they tested whether fish oil without added vitamin E would influence plasma TBARS differently from the use of fish oil added different doses of vitamin E. They showed that fish oil without added vitamin E increased the TBARS level in post-menopausal women, but no change in TBARS was observed when fish oil was added vitamin E (15 g fish oil added doses of 100, 200 and 400 mg α-tocopherol (Wander & Du, 2000).

Some studies have confirmed the concern that LDL enriched with n-3 fatty acids is susceptible to oxidation (Suzukawa *et al.*, 1995; Wander *et al.*, 1996; Turini *et al.*, 2001; Mesa *et al.*, 2004). In the study by Turini *et al*. 9 healthy men were randomly assigned to receive 25 g/day fish oil (4.3 g/day EPA+2.8 g/day DHA) or high oleic sunflower oil (placebo) for 30 days (Turini *et al.*, 2001). The fish oil was supplemented with 1.2 mg α-tocopherol/ g oil. Fish oil supplementation significantly increased the rate of LDL oxidation and significantly reduced tha lag time of oxLDL. Mesa *et al*. studied 42 healthy volunteers who were randomly assigned to receive olive oil (placebo), an EPA-rich oil or a DHA-rich oil for 4 weeks at a dose of 9 g oil/day (Mesa *et al.*, 2004). The EPA group had a daily intake of 4.8 g EPA + 0.2 g DPA + 0.7 g DHA, while the DHA group had a daily intake of 0.8 g EPA + 1.0 g DPA + 4.9 g DHA. All capsules were supplemented with 10 IU (approximately 6.8 mg) mixed natural tocopherols to prevent oxidation. Following supplementation, the EPA treatment significantly increased the formation of conjugated dienes during LDL oxidation compared with baseline, whereas the DHA treatment had no effect. Neither treatment significantly affected the lag time for oxidation, oxidation rate during the propagation phase or maximum diene production. Suzukawa *et al*. studied 20 hypertensive subjects in a randomised double-blind cross-over study receiving a daily dose of 1.6 g EPA + 1.2 g DHA or corn oil (Suzukawa *et al.*, 1995). All capsules were added 20 mg/ml of α-tocopherols to prevent oxidation. Supplementation with fish oil significantly reduced the lag time and the propagation rate which is a paradox since a reduction in propagation rate is an indicator of decreased oxidation. Supplementation with fish oil also significantly increased TBARS after Cu^{2+} incubation compared with baseline and placebo. In a study by Wander *et al*. they tested whether fish oil without added vitamin E would influence the lag time of oxLDL differently from the use of fish oil with different doses of vitamin E (Wander *et al.*, 1996). This study is identical to the one described by Wander and Du (2000) above. They showed that fish oil supplemented with doses as low as 100 mg α-tocopheryl acetate/day increased the resistance of LDL to oxidation.

Others have however not observed increase in susceptibility of LDL to oxidative modification by supplementation with ≤2.5 g EPA and DHA per day (Nenseter *et al.*, 1992; Bonanome *et al.*, 1996; Brude *et al.*, 1997; Higgins *et al.*, 2001). In these studies the study subjects were 12 chronic renal failure patients (daily dose of 2.5 g EPA and DHA as ethyl ester for 2 months) and 62 healthy persons (daily dose of max. 0.9 g EPA and DHA as fish oil for 16 weeks).

Studies using higher doses of marine n-3 fatty acids have also reported no effect on the susceptibility of LDL to oxidative modification. In these studies the number of subjects were 23 normolipidemic persons (daily dose of 5.1 g EPA and DHA as ethyl ester for 4 months) (Nenseter *et al.*, 1992), 42 male smokers with hyperlipidemia (daily dose of 5 g EPA and DHA from fish oil for 6 weeks) (Brude *et al.*, 1997), 28 hypertriglyceridemic patients (daily dose of 4 g EPA and DHA as ethyl ester for 12 weeks) (Stalenhoef *et al.*, 2000), 16 healthy subjects (daily dose of 6 g fish oil for 8 weeks) (Piolot *et al.*, 2003) and 14 exercised-trained subjects (daily dose of 4g EPA and DHA for 6 weeks) (Bloomer *et al.*, 2009).

In summary, a limited number of papers have reported lipid peroxidation as determined by the use of biomarkers following intake of DHA and EPA. In some of the studies antioxidants were added to the oils. None of the studies reported increase in conjugated dienes, total lipid peroxidation or F_2-isoprostanes in plasma or urine. A few studies have shown that LDL enriched with n-3 fatty acids is susceptible to oxidation. However, most of the studies show no increase in the susceptibility of LDL to oxidative modification. One large study with 300 myocardial infarction patients taking 3.5 g EPA and DHA/day as ethyl ester for one year showed increased plasma TBARS.

Comment: The duration of the trials might explain some of the differences in the susceptibility of LDL to oxidative modification since trials with supplementation periods of 4-6 weeks showed increased susceptibility of oxidative modification of LDL (Suzukawa *et al.*, 1995; Wander *et al.*, 1996; Turini *et al.*, 2001) whereas studies with longer supplementation periods of 6, 8, 12 and 16 weeks showed no effect (Nenseter *et al.*, 1992; Bonanome *et al.*, 1996; Brude *et al.*, 1997; Stalenhoef *et al.*, 2000; Higgins *et al.*, 2001; Piolot *et al.*, 2003; Bloomer *et al.*, 2009).

The clinical relevance of lipid peroxidation is not clear since none of the oxidative stress biomarkers analyzed in the present evaluation are defined as risk factors predicting risk of disease. Therefore, more research is needed to develop better approaches to understand the role of oxidative stress and disease. This should include directly assessing the generation of reactants at the tissue level, the use of multiple biomarkers for a more comprehensive assessment of the pathways involved and longitudinal cohort studies investigating how changes in these measures over time are related to survival and disease.

An increase in oxidative stress *in vivo* due to lipid peroxidation is influenced by dietary intake of antioxidants. It is therefore important to assess the habitual dietary intake of the study subjects participating in dietary intervention trials. The antioxidant level in the fortified foods, fish oil or plant oil should also be reported in dietary trials since the amount of added tocopherols have shown to influence markers of lipid peroxidation. Also the intake of n-6 fatty acids can affect the results since some fatty acids are more likely than others to form a particular decomposition product, such as malondialdehyde, whereas other oxidation products, such as the commonly used marker of oxidative stress, F_2-isoprostanes, are formed by oxidation of only one fatty acid, arachidonic acid. Background diet therefore plays an important role in dietary intervention studies and should be included in the description of study subjects.

6.6 Inflammation and modulated immune function

Inflammation is part of the body's immediate response to infection or injury. However, when it occurs in an uncontrolled or inappropriate manner disease can ensue. Low-grade systemic inflammation play an important role in the pathology of some diseases, such as cardiovascular disease, type 2 diabetes, and neurodegenerative diseases of aging. There is a relationship

between markers of inflammation, such as C-reactive protein (CRP) and cytokines IL-6 and IL-18 and the intercellular adhesion molecules 1 (ICAM-1) and prospective cardiovascular risk in apparently healthy individuals as well as patients with CHD or heart failure (Vasan, 2006), and CRP is used in clinical applications (Libby *et al.*, 2009).

IOM summarised in 2005 that there are some evidence from *in vitro* and *ex vivo* studies in peripheral white blood cells from indivuals provided n-3 PUFAs and that such intake, especially of EPA and DHA, may impair immune function by acting immunosuppressive (IOM, 2005). Such effects were observed at doses ranging from 0.9 to 9.4 g/day of EPA and 0.6 to 6 g/day of DHA.The effects of EPA and DHA on the immune system include decreased expression of cell surface adhesion molecules, cytokines and reduced peripheral white blood cell proliferation. However, it is not known whether these experimentally induced effects are relevant models for modulation of the human immune apparatus *in vivo*. Because of the uncertainties in the interpretation of the *ex vivo* studies on *in vivo* immune function we have chosen to review only the literature on inflammatory markers measured in human plasma or serum. Among the 36 studies gathered in the literature search, 6 reported potential negative effects of EPA and DHA on circulating markers of inflammation.

Johansen *et al*. investigated the effects of EPA and DHA as ethyl esters on the levels of hemostatic markers of atherosclerosis and inflammation in 54 patients with CHD (Johansen *et al.*, 1999b). Twenty-three patients had taken 5.1 g EPA and DHA per day for 6 months and 31 were given corn oil as placebo. For another 4 weeks they all received 5.1 g EPA and DHA per day. The study showed that tissue plasminogen activator antigen and the soluble thrombomodulin decreased during the study period, whereas the soluble E-selectin (sE-selectin) and soluble vascular cell adhesion molecule-1 (sVCAM-1) increased. These results indicate that a highly concentrated supplement with EPA and DHA decreases hemostatic markers of atherosclerosis, whereas markers of inflammation increase. The latter may be the result of lipid peroxidation, as a simultaneous decrease in vitamin E and an increase in TBARS were observed.

An increase in sE-selectin and sVCAM-1 after supplementation with EPA and DHA as ethyl esters, has also been described by Seljeflot *et al*. in a population of 41 healthy men at high risk for atherosclerotic disease (Seljeflot *et al.*, 1998). The study was a placebo controlled intervention with 4.8 g/day EPA and DHA as ethyl esters for 6 weeks.

Increase in VCAM-1 has also been described in a recent study including 275 healthy subjects (Paulo *et al.*, 2008). The subjects were randomised to one of four groups receiving sunflower oil (control), lean fish (3 x 150 g/weeks), fatty fish (3 x 150 g/weeks) or fish oil capsules (1.4 g/day EPA and DHA) for 8 weeks. The diet rich in lean fish significantly decreased the level of sICAM-1, whereas both the intervention with fish oil and fatty fish increased VCAM-1 levels.

Increased sE-selectin in young subjects (< 40 years) and decreased sVCAM-1 in older subjects (> 55 years) were reported in a study of 28 healthy subject receiving fish oil containing 1.2 g EPA and DHA per day for 12 weeks. There was no effect on sICAM-1 (Miles *et al.*, 2001).

Cazzola *et al*. studied the effect of fish oil capsules with TAG formulation using 3, 6 or 9 capsules per day for 12 weeks with corn oil as placebo in healthy young (18-42 years, n= 93) and older (53-70 years, n=62) males. The EPA-level was 45% of the oil, and the DHA-level 9% (Cazzola *et al.*, 2007). The daily intake of EPA was 1.4 g, 2.7 g or 4.1 g. They investigated the effects on sVCAM-1, sICAM-1 and sE-selectin, and reported that 4.1g

EPA/day increased sE-selectin in the young subjects while it tended to decrease sICAM-1 in both the young and old group.

Among the 36 human studies included from the literature search, 19 studies have measured CRP. None of these 19 studies report an increase in CRP after intake of EPA or DHA.

There are a few studies that have demonstrated an elevation of plasma cytokines after marine n-3 supplementation. One study demonstrates that EPA and DHA supplementation in heart transplant patients enhanced TNF-α (Holm *et al.*, 2001). The patients were long-time survivors of heart transplantation, randomised in a double-blind fashion to receive 3.4 g/day EPA and DHA as ethyl ester or placebo for 1 year. In the treatment group, but not in the placebo group, there was a rise in TNF-α, a decrease in the anti-inflammatory cytokine IL-10, and a rise in TNF/IL-10 ratio after 12 months, suggesting a pro-inflammatory net effect.

In summary, supplementation with EPA and DHA as ethyl esters or TAG appears to increase the circulating concentrations of sVCAM-1 (reported in 3 studies) (doses varied from 1.4 to 5.1 g/day EPA and DHA). Among healthy individuals an increase was observed with low doses of EPA and DHA (1.4 g/day), but fatty fish intake in the same study also increased the concentration of sVCAM-1. Elevation of sE-selectin was reported in 4 studies (doses varied from 1.2 to 5.1g/day EPA and DHA or EPA alone). One report showed an increased concentration of the cytokine TNF-α in heart transplanted patients supplemented with EPA and DHA as ethyl ester.

No firm conclusions can be drawn regarding negative health effects from intake of EPA and DHA related to systemic inflammation, and no increase in CRP after intake of marine n-3 fatty acids has been observed. However, there is a concern that EPA and DHA at doses of 5 g/day may activate endothelial cells (increased sVCAM-1 and sE-selectin) among individuals at high risk of cardiovascular diseases and patients with coronary heart disease. The clinical relevance of an increase in low-grade systemic inflammation biomarkers among healthy and diseased people is still uncertain.

Comment: It is important to note that most of the studies included in this section reports no or a positive effect on circulating markers of inflammation after intake of marine n-3 fatty acids and only a few papers reports negative effects on inflammatory markers. The discrepancies in the results may be due to different study populations, doses, the relative content of EPA and DHA and antioxidants in fish oils and forms of n-3 fatty acids (TAG versus ethyl esters) and length of the study periods. It must be noted that many of the studies included in the search involve a limited number of patients and have not been designed to identify potential negative effects. The studies which do not show any effects of marine n-3 fatty acids on the production of pro-inflammatory cytokines might also be underpowered (Fritsche, 2006). In addition, other bioactive compounds in the diet can influence the level of inflammatory markers, and unfortunately the background diet is not reported in many of the studies. It is also important to address the balance between n-3 and n-6 fatty acids in the diet due to the opposite effects on the production of eicosanoids.

6.7 Impaired lipid and glucose metabolism

There has been some concern regarding whether EPA and DHA might impair glycemic control in patients with diabetes mellitus type 2 and increase fasting glucose and insulin levels in healthy subjects. In 2005, IOM stated that individuals who already exhibit glucose intolerance or diabetic conditions that require increased doses of hypoglycemic agents should take supplements containing EPA and DHA with caution due to this concern (IOM, 2005).

Hartweg *et al*. performed a Cochrane Database systematic review to accumulate available information on the effects of EPA and DHA supplementation on cardiovascular outcomes, cholesterol levels and glycemic control in people with type 2 diabetes (Hartweg *et al.*, 2008). The authors concluded that n-3 fatty acid supplementation in type 2 diabetes lowers TAGs and very low-density lipoprotein (VLDL) cholesterol, but may raise LDL-cholesterol. EPA and DHA had no statistically significant effect on glycemic control or fasting insulin. However, one Norwegian study included in Hartweg *et al*.'s systematic review (2008) with 26 type 2 diabetes patients reported increased fasting glucose (p=0.017) and impaired insulin sensitivity (p=0.049) after 9 weeks daily fish oil supplementation (5.9 g total n-3 fatty acids, including 1.8 g EPA + 0.3 g DPA + 3.0 g DHA) compared with corn oil (Mostad *et al.*, 2006).

