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<td>The Role of Omega-3 Fatty Acids and Dietary Fish Consumption in Prevention of Cardiovascular Disease</td>
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The Role of Omega-3 Fatty Acids and Dietary Fish Consumption in Prevention of Cardiovascular Disease

Kirsi Kaups
Abstract

The Role of Omega-3 Fatty Acids and Dietary Fish Consumption in Prevention of Cardiovascular Disease

Kirsi Kaups

Cardiovascular disease (CVD) is a common health problem in developed countries: every year, CVD causes more than 1.9 million deaths in the European Union and more than 4 million deaths all over Europe. Consumption of omega-3 fatty acids (n-3 PUFA) could reduce the risk of CVD by multiple pathways. This paper underlines the complexity of the role of n-3 PUFA and fish consumption in CVD prevention, and provides a literature review on the topic. The results showed that via anti-arrhythmic effects, n-3 PUFA is likely to prevent fatal cardiovascular (CV) events, whereas the effects on non-fatal CV events are unclear. Also, n-3 PUFA intake seems to be more effective in preventing CVD among secondary prevention and high-risk populations. Less evidence exists for primary prevention. Several factors could explain why some studies find n-3 PUFA intake non-effective in CVD prevention. Even though the differences between the effects of n-3 PUFA supplementation and dietary fish consumption are not known, dietary fish consumption should be preferred for many reasons. However, both dietary fish intake and n-3 PUFA supplementation are likely to reduce the risk of fatal CVD.
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1. Introduction

Cardiovascular disease (CVD) is a major cause of morbidity and death, making prevention of CVD a global public health goal (Mendis, Puska & Norrvig, 2011, p.2).

Since the suggestion made in 1970s, that high consumption of fish and seafood is responsible for the low rate of CVD in Greenland Eskimos (Bang, Dyerberg & Sinclair, 1980), numerous studies have been carried out to investigate the association between fish consumption, long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) and the risk of CVD (Barringer, 2012).

Early studies almost uniformly found evidence to support the inverse association between CVD risk, and the consumption of long chain n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), present in fish and seafood. However, more recent evidence stays diverse and even controversial (Ibid).

1.1. Research Question

The aim of this paper is to research if long chain omega-3 polyunsaturated fatty acids from fish and fish oil supplements play a role in prevention of cardiovascular disease. Is there a difference between the preventive effects of dietary fish consumption and n-3 PUFA intake from supplements?

1.2. Structure

This paper is divided into 5 chapters. Chapter 1 is introductory, including research question, delimitations of the paper, and choice of method.

Chapter 2 is mainly theoretical, presenting the risk factors and types of CVD, the dietary sources and biochemistry of n-3 PUFA, and the possible mechanisms for n-3 PUFA protective effects against CVD. This will be used and further discussed in chapter 4, to interpret the research findings.

In chapter 3, the literature search will be described in detail, and in chapter 4, the results of the literature study will be presented and discussed based on theory from chapter 2.
The first part of chapter 4 will look into the evidence about n-3 PUFA and CVD in general, whereas the second part of chapter 4 focuses on the possible differences in preventive effects of dietary fish intake and the use of fish-oil supplements.

Finally, chapter 5 concludes the study, including answers to the research questions, best practice considerations and perspectives.

1.3. Delimitations

A plant-based n-3 PUFA alpha-linolenic acid (ALA) might have similar effects as EPA and DHA in the prevention of CVD (Kromhout, Giltay & Geleijnse, for the Alpha Omega Trial Group, 2010). ALA has lower cost and greater global supply than EPA and DHA (Mozaffarian & Wu, 2011). However, the body of evidence between ALA and CVD is relatively inadequate, and more studies are needed (Ibid). Therefore, this paper will not focus on the effects of ALA on CVD, but will, from here on, use the term n-3 PUFA to refer to EPA and DHA, unless noted otherwise.

Secondly, fish consumption, EPA and DHA intake have a variety of other health benefits (Byrd-Bredbenner, Moe, Beshgetoor & Berning, 2009, p 199). Nevertheless, the discussion will only be based on their effects on CVD and its risk factors.

Further, potential risks of fish consumption have been raised as a topic of discussion. Most health concerns are related to the risk of fish, especially fatty fish, containing methyl mercury and other toxins (Byrd-Bredbenner et al., 2009, p 195). For the general adult-population, risk-benefit analyses have concluded that the potential benefits of modest fish consumption outweigh the possible risks (Mozaffarian & Rimm, 2006). For sensitive subpopulation groups, like pregnant women, recommendations are available to minimize the risks (Ibid). The risks and benefits of fish consumption will not be further discussed in the paper.

Also, as described later in the paper, n-3 PUFA levels in the body are not only influenced by the intake of n-3 PUFA, but also by the ratio of omega – 6 and omega -3 (Bryd-Bredbenner et al., 2006, p 213). Still, this paper will only look into the effects of n-3 PUFA on CVD, and not discuss the influences of n-6 PUFA in the diet.

Furthermore, the paper will not address any specific population subgroups.
1.4. Choice of method

In order to answer the research question, a literature study in Pubmed and Medline databases was carried out. Also, relevant studies were identified from the reference lists and included in the paper. The literature study is described in detail in chapter 3 of this paper.

2. Theory

This chapter firstly introduces CVD by presenting risk factors involved and explaining different types of the disease.

Secondly, the dietary sources of n-3 PUFA will be presented, together with their biochemistry, relevant for understanding the possible cardio-protective effects.

Finally, taking the two sections together, the complexity of the issue will be underlined.

2.1. Cardiovascular Disease

Every year, CVD causes more than 1.9 million deaths in the European Union and more than 4 million deaths all over Europe (European Heart Network, 2012). In the United States, CVD kills nearly 600 000 people a year, which is more than those killed by cancer (National Center for Health Statistics, 2011). In Australia, CVD affects 2 out of 3 families, causing about 50 000 deaths per year (Heart Foundation, 2012). Therefore, CVD is a major global health concern.

2.1.1. Risk factors for CVD

CVD is not one specific disease, but refers to a class of diseases which affect the cardiac system. Even though there are various types of CVD, the risk factors are the same. These can be divided into two main subcategories: unchangeable factors and lifestyle factors. By modifying the latter, people can minimize their likelihood of developing CVD.
Unchangeable risk factors:

- **Age** – the risk of CVD increases with age (Byrd-Bredbenner et al., 2009, pp 214 – 215).

- **Gender** – Male sex is a risk factor. Even after menopause, when women’s risk of death from heart disease increases, it is not as great as the risk which men face.

- **Genetics** – Having family history of CVD is a risk factor. At highest risk are the ones who have genetic defects that block the removal of chylomicrons and triglycerides (fats) from the blood, reduce liver’s ability to remove LDL (bad cholesterol) from the blood, limit the synthesis of HDL (good cholesterol), or increase blood clotting.

- **Race** – For example Africans have more severe hypertension levels and are therefore at greater risk for developing CVD than Caucasians. Hispanic/Latinos, Native Americans, Native Hawaiians and some groups of Native Asians also have a higher risk of CVD, partly due to the high prevalence of obesity and diabetes in these groups. (Ibid)

Lifestyle factors

- High blood cholesterol levels
- High blood triglyceride levels
- Hypertension
- Smoking
- Physical inactivity
- Obesity
- Diabetes
- Liver and kidney disease and low thyroid hormone levels  
  (Ibid)
2.1.2. Types of CVD

**Coronary Artery Disease**

Coronary artery disease (CAD) is the most common type of heart disease, and a cause of heart attacks (see below). CAD is caused by cholesterol plaque building up in the arteries, decreasing their width, and reducing blood flow to the heart (atherosclerosis).

For better understanding of the findings in chapter 4, it is important to note that CAD can also be referred to as ischemic heart disease (IHD) or coronary heart disease (CHD) (Mendis et al., 2011, p. 3).

**Inflammation** has a crucial role in the process of the disease: CVD risk factors are likely to cause a prolonged, chronic inflammation in the body which, in turn, has been found to mediate all the stages of atherosclerosis, from initiation, through progression, and finally to the formation of blood clots, blocking the narrowed artery and leading to heart attack (Libby, Ridker & Masery, 2002).