More recently Hartweg and co-workers performed a systematic review and meta-analysis including 26 studies reporting on lipid, glycemic and hematological risk factors in type 2 diabetes after n-3 fatty acid supplementation (Hartweg *et al.*, 2009). The mean dose of EPA and DHA was approximately 2.4 g/day with duration of 6 months, and 21 studies reported results of glycated HbA1c. The supplementation of EPA and DHA did not change HbA1c (-0.01%) (pooled weighted mean difference for HbA1c in studies on 1409 participants). In 19 studies, fasting glucose was reported in a way that enabled pooled analysis. EPA and DHA supplementation did not change fasting glucose (-0.009 mmol/L, p=0.81). They identified 9 studies including a total of 629 patients with CHD reporting fasting insulin data that could be pooled. Fasting insulin values did not change after n-3 fatty acid supplementation (p=0.10). C-peptide was reported in 4 studies including 111 patients with CHD and the level did not change (p= 0.32). Further, they observed a decrease in TAG, fibrinogen and ADP platelet aggregation. LDL-cholesterol increased with 3% while high-density lipoprotein (HDL) cholesterol, blood pressure and inflammatory markers did not change.

It is well documented that EPA and DHA or EPA as monotherapy in lower doses, from 0.4 to 1.8 g per day, do not increase fasting glucose values or increase the risk of developing type 2 diabetes. This is documented by data from large prospective studies on patients with heart disease (Burr *et al.*, 1989; GISSI-Investigators, 1999; Tavazzi *et al.*, 2008; Kromhout *et al.*, 2010) as well as data from the large JELIS study of 18 645 people without heart disease (Yokoyama *et al.*, 2007).

Several relatively large studies with high doses above 3 g/day EPA and DHA indicate that there is no increased fasting glucose or increased development of type 2 diabetes in patients with CHD without type 2 diabetes. These studies have, however, been of shorter duration than the above-mentioned studies using lower doses. The high dose studies have mainly gone over 6-24 months. Some of them have been performed in Norway (Eritsland *et al.*, 1996; Johansen *et al.*, 1999a; Nilsen *et al.*, 2001) and are interesting because the Norwegian diet on average contains relatively much EPA and DHA.

In the study by Eritsland *et al*. 610 patients with CHD used 3.4 g EPA and DHA as ethyl ester per day for 12 months (Eritsland *et al.*, 1996), in a study by Nilsen and Harris (Nilsen & Harris, 2004) 300 patients with CHD used 4 g EPA and DHA as ethyl esters per day in 12-24 months and in a study by Johansen *et al*. 388 patients with CHD used 5.1 g EPA and DHA as ethyl esters per day for 6 months (Johansen *et al.*, 1999a). These studies allows a conclusion that it is most unlikely that EPA and DHA increase the fasting blood glucose or risk for diabetes in CHD patients even in doses up to 5.1 g per day. In general, it can be assumed that several patients with CHD have type 2 diabetes. Therefore, all the studies on subjects with CHD probably include many type 2 diabetes patients. A negative effect on glycemic control of EPA and DHA has not been reported in these studies. Since these studies were not designed to study glycemic control, no firm conclusion can be drawn. In the future, meta-

analysis on the controlled intervention studies with EPA and DHA in CHD patients should examine the effect of treatment on glycemic control.

Some meta-analyses have reported that EPA and DHA may increase LDL-cholesterol in subjects with type 2 diabetes (Hooper *et al.*, 2006; Hartweg *et al.*, 2009).

The dose of EPA and DHA used is reported to be between 0.7 to 5.0 g/day (Hooper *et al.*, 2006). No dose-response relationship has been found. The increase in LDL-cholesterol has been in the range of 1 to 3% in patients with type 2 diabetes, and has not been observed in patients without diabetes. One likely explantation for this is that patients with type 2 diabetes have higher fasting TAG values due to increased production and subsequntly increased plasma concentration of atherogenic VLDL-cholesterol. EPA and DHA will enhance the tranformation of TAG-rich VLDL lipoproteins to cholesterol-rich LDL lipoproteins and leading to a decrease in fasting TAGs and a slight increase in LDL-cholesterol. This shift would be not associated with any change in apolipoprotein B concentration meaning that the total number of lipoprotein particles remains unchanged. Thus the slightly increased LDL-cholesterol concentration that has been observed in some studies with patients with type 2 diabetes is likely to represent a positive metabolic change in lipid metabolism.

In summary, there has been some controversy concerning the effects of EPA and DHA on the glucose metabolism in patients with type 2 diabetes. Furthermore, the effect on insulin sensitivity and development of type 2 diabetes has been discussed as there are several studies with different conclusions. In subjects with type 2 diabetes, the bulk of evidence indicates that glucose control is not affected. Taken together, the existing data with doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day) indicate that any eventually negative effects from EPA or DHA on glucose homeostasis is too weak to be proven based on the present studies.

Meta-analysis has found no negative effects of EPA and DHA on standardised measurements of glycemic control as HbA1c and fasting glucose.

A minor increase in LDL-cholesterol (1-3%) in subjects with type 2 diabetes has been reported in meta-analyses following supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). No dose response relationship has been reported. However, the clinical relevance in subjects with type 2 diabetes of this minor increase in LDL-cholesterol is unclear because of a concomitant reduction in serum triacylglycerol and unchanged apolipoprotein B in the same subjects. No change in LDL-cholesterol was reported in the large coronary heart intervention trials including both subjects with and without type 2 diabetes.

Comment: New large trials assessing the impact of EPA and DHA on lipid and glucose metabolism in humans are ongoing. Overall, these safety characteristics correspond well with safety data presented for a medical drug consisting of DHA and EPA as ethyl esters (Table 6.1). The relationship between EPA and DHA and LDL-cholesterol may be more directly tested by use of EPA and DHA capsules than by changes in diet toward eating more oily fish.

6.8 Gastrointestinal disturbances

Gastrointestinal disturbances reported in intervention studies with n-3 fatty acids are nausea, vomiting, diarrhea, increased faecal frequency, epigastria and defecation, belching, flatulence, eructation, dyspepsia, fishy aftertaste, loose stools, gastrointestinal discomfort, constipation, upset stomach and abdominal cramps.

In the majority of the studies reviewed, the gastrointestinal disturbances were not specifically associated with the intake of n-3 fatty acids as such, but merely with the intake of relatively

large amounts of oil or an oily substance. In these studies, placebo and test compound caused the same type of gastrointestinal disturbances and no statistically significant differences were observed indicating a no effect dose level for the n-3 fatty acids. However, in a few studies the gastrointestinal disturbances were significantly more frequent in the groups given n-3 fatty acids than in the groups given placebo, indicating a dose level where negative effects may occur. In some studies placebo was not used or not defined.

Bromfield *et al*. found in a randomised controlled trial for refractory epilepsy in 23 subjects, that nausea or diarrhea occurred with the same frequency in the test group given 2.2 g/day of EPA and DHA and placebo group (Bromfield *et al.*, 2008).

In 49 patients with recurrent self-harm a similar no effect level for mild gastric discomfort could be suggested with 2.1 g/day EPA and DHA in a randomised controlled trial (Hallahan *et al.*, 2007).

De Truchis *et al*. found no enhanced minor gastrointestinal disorder at the dose level 1.7 g/day of EPA and DHA in a double-blind phase 4 randomised 2-arm parallel group study on 120 HIV-infected patients (DeTruchis *et al.*, 2007).

Von Schacky *et al*. randomised 111 patients to fish oil and 112 to placebo in a 2-year study of patients with documented CHD (von Schacky C. *et al.*, 1999). The first 3 months the intervention group received 4.0 g fish oil as TAG capsules containing 66.6% n-3 fatty acids (EPA+DPA+DHA as TAG at 2.6 g/day). The remaining 21 months they received half the dose (1.3 gday EPA+DPA+DHA). Mild gastrointestinal discomfort was reported in 3 placebo recipients and four fish oil recipients.

Reis *et al*. investigated 222 patients after PCI. Seventy four patients were randomised to ethyl ester of fatty acids extracted from fish oil, 76 to purified fish oil containing the fatty acids as TAG, and 72 patients to placebo (olive oil). The treatment groups received 6 g/day marine n-3 fatty acid supplementation. Negative effects, primarily gastrointestinal, were reported as common in both fish oil groups (Reis *et al.*, 1989). In this study both ethyl esters and TAG fish oil preparations were used, and the authors reported no differences in gastrointestinal disturbances between the two formulations. Overall, 53% in the treatment groups noted side-effects compared with 22% in the placebo group ($p<0.001$). Women receiving fish oil had gastrointestinal disturbances more commonly than men (67 versus 49%, $p<0.05$). An effect level of 6 g/day for EPA and DHA as both TAG and ethyl esters could be identified for this study. A unique feature of this study is the use of n-3 fatty acid supplementation both as ethyl esters and TAG in the same study.

In a randomised controlled trial in 204 patients with Alzheimer disease, the frequency of gastrointestinal disturbances did not differ between the group receiving 2.3 g/day of EPA and DHA as TAG and the group receiving corn oil as placebo (Freund-Levi *et al.*, 2006).

In a clinical phase 1 study Burns *et al*. identified the maximum tolerated dose of EPA and DHA as ethyl esters in 22 patients with cancer cachexia (Burns *et al.*, 1999). Intake above 4.4 g/day induced belching, flatulence, nausea and vomiting. No effect level could be identified because placebo was not used in the study. The same author performed a phase II clinical study with 43 patients who were given 0.15 g fish oil/kg bw/day (fish oil: ethyl esters 470 mg/g EPA+280 mg/g DHA). Negative effects such as edema, emesis and dyspepsia were reported additionally to the adverse effects reported in the phase I study (Burns *et al.*, 2004), but no placebo was used.

In a randomised controlled trial where the effect of 6 g seal oil was tested on 134 patients with non-alcoholic fatty liver disease, the reports of increased faecal frequency and epigastria and defecation were the same in seal oil and placebo group (Zhu *et al.*, 2008).

In a double-blind, placebo-controlled study in 257 patients after PCI with 5.1 g/day of EPA and DHA as ethyl esters or placebo (olive oil) for 6 months Maresta *et al*. reported mild gastrointestinal symptoms (dyspepsia, epigastralgia, or gastric discomfort) in 4 patients equally distributed between the 2 treatment groups (Maresta *et al.*, 2002). In a 6-month study with 120 patients post-PCI with 3.0 g/day EPA and DHA as TAG, the only adverse events reported were 4 patients with nausea and one with diarrhea (Bellamy *et al.*, 1992). No placebo was given.

In a 12 month study described above on 610 patients post-cardiovascular by-pass surgery receiving 3.4 g/day EPA and DHA as ethyl ester, the investigators reported that "Generally, the fish oil supplementation was well tolerated (Eritsland *et al.*, 1996). Adverse effects attributed to fish oil, mainly gastrointestinal complaints, were usually mild, although in some cases the supplementation had to be withdrawn". However, no placebo was given and the gastrointestinal effect could not be linked to EPA and DHA as such.

In a double-blind randomised controlled trial the safety of a microalgal and fungal oil rich in DHA was tested in 32 healthy adults (Innis & Hansen, 1996). Significant more eructation was observed in the 1.7 g/day group (7/8) than in the canola-oil placebo group (1/8), (p=0.013).

Marangell *et al*. treated 36 depressed patients with 2 g/day of DHA or placebo, and no differences were observed for belching and loose stools (Marangell *et al.*, 2003).

In a double-blind randomised controlled trial conducted to determine the effect of meeting the estimated DHA requirement of preterm infants on neurodevelopment, mothers were given 3 g tuna oil high in DHA to achieve breast milk with DHA 1% of total fatty acids (+ 0.6% arachidonic acid) (Makrides *et al.*, 2009). Compared to placebo (soy oil) there were no differences in reports of gastrointestinal disturbances.

The bioequivalence of two different algal DHA oils (DHASCO-T and DHASCO-S) and algal-DHA-fortified food was studied in a 28-day randomised controlled trial (Arterburn *et al.*, 2007). Significantly more eructation compared with placebo was only observed with DHASCO-T at 0.2 and 0.6 g/day. This effect was apparently not linked to DHA itself, but merely to a particular extract type.

In a randomised controlled trial with 75 patients with bipolar depression, administration of 2 g/day of EPA as ethyl ester did not differ from placebo in terms of reported loose stool and gastrointestinal discomfort (Frangou *et al.*, 2006).

In overweight healthy volunteers, the effects of EPA (1 g/day) as ethyl ester was compared to stearidonic acid (SDA) (3.7 g/day) on erythrocyte EPA and DHA levels in a randomised controlled trial (Harris *et al.*, 2008). The frequency of diarrhea and abdominal cramps of these tratments did not differ from control.

In 135 patients with Huntington disease a randomised controlled trial showed no significant difference in diarrhea and loose stool between EPA as ethyl ester (2 g/day) and placebo (Puri *et al.*, 2005).

Testing the anti-depressive effects of EPA as ethyl ester (4 g/day), Peet and Horrobin found in a randomised controlled trial that gastrointestinal disturbances were linked to 4 g of an oily substance, not to EPA as ethyl ester (Peet & Horrobin, 2002b).

In a randomised controlled trial of EPA as ethyl ester in the treatment of bipolar depression and rapid bipolar disorder the frequency of gastrointestinal side effects did not differ between EPA (6 g/day) and placebo (Keck, Jr. *et al.*, 2006).

Table 6.3: Human studies reporting gastrointestinal disturbances (g/day).

Reference	Patients (n)	Observed effects	PUFA	No effect (Related to EPA and DHA)	Effect (Related to EPA and DHA)
Bromfield *et al*., 2008	Refractory epilepsy (23)	Nausea Fishy taste	EPA and DHA (TAG)	2.2	
Hallahan *et al*., 2007	Recurrent self-harm (49)	Gastric discomfort Fishy taste	EPA and DHA (TAG)	2.1	
De Truchis *et al*., 2007	HIV-infected patients (120)	GI disorder	EPA and DHA (TAG)	1.7	
Von Schacky *et al*., 1999	Coronary heart disease (223)	GI discomfort	EPA and DHA (TAG)	2.6	
Reis *et al*., 1989	PCI (148)	GI events	EPA and DHA (Ethyl ester) EPA and DHA (Ethyl ester)		6.0 6.0
Freund-Levi *et al*., 2006	Alzheimer disease (204)	Diarrhea	EPA and DHA (TAG)	2.3	
Zhu *et al*., 2008	Non-alcoholic fatty liver (134)	Increased faecal frequency Epigastria Defecation	Seal oil	6.0	
Maresta *et al*., 2002	Restenosis (287)	Dyspepsia Epigastralgia Gastric discomfort	EPA and DHA (Ethyl ester)	5.1	
Innis & Hansen, 1996	Healthy adults (32)	Eructation	DHA (TAG, fungal oil)		1.7
Marangell *et al*., 2003	Depressed patients (36)	Belching Loose stools	DHA	2.0	
Makrides *et al*., 2009	Mother of preterm infants (545)	Diarrhea Constipation Nausea Vomiting	Tuna oil high in DHA	3.0	
Arterburn *et al*., 2007	Healthy adults (96)	Eructation	DHA (DHASCA-T, algal) DHA (DHASCA-S, algal)	 1.0	(0.2)
Frangou *et al*., 2006	Bipolar depression (75)	Loose stools GI discomfort	EPA (Ethyl ester)	2.0	
Harris *et al*., 2008	Overweight healthy (33)	Diarrhea Abdominal cramps	EPA (Ethyl ester) SDA (TAG)	1.0 3.7	
Puri *et al*., 2005	Huntington (135)	Diarrhea Loose stool	EPA (Ethyl ester)	2.0	
Peet & Horrobin, 2002	Depression (70)	GI events	EPA (Ethyl ester)	4.0	
Keck Jr *et al*., 2006	Bipolar depression (116)	GI side effects	EPA (Ethyl ester)	6.0	

In summary, gastrointestinal disturbances were frequently associated with intake of an oily substance, but the effects of the marine n-3 fatty acids could not be separated from the effect of placebo. In these studies a dose level with no gastrointestinal effects from the the marine n-3 fatty acids as such appeared to be in the range of 2–6 g/day. However, in one study gastrointestinal effects were more frequent among patients treated with 6 g/day of EPA and DHA than among patients treated with placebo, indicating a possible lowest observable effect level of the marine n-3 fatty acids.

6.9 Conclusions on negative health effects related to n-3 fatty acids

The negative health effects described in the literature reviewed are usually reported as side-effects when beneficial effects are studied, frequently in a population with clinical symptoms. The evaluated compounds were EPA and DHA as fish oil or as ethyl esters. In the majority of studies the purity of the test compounds and the content of potential harmful contaminants were not defined.

Negative health effects regarding bleeding complication in humans have not been confirmed for EPA and DHA. The question regarding bleeding time is still not resolved since there are studies reporting no significant change in bleeding time (EPA and DHA 1.8-5.4 per day). One study reporting a significant increase in bleeding time after intake of 6.9 g/day EPA and DHA in coronary heart disease patients on anticoagulant medication. International normalised ratio (INR) does not seem to be influenced by EPA and DHA according to the limited number of studies on INR. Further, it is also noted that the existing data do not allow for any conclusions with regard to possible impact of n-3 fatty acids on particularly vulnerable individuals like patients with inherited or acquired hemorrhagic diathesis.

A few studies have shown that LDL enriched with n-3 fatty acids is susceptible to oxidation. However, most of the studies show no increase in the susceptibility of LDL to oxidative modification. One study with myocardial infarction patients taking 3.5 g EPA and DHA/day showed increased plasma TBARS. No firm conclusions can be drawn regarding negative health effects related to lipid peroxidation.The evidence that the methods, which are used to measure lipid peroxidation, actually reflect lipid peroxidation *in vivo* is limited. Most of the studies were these methods have been used show no change in lipid peroxidation. No oxidative stress biomarkers are defined as risk factors predicting risk of disease. The clinical relevance of lipid peroxidation is therefore not clear.

No firm conclusions can be drawn regarding negative health effects related to systemic inflammation. However, there are indications from the use of biomarkers that EPA and DHA at doses of 5 g/day might activate endothelial cells among people at high risk of cardiovascular diseases and patients with coronary heart disease.

Some studies with EPA and DHA have reported a slightly impaired glucose control. In subjects with type 2 diabetes, the evidence indicates that glucose control is not affected. Taken together, the existing data with doses ranging from 0.8 to 4.8 g/day of EPA and DHA (mean: 2.4 g/day) show that the effects on glucose homeostasis is small and not biologically significant.

Meta-analysis has not found negative effects of EPA and DHA on standardised units of measurements of glycemic control such as HbA1c and fasting glucose.

A minor increase in LDL-cholesterol (1-3%) in subjects with type 2 diabetes has been reported in meta-analyses following supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). No dose response relationship has been reported. However, the clinical relevance in subjects with type 2 diabetes of this minor increase in LDL-cholesterol is unclear because of a concomitant reduction in serum triacylglycerol and unchanged apolipoprotein B in the same subjects. No change in LDL-cholesterol was reported in the large coronary heart intervention trials including both subjects with and without type 2 diabetes.

Negative health effects regarding gastrointestinal function including abdominal cramps, eructation, flatulence, vomiting and diarrhea have been identified with intake of oily

substances. No negative health effects have been found with doses between 2 and 6 g/day of EPA and DHA compared to the effect of placebo oil.

From the reviewed literature it has not been possible to distinguish negative health effects of EPA and DHA as TAG from those of EPA and DHA as ethyl esters. Ethyl esters of EPA and DHA were developed as a pharmaceutical drug to treat patients with cardiovascular diseases and not for a healthy population. The safety of EPA and DHA ethyl esters has only been evaluated as a drug in clinical settings and no negative health effects have been observed in humans with doses below 6 g/day.

In the literature reviewed in the present evaluation, no negative health effects of ALA have been found.

In the preclinical animal studies with this registered n-3 fatty acid drug it was indicated NOAELs in the range of 50-600 mg/kg bw/day for various toxic effects (8-20 g/day equivalent to human exposure by body surface using a standard body weight of 60 kg). In humans, the only side effect that occurred at a significantly higher rate in the 4 g/day EPA and DHA group compared to placebo was taste perversion, primarily “fishy taste”.

7 Positive health effects related to n-3 fatty acids

The possible positive health effects from n-3 fatty acids studied in the scientific literature are mainly related to the following areas:

- Cardiovascular functions
- Inflammation and immune function
- Central nervous system and mental health function

Some less well documented possible positive effects are described at the end of this chapter as “Other reported positive health effects”.

The following reports, documents and reviews on n-3 fatty acids have been valuable background documents in the assessment of positive health effects from n-3 fatty acid intake.

- Draft food based dietary guidelines to promote public health and prevent chronic diseases in Norway (Nasjonalt råd for ernæring, 2011)
- Fish consumption – risk and benefit (Becker *et al*., 2007)
- Draft report Summary of published research on the beneficial effects of fish consumption and omega-3 fatty acids for certain neurodevelopmental and cardiovascular endpoints (FDA, 2010)
- GISSI (GISSI-Investigators, 1999), JELIS (Yokoyama *et al.*, 2007), DART (Burr *et al.*, 1989), DART-2 (Ness *et al.*, 2003), GISSI-HF (Tavazzi *et al.*, 2008)
- Towards establishing dietary reference intakes for eicosapentaenoic and docosahexaenoic acids (Harris *et al.*, 2009)
- Dietary reference intakes for DHA and EPA (Kris-Etherton *et al.*, 2009)

Knowledge about the significance of dietary n-3 fatty acids in mental health and inflammation and immune functioning is emerging, and the listed reports, documents and reviews do not

cover these areas completely. Additional specified searches were therefore conducted for these areas, see section 7.1.

7.1 Literature search

Publications in section 7.2 (Positive health effects on) inflammation and immune function were found by a PubMed search using the keywords eicosapentaenoic acid OR docosapentaenoic acid OR docosahexaenoic acid OR alpha-linolenic acid OR omega-3 OR fish oil OR cod liver oil AND inflammation with the limitation 2000-2009, Humans, Clinical Trial, English. Studies reporting effect of n-3 fatty acids on circulating inflammation markers on healthy subjects, individuals at high risk for heart disease and subjects with CHD were selected. In addition publications from reference lists in published articles, including systematic reviews, were included.

Articles in section 7.4 (Positive health effects from n-3 fatty acids on) Central nervous system and mental health functions were found by MEDLINE search eicosapentaenoic acid OR docosapentaenoic acid OR docosahexaenoic acid OR alpha-linolenic acid OR omega-3 OR fish oil AND mental health/ophthalmology/neurology/brain/specified disorders with the limitation 2000-2009, Humans, Clinical Trial, English. In addition publications from reference lists in published articles, including systematic reviews, were included.

Articles in section 7.5 Other reported positive health effects were found by PubMed search using the keywords eicosapentaenoic acid OR docosapentaenoic acid OR docosahexaenoic acid OR omega-3 OR fish oil OR cod liver oil AND review AND obesity/preterm delivery/bone health/cancer.

7.2 Cardiovascular functions

Although the mechanisms of action are not fully understood, there is clinical evidence showing that EPA and DHA reduce the risk of cardiovascular disease. Reduced incidence of thrombotic disease, less sudden cardiac death and ventricular arrythmias, less mortality from heart failure, decreased level of plasma TAGs and a slight reduction in blood pressure have been shown in several large scale randomised controlled trials, and the findings are relatively consistent and conducted in patient groups with a typical Western background diet high in linoleic acid and relatively low in ALA (n-6/n-3 ratio ranging from 5 to 9).

WHO, EFSA, FAO and FDA have made several evaluations during the last decades. In a report from an expert consultation in 2008 WHO/FAO conclude that a daily intake of 0.25-2.0 g/day EPA and DHA reduces the risk of fatal CHD events and stroke in secondary prevention (FAO/WHO, 2010). The evidence was judged as convincing out of the four levels of evidence (convincing, probable, possible and insufficient). It was agreed that a specific ratio of n-6 to n-3 fatty acids could not be recommended and it was noted that 3 g/day reduces cardiovascular risk factors without negative health effects of concern in short and intermediate-term randomised trials.

According to a recent EFSA Scientific Opinion (EFSA, 2009b) various statements by authoritative bodies advocate fish intake (1-2 times per week) and/or EPA plus DHA intake (~0.25-0.50 g/day) for the prevention of coronary heart disease. In the scientific opinion EFSA recently concluded that a cause and effect relationship has been established between consumption of 3 g/day EPA and DHA and a reduction in blood pressure. This relates to both hypertensive and normotensive persons. Similarly, they concluded that a cause and effect

relationship has been established between consumption of 2-4 g/day EPA and DHA and a reduction in normal fasting TAG. Both maintenance of normal blood pressure and a reduction in fasting plasma TAGs are considered to be positive cardiovascular health effects.

7.2.1 Studies with fish oils and marine ethyl esters

The strongest data on the effects of n-3 fatty acids (EPA and DHA) in humans derives from the large prospective randomised trials. Of five large randomised controlled trials (GISSI, JELIS, DART, DART-2, GISSI-HF) including more than 43 000 patients in total have shown reduction in cardiovascular events including sudden cardiac death due to intake of marine n-3 fatty acids (Burr *et al.*, 1989; GISSI-Investigators, 1999; Ness *et al.*, 2003; Yokoyama *et al.*, 2007; Tavazzi *et al.*, 2008). Three of 5 studies used supplementation with highly purified EPA or EPA and DHA. The two GISSI trials used supplementation with 0.8 g EPA and DHA as ethyl esters per day and the JELIS trial used 1.8 g EPA as ethyl ester only. Indeed very large and high quality epidemiological studies have also provided significant additional evidence during the last decades, and in these studies the intake of n-3 fatty acids from both fish and vegetables has been studied. Patients in the trials have either been in the marine n-3 group or in a control group. One of these trials (DART-2) found that advice of increased intake of fatty fish led to an increased risk of cardiac death (Ness *et al.*, 2003). In addition to these 5 studies there are several studies on patients undergoing percutaneous coronary intervention (PCI) involving more than 4000 patients in total (Harris, 2007). These PCI studies have been quite short in duration (mostly 6-12 months) and too small to study any effect on mortality, rather restenosis have been the primary endpoint. There have been conflicting data regarding the effect of EPA and DHA on the frequency of restenosis.

In GISSI about 11 324 patients who had previously experienced a myocardial infarction were randomised to supplements with EPA and DHA (0.8 g/day) as ethyl ester with or without vitamin E. A combination of death, myocardial infarction and stroke was the primary endpoint (GISSI-Investigators, 1999). After 3.5 years a 14% reduction in all causes of death was observed in the participants randomised to EPA and DHA with a 17% reduction in CHD death and a 10% reduction in the primary endpoint. All reductions were stastistically significant. Sudden death was reduced with 45% in the n-3 group accounting for an important contributor to the reduction in all cause mortality. The study was not blinded.

In the JELIS study 18 645 patients with a total cholesterol above 6.5 mmol/L, both patients in primary prevention or secondary prevention with stable cardiovascular disease were randomly assigned to receive either 1.8 mg of EPA as ethyl ester daily with statin (EPA group; n=9326) or low dose statin only (controls; n=9319) (Yokoyama *et al.*, 2007). In the EPA group a 19% relative reduction was observed in the primary endpoint, major coronary events, after 4.6 years. Unstable angina and non-fatal coronary events were also significantly reduced in the EPA group but sudden cardiac death and coronary death did not differ between groups.

In the GISSI-HF trial 7046 patients with chronic heart failure were randomly assigned to receive supplemental 0.8 g/day EPA and DHA as ethyl esters or to placebo (Tavazzi *et al.*, 2008). A significant reduction in total mortality of 9% in the n-3 fatty acid group was observed. The mortality was high in this heart failure cohort with a relatively high mean age of 67 years. Ninehundred and fifty five (27%) patients died from any cause in the n-3 fatty acid group and 1014 (29%) in the placebo group. In the per-protocol analysis undertaken on 4994 fully compliant patients, who were defined as those who had taken experimental treatments for at least 80% of the time of observation and without major protocol violations,

the rate of all-cause death was 26% (658 of 2512) in the n-3 fatty acid group and 29% (725 of 2482) in the placebo group, p=0.041.

In the The Diet and Reinfarction Trial (DART trial) 2033 men who had recovered from myocardial infarction were allocated to receive or not to receive advice on each of three dietary factors: a reduction in fat intake and an increase in the ratio of polyunsaturated to saturated fat, an increase in fatty fish intake, and an increase in cereal fiber intake (Burr *et al.*, 1989). The subjects advised to eat fatty fish had a 29% reduction in 2 year all-cause mortality compared with those not so advised. There were 94 deaths in the fish advice group compared to 130 in the no fish advice group. The effect was significant and was not altered by adjusting for ten potential confounding factors. Those who could not eat fish were adviced to take fish oil supplements.

The Diet and Angina Randomized Trial (DART-2) involved 3114 men (mean age 61.1 years) with stable angina, who were followed up for 3-9 years (Ness *et al.*, 2003). Advice to eat fatty fish or take fish oil supplement did not affect all-cause mortality, but was associated with a significant increase in sudden cardiac death (11.5% vs 9%, p=0.018). This effect was largely limited to the subgroup given fish oil capsules. Advice to eat more fruit and vegetables had no effect, but poor compliance was discussed. However, there were quite a high number of deaths and major cardiovascular events in this study due to a relatively long time duration, high age and severity of disease. For this reason, various meta-analyses pay less attention to this study.

Recently, the Alpha Omega Trial was published (Kromhout *et al.*, 2010). In this study 4837 patients with cardiovascular disease were randomised to four groups. One group was given margarine with 0.226 g EPA + 0.15 g DHA per day, another group was given the same amount EPA and DHA plus 1.9 g ALA also in margarine, the third group was given 1.9 g ALA and the fourth group received placebo. The primary endpoint was major cardiovascular outcomes (MACE). Even with as many as 671 MACE during the study periode of 40 months, there was no significant difference in the incidence between the study groups, and there were no differences in negative health effects. The patients were older than in most other studies (mean age 69 years) and the majority of the participants were men (78%) and received state-of-the-art anti-hypertensive, anti-thrombotic and lipid-lowering theraphy in addition. The dosage of EPA and DHA was low (0.4 g/day).

7.2.2 Studies with plant oils

Replacing saturated fat with unsaturated fat in a population has been shown to reduce LDL-cholesterol and the burden of cardiovascular disease in the population. By replacing animal fat with vegetable fat, the intake of saturated fat will be reduced and the intake of ALA will increase. However, vegetable fat sources have highly variable content of ALA.

Within the body, ALA can be converted to EPA, DPA and DHA. Thus, it is possible that an intake of ALA can supply tissues with EPA and DHA. In a recent review, Brenna *et al*. conclude that tracer studies have shown a conversion from ALA to EPA, DPA and DHA. This has been observed in nearly all humans studied from birth through late middle age and in both males and females (Brenna *et al.*, 2009). Since an increased intake of ALA will increase the blood levels of EPA and DHA, an assumption is therefore that an increased intake of ALA will reduce the risk of cardiovascular disease. However, because the most potent biological effects are mediated by EPA and DHA, ALA will exert less effect since only quite small proportion are elongated and desaturated in humans. Only a few systematic reviews have looked closer into this topic regarding cardiovascular diseases.

Wang *et al*. published a systematic review of the literature on the effects of n-3 fatty acids consumed as fish or fish oils rich in EPA and DHA or as ALA (Wang *et al.*, 2006). They identified 14 randomised controlled trials of n-3 fatty acid supplements or of diets high in n-3 fatty acids. The authors concluded that evidence suggests that increased consumption of n-3 fatty acids from fish or fish oil supplements, but not of ALA, reduces the rates of all-cause mortality, cardiac and sudden death, and possibly stroke.

In another systematic review and meta-analysis of randomised controlled trials the effect on concentrations of various risk factors for cardiovascular disease were investigated (Wendland *et al.*, 2006). Treatment with ALA did not significantly modify total cholesterol, TAG, weight, body mass index, LDL, diastolic blood pressure, systolic blood pressure, VLDL and apolipoprotein B. They did, however, find a significant reduction in the concentrations of fibrinogen (0.17 µmol/l) and fasting plasma glucose (0.20 mmol/l). There was a small decrease in HDL (0.01 mmol/l).

In the report "The evidence for dietary prevention and treatment of cardiovascular disease" published in 2008 the American Dietetic Association concludes that it is possible (evidence grade III) that an intake of ALA >1.5 g per day reduces the risk for coronary heart disease and the risk for death of coronary heart disease (VanHorn *et al.*, 2008).

In the more recently published Alpha Omega Trial (Kromhout *et al.*, 2010), ALA (1.9 g/day), as compared with placebo, and EPA (0.23 g/day) or DHA (0.15 g/day), was associated with a tendency of reduction in the rate of major cardiovascular events (a hazard ratio of 0.73 (95% CI 0.51-1.03) (p=0.07)).

There is evidence that the effect of ALA is not the same as for EPA and DHA since ALA does not act through the same mechanisms of action (Sanderson *et al.*, 2002). Moreover, EFSA states that ALA is essential in human nutrition as a precursor for EPA and DHA, and apart from this ALA has no known specific function (EFSA, 2009b). However, the conversion of ALA to EPA and particularly to DHA is limited in humans, underlining that a sufficient intake of EPA and DHA is important for good health.

7.2.3 Conclusions on positive cardiovascular effects

Although the mechanisms of actions are not fully understood, clinical evidence show that EPA and DHA reduce the risk of cardiovascular disease. There is less evidence for primary prevention than secondary prevention.

The probably strongest data on the effects of n-3 fatty acids in humans derives from the large randomised controlled trials involving more than 43 000 study participants suffering from cardiovascular disease. Except in the The Diet and Reinfarction Trial (DART) study, the patients were given either 0.8 g EPA and DHA or 1.8 g of pure EPA as ethyl ester daily. The experimental evidence from these large clinical trials with lower doses is more robust compared to smaller clinical trials with higher doses (ranging from 4 to 7 g EPA and DHA per day).

There is possible evidence that an intake of ALA >1.5 g per day reduces the risk for coronary heart disease and the risk for death of coronary heart disease (VanHorn *et al.*, 2008). The bulk of the present evidence suggests that ALA does not have the same effects as EPA and DHA on the vascular system or on the biochemical factors such as plasma TAG concentration.

Comment: Over the past 40 years and more, the effect of n-3 fatty acids, particularly EPA and DHA, has been more thoroughly studied with respect to cardiovascular disease than any

other biological effects. Several large prospective, controlled studies have been conducted and the data allow fairly reliable conclusions. Some general challenges in the interpretation of the studies should, however, be noted.

Firstly, most controlled studies have been conducted on people with cardiovascular disease. The effect of EPA and DHA has been documented in secondary prevention (for patients with coronary heart disease or severely increased risk for coronary heart disease) but the data is less convincing in primary prophylaxis. This might be due to few available studies in healthy individuals.

Secondly, the preventive treatment of cardiovascular disease has been modernised significantly in the last 20-30 years. A population with cardiovascular disease anno 2010 is treated in a different way than before. This applies to key risk parameters such as cholesterol, blood pressure, diabetes and anti-thrombotic therapy. The drug treatment has become more aggressive. This means that the populations studied previously may not be completely comparable to today's population regarding secondary prophylaxis. Questions may be asked on whether n-3 fatty acids have the same effect on populations who today are optimally treated versus previous study populations using less medication. Therefore, some uncertainty lies in the fact that the positive effects from n-3 fatty acid treatment observed in the GISSI trial and other older studies would have a lower impact in patients using modern optimal post-myocardial infarction treatment.

Thirdly, the optimal dose is not known and the dose question in different populations may vary depending on the basal dietary intakes of n-3 fatty acids and n-6 fatty acids.

7.3 Inflammation and immune function

Epidemiological observational studies have shown an inverse correlation between intake of n-3 PUFAs from fish and/or fish oil and circulating markers of inflammation (Madsen *et al.*, 2001; Lopez-Garcia *et al.*, 2004). However, studies investigating the relationship between dietary fatty acids and the immune response conducted in healthy persons or in patients suffering from immune-related diseases have been inconsistent. EFSA recently stated that our knowledge for the evaluation of effects of ALA and the marine n-3 fatty acids on circulating markers in general is too weak to draw clear conclusions.

Data from animal studies have shown that n-3 fatty acids can have anti-inflammatory and immunomodulatory activities in a wide array of diseases e.g. autoimmunity, arthritis and infection (Fritsche, 2006). Mechanistic studies conducted *in vitro*, indicates that n-3 fatty acids may affect immune function by acting on phagocytosis and inhibiting the production of cytokines by reducing the T-cell response (Calder, 2008c).

7.3.1 Studies with fish oils and marine ethyl esters

Circulating markers of low-grade systemic inflammation

The literature review identified thirteen studies in which circulating markers of inflammation were assessed in healthy individuals. Six of these studies report no significant effect of intake of EPA and DHA on C-reactive protein (CRP), cytokines or adhesion molecules with doses from 0.9 to 5.9 g/day (Madsen *et al.*, 2003; Geelen *et al.*, 2004; Vega-Lopez *et al.*, 2004; Fujioka *et al.*, 2006; Michaeli *et al.*, 2007; Pot *et al.*, 2009). A beneficial effect was observed in 6 studies and were related to a significant decrease in CRP, the cytokine IL-6, soluble intercellular adhesion molecule (sICAM-1) or soluble vascular adhesion molecule (sVCAM-

1) compared to the control group (Miles *et al.*, 2001; Thies *et al.*, 2001; Ciubotaru *et al.*, 2003; Paulo *et al.*, 2008; Yusof *et al.*, 2008; Tsitouras *et al.*, 2008). The beneficial effects were seen with both high and low doses (1-5 g/day) EPA and DHA. Cazolla *et al*. observed an increase in sE-selectin after intake of 4.05 g EPA while the same concentration of EPA tended to decrease s ICAM-1 (Cazzola *et al.*, 2007). This study is described in section 6.6.

Fifteen studies have assessed circulating markers of inflammation after intake of marine n-3 fatty acids in individuals at high risk of developing CHD. No significant effects on circulating markers were determined in nine of these studies in which EPA or DHA (4 g/day ethyl esters) or a combination of EPA and DHA (1-3.2 g/day) were given (Sampson *et al.*, 2001; Chan *et al.*, 2002; Mori *et al.*, 2003; Jellema *et al.*, 2004; Krebs *et al.*, 2006; Plat *et al.*, 2007; Browning *et al.*, 2007; Murphy *et al.*, 2007; Kabir *et al.*, 2007). A significant decrease in CRP, cytokines (IL-18, IL-6 or TNF-α) or adhesion molecules (sE-selectin and/or sICAM-1) compared to the control group were reported in 4 studies with doses ranging from 1.2 to 1.4 g/day of EPA and DHA (Berstad *et al.*, 2003; Hjerkinn *et al.*, 2005; Accinni *et al.*, 2006; Troseid *et al.*, 2009), and a decrease in IL-6 in one study with 3 g DHA per day (Kelley *et al.*, 2009). In contrast, one study reports an increase sE-selectin and sVCAM-1 after intake of 4.8 g/day EPA and DHA (ethyl ester) in hyperlipidemic male smokers (Seljeflot *et al.*, 1998). This study is further described in section 6.6.

There are also intervention studies investigating the effect of EPA and DHA in patients with coronary heart related disease. Five out of 8 studies do not show any effect on CRP, cytokines or adhesion molecules in the circulation with doses ranging from 0.8 to 3.4 g/day EPA and DHA as ethyl esters or 4.3 g/day EPA and DHA (Grundt *et al.*, 2003a; Lee *et al.*, 2006; Madsen *et al.*, 2007; Schiano *et al.*, 2008) or adhesion molecules in plaques from carotid stenosis with 1.4 g/day EPA and DHA (Thies *et al.*, 2003). A potential beneficial effect on inflammation was found in one study in which a reduction in sVCAM-1 and IL-6 was observed with salmon containing high levels (2.9 g/day) of EPA and DHA (Seierstad *et al.*, 2005). Two studies report elevation of circulating markers of inflammation. In a study by Johansen *et al.*, sE-selectin and sVCAM-1 increased by the intake of 5.1 g/day EPA and DHA (Johansen *et al.*, 1999b). EPA and DHA supplementation in heart transplanted patients enhanced the TNF/IL-10 ratio indicative of a pro-inflammatory effect (Holm *et al.*, 2001). These studies are further described in section 6.6.

Inflammatory diseases

A meta-analysis of nine randomised controlled trials with children (> 2 years) and adults with asthma receiving fish oil for more than 4 weeks did not show any significant effects on asthma symptom scores, lung function, asthma medication or bronchial hyper-responsiveness (Woods *et al.*, 2002) (Cochrane database of systematic reviews and references therein). Not all endpoints were studied in each trial. Doses used varied between 1 g/day of EPA and DHA to 3.2 g EPA+2.2 g DHA per day (18 capsules per day) or 3.6 g EPA+2.3 g DHA per day (20 ml fish oil per day), and only EPA supplementation (4 g EPA/day). There was no consistent effect on any of the investigated parameters and no adverse effects were associated with fish oil supplements.

A number of randomised placebo controlled, double blinded trials of fish oil treatment (daily doses ranging from 1.4 g EPA+0.2 g DHA to 4.6 g EPA+2.5 g DHA) in patients with rheumatoid arthritis (RA) have been reviewed by Calder in 2008. Almost all the reviewed studies showed beneficial effects of fish oil (Calder, 2008b). Both subjective and objective symptoms improved, including reduced duration of morning stiffness, number of tender or

swollen joints, joint pain, and time of fatigue and grip strength. Of particular importance was the observed reduction in the use of anti-inflammatory drugs. One meta-analysis from 1995 based on results from trials published in the period 1985-1992 concluded that dietary fish oil supplementation for 3 months reduced joint symptoms and morning stiffness (Fortin *et al.*, 1995). Another meta-analysis based on 17 randomised controlled trials conducted with Cochrane review Manager for 6 separate outcomes, assessed the pain-relieving effects of marine n-3 fatty acids in patients with rheumatoid arthritis or joint pain secondary to inflammatory bowel disease (IBD) and dysmenorrhoea. It showed that supplementation for 3-4 months reduced joint pain intensity, morning stiffness and number of painful and/or tender joints. However, significant effects were not detected for physician-assessed pain at 3-4 months (Goldberg & Katz, 2007).

The effect of fish oil (2.7-5.6 g/day) on IBD such as ulcerative colitis and Crohn's disease has been studied in randomised placebo-controlled double blinded trials (Calder, 2008a). The dose of EPA and DHA varied mainly from 1.6 g EPA+1.1 g DHA to 3.2 g EPA+2.4 g DHA per day. Reviews of fish oil and IBD generally conclude that there is a small but not significant improvement, but some studies have given contradictory results (Belluzzi *et al.*, 2000; Belluzzi, 2002; MacLean *et al.*, 2005; Becker *et al.*, 2007; Calder, 2008a). The conclusion from a meta-analysis which identified 13 randomised controlled trials in Crohn's disease and ulcerative colitis was that the available data are insufficient to draw conclusions about the effects of marine n-3 fatty acids on clinical score, remission and relapse rate (MacLean *et al.*, 2005). Consistent across 3 studies was the finding that marine n-3 fatty acids reduce corticosteroid requirements, although statistical significance was shown in only one of these studies (MacLean *et al.*, 2005). In a study by Belluzzi *et al*. patients with Crohn's disease (n=78) were treated daily for 12 months with fish oil capsules containing a new marine lipid concentrate in free fatty acid form resulting in a daily dose of 1.8 g EPA+0.9 g DHA or placebo-oil (Belluzzi *et al.*, 1996). In this study they showed that fish oil significantly reduced the rate of relapse. In contrast, two large multi-centre randomised, double blinded, placebo-controlled studies did not demonstrate any effect of marine n-3 free fatty acids among patients with Crohn's disease activity index score of less than 150 (Feagan *et al.*, 2008). These two studies have been included in a recent Cochrane review (in total 6 studies were eligible for inclusion), and the conclusion from this review was that intake of EPA and DHA is safe but probably ineffective for maintenance of remission in Crohn's disease (Turner *et al.*, 2009). The doses varied from 1.2 g EPA + 0.6 g DHA per day as TAG to 3.3 g EPA + 1.8 g DHA per day as ethyl esters.

7.3.2 Studies with plant oils

Only a few studies of the effect of ALA on the immune function have been performed (Nordstrom *et al.*, 1995; Rallidis *et al.*, 2003; Bemelmans *et al.*, 2004; Zhao *et al.*, 2004).

A randomised controlled 3-diet study with crossover design (6-week intervention and 3 week wash-out) was conducted in order to study inflammatory markers in hypercholesterolemic subjects (n=23) (Zhao *et al.*, 2004). The diets consisted of either a control (an average American diet consisting of 0.8 g ALA per day, linoleic acid:ALA ratio 10:1), a diet high in ALA and PUFA (5.2 g ALA per day, linoleic acid:ALA ratio 2:1) and a diet high in linoleic acid and PUFA (2.7 g ALA, linoleic acid:ALA ratio 4:1). Total fat was approximately 35 E% in all three diets. The level of CRP was significantly reduced in the ALA group ($p<0.01$) but a reduction trend was also observed in the linoleic acid group ($p=0.08$). The level of ICAM-1 was significantly reduced in both the ALA ($p<0.01$) and the linoleic acid ($p<0.01$) group compared to the average American diet. Furthermore, they showed that the ALA diet

significantly reduced VCAM-1 (p<0.01) and E-selectin (p<0.01) more than the linoleic acid diet.

Rallidis *et al*. reported a significant reduction within the group in CRP (p=0.0008) and the IL-6 (p=0.01) after supplementation with 15 ml linseed oil (8 g ALA per day) in dyslipidaemic male patients (n=76) for 3 months. No significant effects were observed in the group who received 15 ml safflower oil (11 g linoleic acid per day). The n-6:n-3 ratio was 1.3 in the linseed group and 13.2 in the safflower group (Rallidis *et al.*, 2003).

A randomised double-blind placebo-controlled trial in moderately hypercholesterolemic (n=103) men and women was performed (Bemelmans *et al.*, 2004). The participants were assigned to a group with margarine enriched with ALA (fatty acid composition 46% ALA, 15% linoleic acid) or linoleic acid (58% linoleic acid and 0.3% ALA) for 2 years. The daily intake of ALA was 2.3 E% in the ALA group and 0.4 E% in the linoleic acid group. A significant lowering effect of CRP levels (p<0.05) in the ALA-enriched margarine group compared to the linoleic acid-enriched margarine group, but no effect on IL-6, IL-10 and sICAM-1 was found.

In patients with rheumatoid arthritis one randomised double-blind placebo-controlled trial showed no effect of ALA (Nordstrom *et al.*, 1995).

7.3.3 Conclusions on positive effects on inflammation and immune function

Our knowledge basis for the evaluation of effects of ALA and EPA and DHA on circulating markers related to low-grade systemic inflammation is in general too weak to draw clear conclusions.

No conclusion for patients with chronic inflammatory diseases can be stated based on information of effects of intake of ALA gathered in this evaluation.

No definite conclusion can be drawn regarding the efficacy of n-3 fatty acid supplementation as a treatment for asthma in children and adults using doses from 1 to 5.4 g/day EPA and DHA.

N-3 fatty acids are probably ineffective for maintenance of remission in Crohn's disease even when using doses up to 5.6 g EPA and DHA per day as fish oil, and there is insufficient evidence of benefit of EPA and DHA in ulcerative colitis.

In patients with rheumatoid arthritis the conclusion is that even if some studies have not shown any benefits of n-3 fatty acids, most studies show that an intake of fish oil (ranging from 1.6 to 7.1g/day EPA and DHA) can lessen symptoms or reduce the use of anti-inflammatory drugs.

EFSA has stated that a cause and effect relationship has not been established between the consumption of EPA and DHA and the maintenance of normal joints (EFSA, 2009a).

7.4 Central nervous system (CNS) and mental health functions

The field of research relating fatty acids to the central nervous system (CNS) is relatively new, most trials being performed during the last 15 years. The complexity of this field, reflecting the complexity of CNS and how CNS changes during life, implies that many small-sized trials have been run with different conditions and response parameters. This makes them difficult to compare. The evidence of positive effects of EPA and DHA on the CNS in

humans stems both from observational and experimental studies. In general, subjects have been patients, though a few trials have been run in healthy groups.

Efforts to evaluate and summarise the findings have been made by The National Food Administration in Sweden (Becker *et al.*, 2007), the US FDA (FDA, 2010) and The Norwegian National Council of Nutrition (Nasjonalt råd for ernæring, 2011). In the present evaluation, 56 double-blind randomised clinical trials and three reviews concerning CNS function in healthy subjects or in patients with CNS disorders have been included. The conclusions regarding some endpoints in previous reports have been modified based upon a more extended literature search and new studies.

Briefly, the previous reports acknowledge a positive relationship between intake of fish or fish oil and early neurodevelopment. They do not mention trials with fatty acids in healthy subjects, and only the Swedish report mentions disorders of childhood and adolescence. They conclude that the current evidence of a clinical effect from n-3 fatty acid supplementation in patients with depression and schizophrenia is insufficient. As for dementia, the Swedish report states that n-3 fatty acids may be preventive in the early development of Alzheimer's disease (AD), whereas the Norwegian report states that no evidence exists that n-3 fatty acids inhibit the progression of dementia.

7.4.1 Studies with fish oils and marine ethyl esters

7.4.1.1 *Neurodevelopment during pregnancy and infancy*

Several epidemiological studies and trials have been reviewed by Makrides *et al.* indicating a positive relationship between the amount of fish oil consumed and neurodevelopmental effect (Makrides, 2008). This is in line with conclusions in previous reports. Recently, Makrides *et al*. (2009) showed that girls' mental development at 18 months was significantly better if their lactating mothers had received high (3 g/day of tunafish oil rich in DHA) versus standard doses of DHA supplementation. For the total group there were fewer infants with significant mental delay in the high-DHA group (Makrides *et al.*, 2009). Gibson *et al.* did not find significant differences related to neurodevelopment between groups of healthy term infants receiving 0.6 g PUFA (mainly arachidonic acid and DHA) or placebo (Gibson *et al.*, 2009). More research is needed to establish dose-effects relationships and persistence of effects. EFSA has recently proposed an AI of 0.10 g/day DHA for infants (>6 months of age) and small children below 24 months based on visual function.

7.4.1.2 *CNS functioning in healthy subjects*

Randomised placebo-controlled trials have shown that DHA-rich fish oil reduces aggression in healthy subjects (Hamazaki *et al.*, 1998; Itomura *et al.*, 2005). Fontani *et al*. showed that 2.8 g/day EPA-rich fish oil, compared to olive oil, had a significant positive effect on a broad range of mental functions in healthy volunteers (Fontani *et al.*, 2005). In contrast, Antypa *et al*. showed that 2.3 g/day EPA-rich supplements had limited CNS effects among 54 healthy students, possibly because of a ceiling effect, i.e. no further effect above 2.3 g/day (Antypa *et al.*, 2009).

7.4.1.3 *CNS disorders*

Behavioural and mental disorders in childhood and adolescence

Several randomised trials using EPA-rich fish oil have shown significant positive effects on ADHD-related symptoms with 0.5-0.7 g/day EPA and DHA or EPA alone (Richardson & Puri, 2002; Richardson & Montgomery, 2005; Sinn & Bryan, 2007; Johnson *et al.*, 2009; Gustafsson *et al.*, 2010). In contrast, the effect of DHA-rich oils (EPA:DHA ratio less than 1:3) on ADHD symptoms did not differ significantly from placebo (Voigt *et al.*, 2001; Stevens *et al.*, 2003; Hirayama *et al.*, 2004).

Schizophrenia and other psychotic disorders

Studies published before 2006 (Fenton *et al.*, 2001; Peet *et al.*, 2001; Emsley *et al.*, 2002; Peet & Horrobin, 2002a) are included in the previous reports (Becker *et al.*, 2007; FDA, 2010; Nasjonalt råd for ernæring, 2011). In trials published later than 2006 only EPA as ethyl esters or EPA-enriched fish oil have been used, mostly combined with ordinary anti-psychotic drugs (Emsley *et al.*, 2006; Berger *et al.*, 2007). The effect on symptoms has differed widely from study to study, from beneficial to detrimental.

In one study 81 non-psychotic ultra high-risk adolescents received supplements with 700 mg EPA + 480 mg DHA or placebo as the only medication for 12 weeks. At 12 months follow-up, about 4.9% in the fish oil group and about 27.5% in the placebo group had transitioned to psychosis (p=0.004). The treatment with EPA and DHA had a significant beneficial effect on psychiatric symptoms and overall functioning (Amminger *et al.*, 2010).

Mood disorders

In a meta-analysis by Appleton *et al*. on the effect of n-3 fatty acids in depressive symptoms, all trials were analysed together, not taking into consideration the different types of n-3 fatty acids (Appleton *et al.*, 2006). However, all the studies included in the meta-analysis using pure EPA or EPA >DHA in dosages 1-2 g/day (0.6 g/day in the children study) showed a significant beneficial effect (Nemets *et al.*, 2002; Peet & Horrobin, 2002c; Su *et al.*, 2003; Nemets *et al.*, 2006; Frangou *et al.*, 2006). None of the studies included in the meta-analysis using supplements with pure DHA or DHA > EPA showed a significant difference between n-3 fatty acids and placebo (Marangell *et al.*, 2003; Silvers *et al.*, 2005).

All studies published after the meta-analysis by Appleton *et al*. confirm the validity of this distinction between EPA and DHA (Hallahan *et al.*, 2007; Mischoulon *et al.*, 2008; Jazayeri *et al.*, 2008; Carney *et al.*, 2009). A recent meta-analysis of 28 trials of n-3 fatty acids where depressive symptoms were a reported outcome, concluded: "..there is substantial evidence both from the current meta-analysis, and from the studies outlined above, that EPA and not DHA may be effective in depressive disorders. However, further studies are required of sufficient methodological quality, duration, and sample size to confirm these findings" (Martins, 2009). Symptoms of depression were significantly reduced in 13 studies using supplements with more than 50% EPA (p =0.005) and in 8 studies using pure EPA as ethyl ester (p=0.002).

Dementia and cognitive deficits in the elderly

In one trial in patients with mild to moderate Alzheimer's disease (n=32) n-3 fatty acids (600 mg EPA + 1720 mg DHA daily as TAG) for 26 weeks reduced the decline in cognitive functioning more than placebo (corn oil) (p=0.02) (Freund-Levi *et al.*, 2006).

Cole *et al*. stated that DHA alone may be beneficial in mild to moderate-severe Alzheimer's disease, but may not work well by itself in cases of established Alzheimer disease (Cole *et al.*, 2009). However, it may be effective even in established Alzheimer disease in combination with an antioxidant.

Cunnane *et al*. stated in a review that it is unclear whether DHA itself has any effect once aging-associated cognitive decline or Alzheimer's disease is clinically evident (Cunnane *et al.*, 2009). The authors also focused on the span of doses of DHA used in these studies covering a 36-fold range from 120 mg/day to 4.3 g/day without identifying any dose-related effects on cognition. According to Cunnane *et al*. this might be due to the fact that supplemental DHA may be beneficial when cognitive decline is mild, but that supplemental DHA may not necessarily be recommended when the decline is more severe, possibly because additional DHA might contribute to degenerative processes in the brain related to lipid peroxidation. However, the authors in this review did not distinguish systematically between EPA and DHA.

Other CNS disorders

EPA has been beneficial in trials on borderline personality disorder (Zanarini & Frankenburg, 2003) and substance abuse (Buydens-Branch *et al.*, 2008). Overall, studies are too few for conclusions to be drawn regarding the effect of n-3 fatty acids in these mental disorders. There is no robust evidence of beneficial effects of n-3 fatty acids in neurology (Vaddadi *et al.*, 2002; Puri *et al.*, 2002; Puri *et al.*, 2005; Weinstock-Guttman *et al.*, 2005; Yuen *et al.*, 2005; DeGiorgio *et al.*, 2008; Bromfield *et al.*, 2008; The Huntington Study Group, 2008; Shinto *et al.*, 2009).

There is also insufficient evidence for drawing conclusions regarding the effect of n-3 fatty acids in ophthalmology (Hoffman *et al.*, 2004; Hodge *et al.*, 2007).

7.4.2 Studies with plant oils

No controlled trials using plant oils for improving CNS functioning or for preventing or treating mental health functions have been identified.

7.4.3 Conclusions on positive effects on CNS and mental health functions

There is emerging evidence indicating that EPA and DHA have positive effects on CNS and mental health functions. At present there are a limited number of studies but this is a topic gaining increased attention.

The evidence of a beneficial effect of supplementation with EPA and DHA on neurodevelopment seems to be greatest for preterm infants and for supplementation via the mother during the last half of pregnancy. However, no firm conclusion can be made for postnatal supplementation to term infants. This is in line with previous reports (Becker *et al.*, 2007; FDA, 2010; Nasjonalt råd for ernæring, 2011).

There is some evidence of a beneficial effect of 0.6-2.8 g/day of EPA and DHA on mood, aggression and cognition in healthy subjects, but studies are few and no firm conclusions can be made.

Overall, in CNS disorders, the most consistent effects are seen when EPA is used alone or mixed with DHA in a proportion of EPA:DHA>3:2. There is some evidence of a beneficial effect of EPA-rich supplements in children-adolescent disorders, especially ADHD (dose range 0.5-0.7 g/day). So far, the best evidence within adult psychiatry is for mood disorders, mainly showing effects at 1-2 g/day of EPA alone or combined with DHA. However, in most of these studies EPA and DHA have been added to ordinary medication.

No firm conclusions can be made regarding the effect of EPA and DHA in schizophrenia or dementia or other CNS and eye disorders.

Comment: Several problems pertain to judging health effects of EPA and DHA on the CNS (mainly the brain). Trials have been quite few and samples have been relatively small (mostly < 100 subjects). The resulting lack of statistical power implies that the true effect of EPA and DHA will be underestimated. Few and small studies increase the risk of biased publication, entailing an overestimation of beneficial effects. Smaller sample sizes imply that less can be said about effect modifiers and confounding factors. Early neurodevelopment (infancy and early childhood) and affective disorders (depression) are the fields best studied. Meta-analyses have been published in several fields, including early neurodevelopment, autism, affective disorders, schizophrenia, borderline personality disorder and dementia. Only when meta-analyses are performed, more conclusive statements about effects should be expressed.

Most authors of systematic reviews do not distinguish between EPA and DHA, and there is increasing evidence that the effect of EPA and DHA differ considerably in the CNS. EPA seems to have a stronger pharmacological effect in the CNS than DHA. It is therefore the essential component when treating CNS disorders. DHA is, on the other hand, much more prevalent in the brain than EPA. Reviewers who do not distinguish between EPA and DHA usually will conclude that “n-3 fatty acids” have a minor effect, whereas those who report separately results from trials on EPA-rich and DHA-rich agents more often conclude that EPA yields major beneficial effects, whereas DHA does not (e.g. Appleton *et al*., 2010 vs Martins *et al*., 2009).

For many disorders, especially schizophrenia and depression, EPA and DHA are used as supplements to ordinary medication in the majority of trials. Thus, we will not know the effect of EPA and DHA alone, which is more relevant for judging their health effects as food supplements in the general population.

It is not known how the background diet modifies the effect of EPA and DHA taken as food supplements. Probably the effect of the supplement on the CNS will differ according to the dietary intake of EPA and DHA.

7.5 Other reported positive health effects

In addition to the reported effects on inflammation and immunity, cardiovascular diseases, and in various mental illnesses which have been discussed in the previous chapter, there are also indications of a positive influence of the n-3 fatty acids on obesity, preterm delivery, bone health, cancer and fertility. However, the documentation for these lifestyle diseases is scarce and only some of them will be mentioned briefly in the present chapter.

7.5.1 Metabolic syndrome, obesity and insulin resistance

Metabolic syndrome is a condition including glucose intolerance, insulin resistance or diabetes, in addition to at least two of the following risk factors; abdominal obesity, dyslipidemia, hypertension or albuminuria. Obese people in general are at elevated risk for diabetes, a condition in which normal amounts of insulin fail to maintain normal blood glucose levels. Some epidemiological studies on inuits and Alaskan eskimos have found a positive association between serum concentrations of marine n-3 fatty acids and protection against such insulin resistance and thereby the development of type 2 diabetes (Ebbesson *et al.*, 2005; Thorseng *et al.*, 2009). Other epidemiological studies have found the opposite effect (Djousse *et al.*, 2010). The basis for the conflict between results in epidemiological studies have not yet been clarified e.g. in intervention studies. The numbers of human intervention studies are limited and partly inconclusive (Fedor & Kelley, 2009). Studies conducted on healthy individuals whose glucose and insulin levels are within the normal range, indicate minor or no improvement of insulin resistance (Itoh *et al.*, 2007; Giacco *et al.*, 2007). In subjects with increased inflammation or with high degree of obesity, the insulin resistance may be negatively associated with the marine n-3 status (Haugaard *et al.*, 2006).

7.5.2 Preterm birth

The marine n-3 fatty acids are important to fetal and infant growth and development. It has also been suggested that a greater intake of marine n-3 fatty acids are improving pregnancy outcomes, i.e. preventing preeclampsia, prolonging gestation and enhancing pregnancy duration (Olsen, 2004). In a meta-analysis by Szajewska *et al*., n-3 fatty acid supplementation was associated with a small, but significant increase in duration of pregnancy and in head circumference, compared to control. However, no significant differences were found in the percentage of preterm deliveries or in the birth weight (Szajewska *et al.*, 2006). It has been speculated that the effect of fish oil on gestational age has been underestimated due to limited effect of intervention in high fish intake population (Secher, 2007). Thus the question whether marine n-3 fatty acids can affect pregnancy outcomes and birth weight deserves further attention.

7.5.3 Bone health

Postmenopausal women are at greatest risk of developing osteoporosis, which is a condition with decreased bone mass and increased risk for fractures. No cure exist for this disease, thus the best strategy for women is to maximize their peak bone mass before the age of 30 and to decrease the bone resorption after menopause. The maximum bone development may be obtained by a well-balanced diet including vitamin D, calcium, and n-6 and n-3 fatty acids. So far, the evidence for a direct effect of marine n-3 fatty acids on osteoporosis is still lacking, however, there is emerging evidence of beneficial effects of marine n-3 fatty acids on bone metabolism and bone disease, partly due to its antagonistic effect to arachidonic acid in prostaglandin synthesis and its suppression of inflammatory cytokines (Watkins *et al.*, 2001; Kruger *et al.*, 2010). Evidence emerging from human studies support the premise that dietary marine n-3 fatty acids and the n-6:n-3 ratio influence bone metabolism and bone disease, and the underlying mechanisms might be attributed to increased intestinal calcium absorption, increased calcium deposition in bone, increased collagen synthesis, and reduced bone resorption due to decreased urinary calcium excretion (Watkins *et al.*, 2003).

7.5.4 Cancer

A positive correlation has been found between the incidence of various cancers (breast, colon and lung) and high intake of fat (World Cancer Research Fund/American Institute for Cancer Research, 2007). However, diets rich in marine n-3 fatty acids are inversely correlated with the development of colorectal cancer (Riediger *et al.*, 2009; Fetterman, Jr. & Zdanowicz, 2009). So far the exact mechanism behind any prevention of cancer is unknown. It may be related to the link between a reduction in n-6:n-3 ratio, a shift in the production of eicosanoids towards the 3-series of prostaglandins and a subsequent suppression of cell proliferation (Courtney *et al.*, 2007). However, the evidence for a protective effect against cancer of n-3 fatty acids is still inconclusive (Foley *et al.*, 2004). Randomised, placebo-controlled, clinical trials are required to document the possible anti-cancer effect of the dietary n-3 fatty acids.

8 Intake assessment

The calculations on intake of n-3 fatty acids are based on consumption data from the nationally representative dietary surveys Norkost, Ungkost, Småbarnskost and Spedkost. The concentration levels of the different n-3 fatty acids in regular foods and drinks used in the calculations are based on data from the Norwegian Food Composition Table 1995 (Matvaretabellen, 1995), including an annex with fatty acids in 400 foods and drinks. It is however worth mentioning that the n-3 fatty acids in margarines, n-3 supplements and farmed trout and salamon have changed since the early nineties. Composition data on food supplements and particularly fortified foods are limited in the national dietary surveys, as is the knowledge about frequency of use of n-3 supplements and consumption patterns of foods containing n-3 fatty acids including fortified foods in various age groups.

The food supplements included in the intake from regular foods (scenario 1 in section 8.1) are mainly cod liver oils.

The concentration levels of n-3 fatty acids in fortified foods used in the “estimated intake” (scenario 2 in section 8.2) were collected from the manufacturers (see page 58), and the concentration levels in food supplements in scenario 2 are a calculated weighed average of n-3 fatty acid supplements used in the Norwegian Mother and Child Cohort Study (Brantsaeter *et al.*, 2007).

The following consumption surveys are used in the intake calculations:

- Adults; Norkost 1997 is based on a quantitative frequency questionnaire that was answered by 1291 males and 1381 females aged 16-79 years (Johansson L & Solvoll K, 1999).
- 9- and 13-year old children/adolescents; Ungkost 2000 is based on a 4-day food intake registration (9-year olds 815 children, 13-year olds 1009 adolescents). Food amounts were presented in predefined household units or as portions estimated from photographs (Øverby & Andersen, 2002).
- 4-year old children; Ungkost 2000 (study conducted in 2001) is based on a 4-day food intake registration (391 children). Food amounts were presented in predefined household units or as portions estimated from photographs (Pollestad *et al.*, 2002).
- 2-year old children; Småbarnskost 1998/1999 is based on a semi-quantitative food frequency questionnaire (FFQ) answered by 868 males and 852 females (Lande & Andersen, 2005a).

- 1-year old children; Spedkost 1998/1999 is based on a semi-quantitative FFQ answered by 1022 males and 910 females (Lande & Andersen, 2005b). Only those children who were not breastfed were included in the intake assessment (674 males and 557 females).

These studies were conducted from 1997 to 2001, and are the only nationally representative studies where n-3 fatty acids intake has been estimated. It is however important to notice that there is a timelag of 10 years since these studies were conducted and that eating habits including supplement use with n-3 fatty acids might have changed in this time period.

Food supplement concentrations in the calculations in scenario 2 are collected from MoBa (Haugen *et al.*, 2008). The study is based on 40 108 women participating in MoBa. The women had filled in version 2 of the FFQ in MoBa between February 2002 and February 2005.

- The Norwegian Mother and Child Cohort Study (MoBa) is a cohort study, initiated by and maintained at the Norwegian Institute of Public Health (Magnus *et al.*, 2006). In brief, MoBa is a nation-wide pregnancy cohort that in the years 1999-2009 has included more than 100 000 pregnancies. Women were recruited to the study through a postal invitation in connection with a routine ultrasound examination offered to all pregnant women in Norway during weeks 17–18 of gestation. The participation rate was 43% (Magnus *et al.*, 2006).

8.1 Intake from regular foods and the food supplements included in Norkost, Ungkost, Småbarnskost and Spedkost–scenario 1

The intakes of n-3 fatty acids in different age groups of the Norwegian population excluding intake from fortified products (scenario 1) are shown in Table 8.1 and described below. Recommended intake of n-3 fatty acids is at least 0.5 E% for children from 2 years of age and adults, and at least 1 E% for infants 6-11 months and pregnant and lactating women. No specific recommendations related to the intake of EPA, DPA or DHA are given to the Norwegian population (Sosial- og helsedirektoratet, 2005). EFSA recommends 0.10 g/day DHA for children from 6 to 24 months, and 0.25 g/day EPA and DHA for children, adolescents and adults above 2 years.

The average intake of total n-3 fatty acids for 1 year olds who take supplements is 1.2 g/day (0.8 E%), i.e. below the recommended level of 1 E%. At the 95th percentile intake is two-fold higher (2.5 g/day and 1.7 E%). The intake at the 5th percentile is 0.2 E% (supplements included). The average intake of EPA+DPA+DHA including supplements is 0.4 g/day. The lowest intake of EPA+DPA+DHA in this age group is approximately zero (5th percentile with or without supplements) and the highest intake is 1.4 g/day (95th percentile with supplements).

The average intake of total n-3 fatty acids in the age groups 2, 4, 9 and 13 years who take supplements is 1.6 – 1.8 g/day (0.7 – 1.1 E%), and the 2 year olds have the highest E%. The intake of total n-3 fatty acids at the 95th percentile (supplements included) is 3.0 – 3.5 g/day (1.3 – 2.0 E%) for the same age groups.

Table 8.1: Intake of n-3 fatty acids from national dietary surveys – scenario 1 (all numbers are rounded off).

		ALA (g/day)		**(EPA+DPA+DHA)** (g/day)		**Total n-3** (g/day)		**E% n-3**	
		Incl. Suppl.	*No suppl.*	*Incl. suppl.*	*No suppl.*	*Incl. suppl.*	*No suppl.*	*Incl. suppl.*	*No suppl.*
16-79 year olds	Average	1.9	1.8	0.9	0.6	2.7	2.4	1.1	1.0
	5th percentile	0.7	0.6	0.1	0.1	0.9	0.9	0.5	0.5
	95th percentile	4.1	4.1	2.7	1.4	5.7	5.1	2.0	1.5
13-year olds	Average	1.4	1.4	0.3	0.2	1.6	1.5	0.7	0.7
	5th percentile	0.5	0.5	0.0	0.0	0.6	0.6	0.3	0.3
	95th percentile	2.8	2.8	1.1	0.7	3.5	3.2	1.3	1.2
9-year olds	Average	1.4	1.4	0.3	0.2	1.8	1.6	0.8	0.7
	5th percentile	0.6	0.6	0.0	0.0	0.7	0.7	0.4	0.4
	95th percentile	2.6	2.6	1.2	0.7	3.3	3.0	1.4	1.2
4-year olds	Average	1.2	1.2	0.4	0.2	1.6	1.3	1.0	0.8
	5th percentile	0.6	0.6	0.0	0.0	0.7	0.6	0.5	0.4
	95th percentile	2.1	2.1	1.4	0.6	3.0	2.4	1.6	1.3
2-year olds	Average	1.1	1.0	0.6	0.2	1.7	1.2	1.1	0.8
	5th percentile	0.5	0.5	0.0	0.0	0.6	0.5	0.4	0.4
	95th percentile	1.9	1.9	1.7	0.7	3.3	2.3	2.0	1.3
1-year olds[1]	Average	0.7	0.7	0.4	0.1	1.2	0.8	0.8	0.6
	5th percentile	0.2	0.2	0.0	0.0	0.2	0.2	0.2	0.2
	95th percentile	1.7	1.6	1.4	0.4	2.5	1.9	1.7	1.2

[1]Only the non-breastfed infants are included.

The intake at the 5th percentile and for those who do not take supplements is below the recommendations (0.5 E%) for all age groups, and at the recommendations for 4 year olds who take supplements. The average intake of EPA+DPA+DHA without supplements is 0.2 g/day in the age groups 2, 4, 9 and 13 years and below the EFSA recommendations at 0.25 g EPA and DHA per day. The intakes in the same groups, but including supplements, are in the range 0.3-0.6 g/day. The lowest intake in this age group is approximately zero (5th percentile with or without supplements) and the highest intake is 1.7 g/day (95th percentile for 2-years olds with supplements).

The intake of total n-3 fatty acids for adults who take supplements is 2.7 g/day (1.1E%) at average and 5.7 g/day (2.0 E%) at the 95th percentile (supplements included). The intake at the 5th percentile is 0.5 E%. The average intake of EPA+DPA+DHA without supplements is 0.6 g/day. The lowest intake of EPA+DPA+DHA in adults is 0.1g/day (5th percentile with or without supplements) and below the EFSA recommendation. The highest intake is 2.7 g/day (95th percentile with supplements).

8.2 Estimated intake assuming consumption of n-3 fortified foods and n-3 food supplement-scenario 2

The following products fortified with marine oils (or EPA and DHA) and plant oils are included in the intake estimations in scenario 2: Fruit juices/drinks (Smartfish), fruit drinks (Mills), TINE fruit yoghurts, Litago yoghurts for children, Brelett light margarine (Fjordland), liverpate (light) (Stabburet and Gilde), bread (>50% wholemeal) (Goman Kystbrød, Bakers Naturlig sunt and Kompis) and spreadable caviar (Mills and Stabburet). Data was collected in May 2009.

Three Smartfish drinks were available in Norway at the time of data collection. The average concentration levels of EPA, DPA and DHA in the three available drinks are used in the intake estimation. In the calculations, all juices and apple nectar are replaced with fortified Smartfish drinks. The concentration levels of EPA, DPA, DHA and total n-3 are the same in TINE n-3 yoghurts and Litago yoghurts. When estimating the intake including the fortified foods, all Tine fruit yoghurts except musli yoghurts were replaced by the fortified versions. All light margarines were replaced by Brelett omega. The average concentration levels of n-3 fatty acids from the two available fortified fat-reduced liver pates, the two available fortified spreadable caviars and the three available fortified breads replaced the content of these substances in all fat-reduced liver pates, spreadable caviars and all breads with >50% wholemeal, respectively.

The fat-reduced Vita Hjertego' hot dog wiener and the fat-reduced Vita Hjertego' hot dog grill are added with plant oil and have an enhanced content of ALA compared to the fat-reduced alternatives in the Norwegian Food Composition Table. These are also included in scenario 2, replacing all fat-reduced hot dogs.

The estimated intake of n-3 fatty acids including fortified foods and weighed average values of fatty acids from a calculated food supplement in scenario 2 is shown in Table 8.2 and described below.

The average estimated intake of total n-3 fatty acids for 1-year olds who take supplements is 1.7 g/day (1.2 E%) and 3.0 g/day (2.0 E%) at the 95th percentile (supplements included). The intake at the 5th percentile is 0.2 E% if supplements are not included. The average intake of EPA+DPA+DHA including supplements is 1.0 g/day. The lowest intake of EPA+DPA+DHA in this age group is approximately zero (5th percentile without supplements) and the highest intake is 1.6 g/day (95th percentile with supplements).

The average estimated intake of total n-3 fatty acids among children and adolescent 2, 4, 9 and 13 years who take supplements is 2.3-2.6 g/day (1.2-1.5 E%), and the 2 year olds have the highest E%. The estimated intake of total n-3 fatty acids at the 95th percentile (supplements included) is 3.6-4.7 g/day (1.8-2.2 E%) for the same age groups. The estimated intake at the 5th percentile in these age groups is below recommended level only in 13 year olds who do not take supplements (0.4 E%). The average intake of EPA+DPA+DHA without supplements is 0.5-0.6 g/day in the age groups 2, 4, 9 and 13 years and with supplements 1.1-1.2 g/day).

The lowest intake of EPA+DPA+DHA in these age groups is approximately 0-0.1g/day and below EFSA's recommendation at 0.25 g/day EPA and DHA (5th percentile without supplements) and the highest intake is 2.1 g/day (95th percentile 2-years old and 13-year olds with supplements).

Table 8.2: Estimated intake of n-3 fatty acids including fortified products and food supplement with weighed average values of n-3 fatty acids – scenario 2 (all numbers are rounded off).

		ALA (g/day)		**(EPA+DPA+DHA)** (g/day)		**Total n-3** (g/day)		**E% n-3**	
		Incl. Suppl.	*No suppl.*	*Incl. suppl.*	*No suppl.*	*Incl. suppl.*	*No suppl.*	*Incl. suppl.*	*No suppl.*
16-79 year olds	Average	1.9	1.9	1.5	0.9	3.4	2.8	1.4	1.1
	5th percentile	0.7	0.7	0.8	0.2	1.6	1.0	0.9	0.6
	95th percentile	4.1	4.1	2.7	2.0	6.3	5.6	2.0	1.8
13-year olds	Average	1.4	1.4	1.1	0.5	2.6	1.9	1.2	0.8
	5th percentile	0.6	0.5	0.7	0.0	1.3	0.7	0.7	0.4
	95th percentile	2.9	2.9	2.1	1.4	4.7	4.0	1.8	1.5
9-year olds	Average	1.5	1.5	1.1	0.5	2.6	2.0	1.2	0.9
	5th percentile	0.7	0.6	0.7	0.1	1.5	0.9	0.8	0.5
	95th percentile	2.7	2.7	2.0	1.3	4.3	3.6	1.9	1.6
4-year olds	Average	1.2	1.2	1.1	0.5	2.3	1.7	1.4	1.0
	5th percentile	0.6	0.6	0.7	0.1	1.4	0.8	0.9	0.5
	95th percentile	2.1	2.1	1.8	1.2	3.6	3.0	2.1	1.7
2-year olds	Average	1.1	1.1	1.2	0.6	2.3	1.6	1.5	1.1
	5th percentile	0.5	0.5	0.7	0.1	1.3	0.7	1.0	0.6
	95th percentile	2.0	2.0	2.1	1.5	3.7	3.1	2.2	1.8
1-year olds[1]	Average	0.7	0.7	1.0	0.3	1.7	1.1	1.2	0.7
	5th percentile	0.2	0.2	0.7	0.0	0.9	0.3	0.7	0.2
	95th percentile	1.7	1.7	1.6	1.0	3.0	2.4	2.0	1.5

[1]Only the non-breastfed infants are included.

The estimated intake of total n-3 fatty acids for adults who take supplements is 3.4 g/day (1.4 E%) at average and 6.3 g/day (2.0 E%) at the 95th percentile (supplements included). The estimated intake at the 5th percentile is 0.6 E% if supplements are not included.

The average intake of EPA+DPA+DHA without supplements in adults is 0.9 g/day. The lowest intake in adults is 0.2 g/day and below EFSA's recommendation at 0.25 g/day EPA and DHA (5th percentile without supplements) and the highest intake is 2.7 g/day (95th percentile with supplements).

8.3 Comparison of the two different scenarios

The consumers have three identified available sources of n-3 fatty acids; from regular foods, from fortified foods and from supplements. The lowest consumption of EPA+DPA+DHA is recorded among people with a low intake from regular foods and no fortified products or supplements, whereas the highest intake of EPA+DPA+DHA is recorded among people with a high estimated intake from regular and fortified foods who also takes supplements. To visualize these two variations in intake, they are compared in Figure 8.1 where the 5th percentile intake of EPA, DPA and DHA in the groups without supplementation and fortification is approximately zero. In contrast the 95th percentile from the group with supplementation and fortification is close to 2 g/day (1.6-2.7 g/day). In addition, the average intake without supplementation and fortification, and the average intake with supplementation but not fortification are also included. No upper safe limit exists for total daily n-3 intake (ALA+EPA+DPA+DHA).

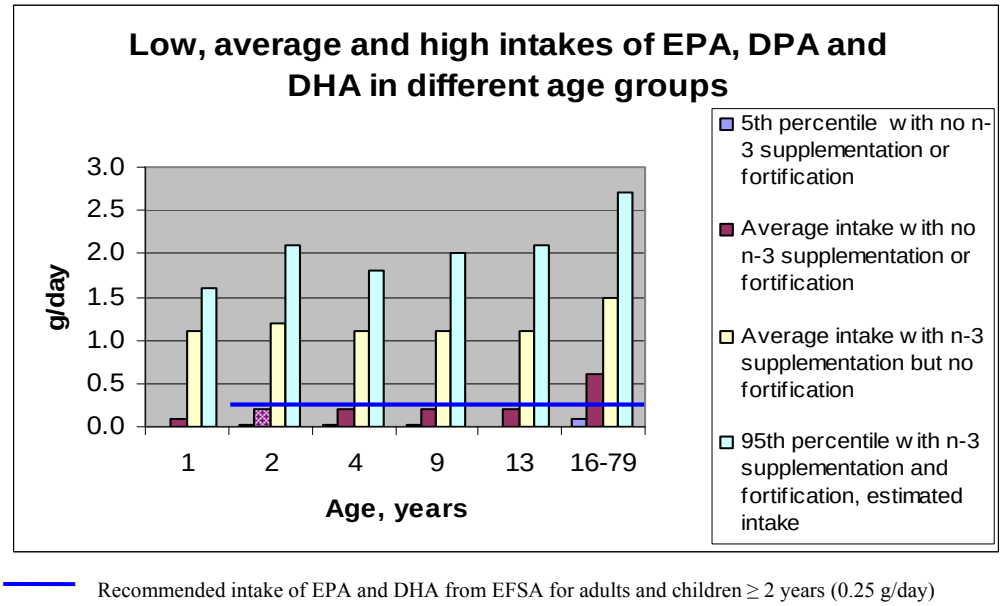


Figure 8.1: Low, average and high intake of EPA, DPA, DHA and total n-3 fatty acids in different age groups (g/day).

In Figure 8.2 the low, average and high intake of total n-3 fatty acids are compared and related to the recommended intake of 0.5E% for children and adults, indicated by the horizontal line. Most of the population has an intake that meets the recommandations for total n-3 fatty acids. However, the individual n-3 fatty acids have different physiological effects. The major part of the documentation on positive health effects is obtained from studies with EPA and DHA. Furthermore, the conversion of ALA to DHA is in the range of 0.5% (Plourde & Cunnane, 2007). During the last decade there are increasing numbers of recommedations for EPA and DHA for health promotion and reduced risk of cardiovascular diseases (Table 5.1 and 5.2).

Figures 8.1 shows low, average and high intake of EPA, DPA and DHA as g/day, and figure 8.2 shows low, average and high intake of total n-3 fatty acid i.e. ALA, EPA, DPA and DHA as E%.

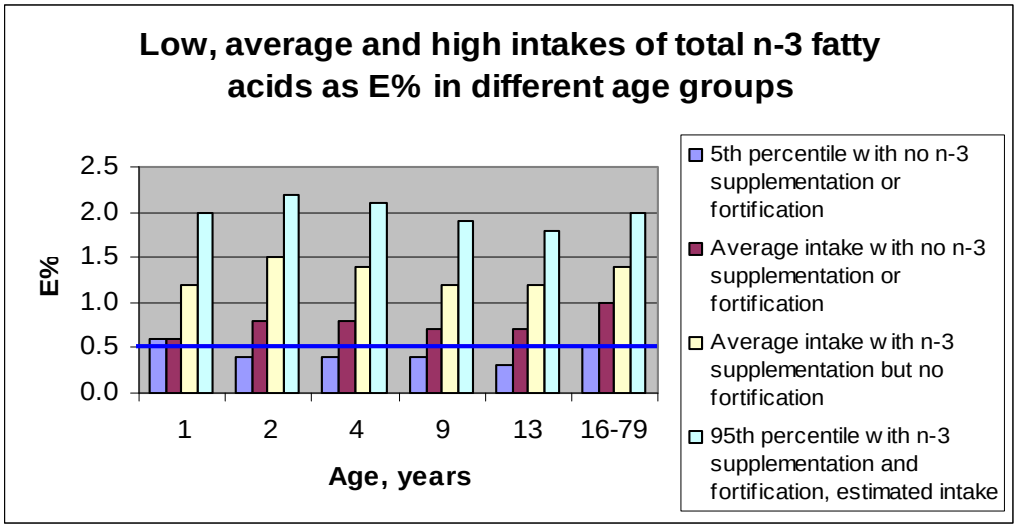


Figure 8.2: Low, average and high intake of ALA, EPA, DPA, DHA and total n-3 fatty acids in different age groups (E%).

9 Knowledge gaps

The main knowledge gaps identified during work with this evaluation are summarised below:

- Very few studies, including large interventions trials, have systematically addressed possible negative health effects from EPA and DHA, and there are limited data on healthy subjects including children and pregnant women.
- There are very few human studies available regarding possible negative health effects from lipid peroxidation following EPA and DHA supplementation. Oxidative stress biomarkers are not yet established as defined risk factors for disease. Studies investigating the relationship between changes in multiple oxidative stress biomarkers and disease and survival are needed.
- Appropriate intervention trials assessing the dose response effects of EPA and DHA on glucose metabolism, insulin resistance, LDL-cholesterol and VLDL triacylglycerol in subjects with type 2 diabetes.
- There is a lack of data comparing health effects following supplementation with ALA versus EPA versus DHA.
- Appropriate intervention trials assessing the dose response effects of EPA and DHA on CNS and mental health disorders.
- The data on content of individual n-3 fatty acids in the Norwegian Food Composition Table and the currently available food supplements are insufficient, and should be completed. Moreover, there is a need for updated and more detailed consumptions surveys including fortified foods and frequency of use of different supplements.

10 Answers to the terms of reference and conclusion

In general, only a few studies investigating EPA, DHA and ALA as food supplements in primary prevention have controlled for the n-3 fatty acids in background diets or compared different sources of n-3 fatty acids.

Most of the studies reviewed have been conducted in various patient groups, especially interventions with n-3 fatty acid supplements as secondary prevention in coronary heart diseases, inflammation conditions or mental health disorders.

The background diet is not often described in the reviewed literature, but is presumed to be a typical Western diet high in linoleic acid and relatively low in ALA (n-6/n-3 ratio ranging from 5 to 9). Food based dietary guidance (e.g. to eat more fatty fish and fruit and vegetables) is included in some of the large intervention trials of secondary prevention in coronary heart diseases.

Most of the studies include EPA and DHA alone or in combinations, mainly as triacylglycerols or as ethyl esters, and several studies include EPA and DHA ethyl esters registered as drug. In many studies where combinations of EPA and DHA have been used, only the total amounts of EPA and DHA and not the amounts or ratio of the individual fatty acids were specified.

ALA as food supplement has been much less studied.

What are the negative health effects of n-3 fatty acids?

The following negative health effects have been identified in studies with EPA and DHA; bleeding tendency, lipid peroxidation, impaired inflammation and other immune function, impaired lipid and glucose metabolism and gastrointestinal disturbances.

An increased bleeding time has been found after intake of 6.9 g/day EPA and DHA in coronary heart disease patients on anti-coagulant medication. However, no negative health effects regarding bleeding complication in connection with EPA and DHA supplementations have been reported.

A limited number of studies have reported data on lipid peroxidation following n-3 fatty acid supplementation. Most of these did not show any increase in lipid peroxidation biomarkers. One large study with myocardial infarction patients taking 3.5 g EPA and DHA per day (EPA/DHA ratio not given) as ethyl ester showed increased thiobarbituric acid reactive substances (TBARS) in plasma. The relationship between *in vivo* lipid peroxidation and TBARS is uncertain. Moreover, none of the oxidative stress biomarkers are presently defined as risk factors of disease. The clinical relevance of lipid peroxidation is therefore unclear.

Several studies have measured biomarkers of systemic inflammation in healthy subjects and different patient groups supplemented with n-3 fatty acids. No increase in CRP after intake of marine n-3 fatty acids has been observed. EPA and DHA at doses of 5 g/day have been shown to activate endothelial cells (increased sVCAM-1 and sE-selectin) among individuals at high risk of cardiovascular diseases and in patients with coronary heart disease. Although low-grade systemic inflammation plays an important role in the pathology of some diseases, such as cardiovascular disease and type 2 diabetes, the clinical relevance of an increase of low-grade systemic inflammation is still uncertain.

Regarding effects on glucose control, the evidence indicates no effect in subjects with type 2 diabetes of supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day).

A minor increase in LDL-cholesterol (1-3%) in subjects with type 2 diabetes has been reported in meta-analyses following supplementation with EPA and DHA in doses ranging from 0.8 to 4.8 g/day (mean: 2.4 g/day). No dose response relationship has been reported. However, the clinical relevance in subjects with type 2 diabetes of this minor increase in LDL-cholesterol is unclear because of a concomitant reduction in serum triacylglycerol and unchanged apolipoprotein B in the same subjects. No change in LDL-cholesterol was reported in the large coronary heart intervention trials including both subjects with and without type 2 diabetes.

Negative health effects regarding gastrointestinal function, including abdominal cramps, flatulence, eructation, vomiting and diarrhea, have been reported, but seem to be associated with intake of an oily substance and not ascribed specifically to EPA and/or DHA.

It has not been possible to distinguish between negative health effects of triacylglycerols and ethyl esters of EPA and DHA.

In the studies investigating ALA, no negative health effects have been observed.

Is it possible to set tolerable upper intake levels (ULs) for the n-3 fatty acids?

There is no evidence that an intake of ALA from linseed oil and margarine up to 8 g/day in addition to the contribution from a Western diet has any negative health effects and it is therefore no rationale to set a tolerable upper intake level for ALA. A high intake (95th percentile) of ALA from foods, including supplements, is 4 g/day for adults.

Possible negative health effects of EPA and DHA have been reported in various patient groups at the following doses:

- Increased bleeding time, but with no reported bleeding complications, following supplementation with 6.9 g/day of EPA and DHA as ethyl esters in patients after coronary angioplasty (patients were using aspirine).
- Increased TBARS of uncertain clinical relevance following supplementation with 3.5 g/day of EPA and DHA as ethyl esters in myocardial infarction patients.
- Increased sVCAM-1 and sE-selectin with uncertain clinical relevance with supplemental 5 g/day of EPA and DHA as ethyl esters among people at high risk of cardiovascular diseases and patients with coronary heart disease.
- A minor increase in LDL-cholesterol (1-3%) with unchanged apolipoprotein B and concominant reduction in triacylglycerol in subjects with type 2 diabetes at doses of 0.7-5.0 g/day EPA and DHA has been reported in meta-analyses. However, this increase is of uncertain clinical relevance and no dose-response relationship has been reported.

Based on the reviewed literature, it is not possible to identify clear adverse effects from EPA and DHA, which can be used for setting tolerable upper intake levels.

What are the health consequences of using n-3 fatty acids as ethyl esters?

Fatty acid ethyl esters are synthetic compounds not naturally present in any foods. Ethyl esters of EPA and DHA were developed as a pharmaceutical to treat patients with cardiovascular diseases and not for a healthy population. However, such esters are currently in use as food supplements.

The safety of EPA and DHA ethyl esters has only been evaluated as a drug in clinical settings. From the reviewed literature it has not been possible to distinguish the health effects from EPA and DHA as TAG from those of EPA and DHA as ethyl esters.

What are the positive health effects of n-3 fatty acids?

Positive health effects have been evaluated in the following domains; cardiovascular diseases, inflammation and immune function, CNS and mental health functioning. The studies have investigated EPA and DHA mainly as fish oils or as ethyl esters. The few studies investigating ALA have also been included.

Coronary heart diseases

The strongest evidence for possible beneficial effects of n-3 fatty acid supplementation in humans is provided by large randomised controlled trials involving more than 43 000 study participants suffering from cardiovascular disease (secondary prevention). In patients given either 0.8 g EPA and DHA or 1.8 g of EPA as ethyl ester daily the risk of cardiovascular events and mortality was reduced.

Primary prevention from EPA and DHA supplementation has been less studied. However, EFSA has based its recommendation for adults on scientific evidence indicating that oily fish consumption (1-2 meals per week or dietary supplements containing EPA and DHA and equivalent to a range of 0.25 to 0.50 g of EPA and DHA daily) decrease the risk of mortality from coronary heart disease and sudden cardiac death (EFSA, 2010b).

The present evidence suggests that supplemental ALA given to individuals on a Western diet does not have the same beneficial effects as EPA and DHA on the vascular system or on the biomarkers of disease risk e.g. serum triacylglycerols.

Inflammation and immune function

The evidence for possible beneficial effects of ALA and EPA and DHA on circulating markers related to low-grade systemic inflammation is in general too weak to draw clear conclusions.

There is no conclusive evidence for beneficial effects of supplemental ALA for patients with chronic inflammatory diseases.

Asthma

No definite conclusion can be drawn regarding beneficial effects of n-3 fatty acid supplementation as an adjuvant treatment for asthma in children and adults. Doses from 1 to 5.4 g/day EPA and DHA have been tested.

Inflammatory bowel diseases

Present documentation indicates that n-3 fatty acids are probably ineffective for maintenance of remission in Crohn's disease for doses up to 5.6 g EPA and DHA per day as fish oil. There is insufficient evidence of benefit of EPA and DHA in ulcerative colitis.

Rheumatoid arthritis

Evidence suggests that intake of fish oil (containing from 1.6 to 7.1g/day EPA and DHA) might lessen symptoms or reduce the use of anti-inflammatory drugs in patients with rheumatoid arthritis.

Central nervous system and mental health functions

EPA and DHA have been observed to give positive effects on early neurodevelopment, especially supplementation to preterm infants, and given to the pregnant women during the last half of pregnancy.

There are few studies investigating effects of EPA and DHA on mental functioning in healthy individuals. No conclusions can be drawn as yet.

Positive effects in various CNS disorders are reported from EPA and DHA with doses ranging from 0.5 to 2.8 g/day. The most consistent effects are seen using EPA alone or in combination with DHA in a proportion >3:2. Intake of 0.5-0.7 g/day EPA and DHA or EPA alone indicates significant positive effects on ADHD-related symptoms. Result from a meta-analysis of clinical trials on depressive symptoms indicates that intake of EPA but not DHA reduces depressive disorders.

There is no conclusive evidence of a possible beneficial effect of EPA and DHA as supplements on schizophrenia or other disorders of psychiatry.

There is no conclusive evidence of a possible beneficial effect of EPA and DHA as supplements on neurology and ophthalmology.

What is the intake of n-3 fatty acids in the Norwegian population and the status according to potential negative or positive health effects of n-3 fatty acids?

Intakes in two different scenarios have been calculated.

- Scenario 1 includes intake of n-3 fatty acids from regular foods, and n-3 supplements mainly as cod liver oils.
- Scenario 2 includes intake of n-3 fatty acids from regular foods, fortified foods and a weighted average of n-3 fatty acid supplements.

In neither of the scenarios the intake of EPA and DHA exceed the doses associated with increased bleeding time, bleeding complications, or, although of uncertain significance as risk factors of disease, markers of lipid peroxidation and endothelial activation (increase in TBARS or sVCAM) as reported in the reviewed studies. The intake of ALA in both scenarios was well below an amount considered safe (< 8g/day).

In scenario 1, the intake of total n-3 fatty acids in the 5th percentile in 2, 9 and 13 year olds with or without supplements and 4 year olds without supplements were below both the

Norwegian recommendations for intake of total n-3 fatty acids (0.5 E%) and the EFSA recommendations for daily intake of EPA and DHA (0.25 g/day). For the remaining population, the intakes of total n-3 fatty acids in scenario 1 meet the Norwegian recommendations. A large part (39-51%) of 1 and 2 year old children has an intake of DHA below the EFSA recommendation at 0.1 g/day, even when supplements are included. Among the other children and adolescents (age 4-13 years) approximately 58-78% have an intake of EPA and DHA below the EFSA recommendation at 0.25 g/day.

In scenario 2, the estimated average intake of total n-3 fatty acids in 1 year olds without supplements and also in the 5th percentile (with or without supplements) was below the Norwegian recommendations. The total n-3 intake in the 5th percentile in the 13 year olds without supplements was also below the Norwegian recommendation. For the remaining population the intakes of total n-3 fatty acids in scenario 2 meet the Norwegian recommendations. In this scenario, 9-44% of the children and adults in the different age groups, who do not use n-3 supplements, have an intake of EPA and DHA below the EFSA recommendations. If n-3 supplements are included, all age groups meet the EFSA recommendation on EPA and DHA.

Based on these scenarios it is evident that the main dietary n-3 fatty acid in the Norwegian population is ALA and average intakes of ALA without or with supplements are 0.7-1.8 g/day and 0.7-1.9 g/day, respectively. The main source of EPA, DPA and DHA is food supplements. The average intakes of EPA, DPA and DHA range between 0.1-0.6 g/day without supplements and 0.3-0.9 g/day with supplements. Both scenarios show that the intake of EPA and DHA among children is low.

Concluding remarks

It was not possible to identify clear adverse effects from EPA and DHA up to the dosage 6.9 g/day, and no tolerable upper intake level could be established. Other possible negative health effects of EPA and DHA have been reported in various patient groups at doses above 3.5 g/day, i.a. increases in biomarkers indicative of lipid peroxidation and endothelial activation. However, these effects are not established risk factors of disease, and their significance is uncertain and should be further investigated. The intake scenarios show that 95% of the population is well below 3.5 g EPA and DHA per day.

The evidence presented above show that it is possible to obtain positive health effects in the Norwegian population from intake of EPA and DHA, including from food supplements, without any appreciable risk of negative or adverse health effects.

The Scientific Steering Committee notes that the intake of EPA and DHA is below the EFSA recommendation in a large fraction of children and adolescents. An intake below the EFSA recommendation may miss the opportunity of positive effects from EPA and DHA on neurodevelopment and prevention of coronary heart disease.

This evaluation has shown that given a Western diet, the positive health effects are linked to EPA and DHA and not ALA. Therefore, the Scientific Steering Committee recommends that conciderations on adequate intakes of n-3 fatty acids should be specific on ALA, on EPA and on DHA.

Annex 1

The weighted average values for the different fatty acids in food supplements used in Table 8.2 are calculated based on information about actual content in food supplements used in the Norwegian Mother and Child Cohort Study and frequency of use reported by the cohort participants (Haugen *et al.*, 2008).

Product	**Concentration substance X, g**	**Frequency of use**	**Concentration substance X * frequency**
A	12	6875	82 500
B	42	3797	159 474
C	37	11	407
Sum		*10 683*	*242 381*
Weighted average	22.69[1]		

[1]242 381/10 683

ALA:
Sum concentration substance X * frequency = 214.07
Sum frequency of use = 17 059
Weighted average = 0.01g

DHA:
Sum concentration substance X * frequency = 4575.99
Sum frequency of use = 17 059
Weighted average = 0.27g

DPA:
Sum concentration substance X * frequency = 11.40
Sum frequency of use = 17 059
Weighted average ~ 0

EPA:
Sum concentration substance X * frequency = 4212.01
Sum frequency of use = 17 059
Weighted average = 0.25g

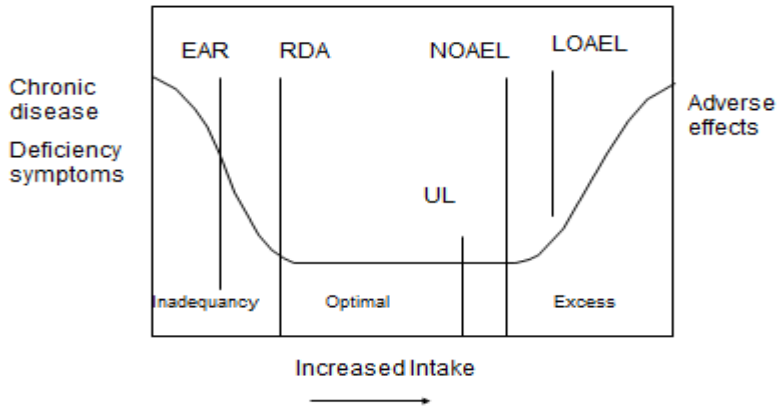
n-3 fatty acids:
Sum concentration substance X * frequency = 10484.81
Sum frequency of use = 17 059
Weighted average = 0.62

n-6 fatty acids:
Sum concentration substance X * frequency=93.50
Sum frequency of use=17 059
Weighted average=0.01 g

Annex 2

Methods for establishment of dietary reference intake (DRI)

Establishment of dietary reference values for nutrients requires a set of reliable data, including data on nutrient needs to avoid deficiencies and data for setting safe upper levels. In addition, the population for whom these values apply must be carefully defined.



The figure shows how the dietary reference intakes are related to the U-shaped curve for the risk of developing health problems along the intake scale of a nutrient. The left part of the curve indicates how the risk of nutrient inadequacy and chronic disease may increase when the intake decreases below an optimal level. Estimated Average Requirement (EAR) is the intake at which the risk of inadequacy is estimated to be 50% for an individual. The Recommended Dietary Allowance (RDA) is the intake at which the risk of inadequacy would be very small, only 2 to 3 percent. Setting the reference values to prevent chronic disease as a consequence of inadequacy of n-3 fatty acids is a continuous process, which takes into account all available scientific data and new approaches (Harris *et al.*, 2009; Kris-Etherton *et al.*, 2009). Our review of positive health effects of n-3 fatty acids in humans (Chapter 7) will contribute to this work.

At intakes between the RDA and the Tolerable Upper Intake Level (UL), the risk of inadequacy and chronic disease is estimated to be close to zero. The optimal intake level defines an intake range with no risk of negative health effects. The right part of the curve indicates how the risk of adverse events increase with increased excess intake. It is assumed that the adverse events occur above a threshold intake level. Because the accurate threshold dose is difficult to determine, surrogate measures such as No Observed Adverse Effect Level (NOAEL) and Lowest Adverse Effect Level (LOAEL) are used. UL is the highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population throughout life. As intake increases above the UL, the potential risk of adverse effects may increase. An UL is set taking into account the scientific uncertainties in the data by dividing the NOAEL by an uncertainty factor. This factor accounts for uncertainties in human inter-variability and extrapolation of data from animals to humans, as well as other uncertainties in the data.

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