**Angina**

Angina is a chest pain resulting from decreased blood oxygen supply to a part of a heart muscle, which can be (but not necessarily is) caused by CAD (Mendis et al., 2011, pp 14-15).

**Myocardial Infarction** (also known as heart attack).

Myocardial infarction (MI) forms when a blood clot completely blocks a coronary artery which supplies heart with blood. The blood clot usually forms at the site of rupture of the atherosclerotic, cholesterol plaque on the inner wall of the artery (i.e. site effected by CAD). The most common complications of MI are heart failure and ventricular fibrillation (see below) (Ibid).

**Stroke** (also known as cerebrovascular event)

Stroke occurs when an artery, leading blood to the brain, becomes blocked due to the rupture of the cholesterol plaque. The brain no longer gets the necessary supply of oxygen and other nutrients, causing the brain cells to die. (Ibid)
Heart Failure

Heart failure means that the heart has become weakened, being unable to pump enough blood to supply the body with necessary oxygen and nutrients. In attempt to overcome the lack of capacity, the chambers of the heart either stretch, to allow for larger amounts of blood to be pumped through the heart, or become more stiff and thick. Described adaption might work for a short while, but eventually, the kidneys are likely to start making the body to retain fluid and sodium, which builds up in the limbs and organs. The body becomes congested and congestive heart failure occurs. People with heart failure are also at risk for arrhythmias (Mendis et al., 2011, p 3).

Ventricular Fibrillation

Ventricular fibrillation is a type of arrhythmia, occurring if the ventricles of the heart do not have a coordinated electrical pattern. For example, when the heart cells receive less oxygen than needed (e.g. due to ischemia in MI), they become overloaded with Calcium, which in turn induces electrical and mechanical changes in the heart tissues. Ventricular fibrillation is one of the most common causes of sudden death (or sudden cardiac arrest), which is a term used when the heart suddenly stops beating (Mendis et al., 2011, pp 14-15).

As seen, the pathophysiology of CVD can be sequenced as follows: via unhealthy lifestyle, people can develop risk factors for CVD. If cholesterol plaque starts building up in the blood vessels, CAD forms. People are at risk for a heart attack (MI), which occurs if a blood clot completely blocks a coronary artery; or at risk for stroke, when the vessel is blocked in the brain. Inflammation plays an important role in the entire process from developing CAD, until the formation of blood clots. CVD risk factors can also weaken the heart and cause heart failure. Both MI and heart failure can, via arrhythmias, lead to sudden death.
2.2. Omega – 3 polyunsaturated fatty acids

2.2.1. Dietary sources

Omega fatty acids cannot be synthesized in human body (Denniston, Topping & Caret, 2011, p 570). Therefore, they are essential in the diet.

The main source of n-3 PUFA is fish and seafood. A plant-based n-3 PUFA - alpha-linolenic acid (ALA), can also be converted into EPA and DHA in the body, but the conversion rates are low, and therefore, the tissue levels of EPA and DHA are mainly dependent on their direct dietary intake – i.e. seafood or n-3 PUFA supplement consumption (Mozaffarian & Wu, 2011).

Fish consumption has many benefits which n-3 PUFA supplementation does not have, and which may play a role in CVD prevention:

1) Fish contains other cardio-protective and otherwise essential nutrients like vitamin D, selenium, antioxidants and fish protein, which are not present in supplements.

2) Fish can substitute red meats, thereby reducing saturated fat intake and increasing the consumption of unsaturated fats.

3) Fish is often served together with other healthy food items, for example various vegetables, dill, lemon etc. in various cuisines.

4) Fish might be an indicator of other healthy lifestyle-behaviors. For example, regular consumption of fish has been associated with higher intake of chicken, fruits, vegetables and dairy, and lower intake of red meat and prevalence of smoking. (Hu et al., 2003)

2.2.2. Biochemical pathways

In the human body, n-3 and n-6 PUFA act mainly via hormone-like molecules eicosanoids (Nordic Council, 2004). A simplified illustration of the pathways and the physiological effects of n-3 and n-6 PUFA is provided in Figure 1, and described below the figure.
N-6 PUFA linoleic acid is necessary for the synthesis of arachidonic acid (AA), and n-3 PUFA alpha-linolenic acid is necessary for the synthesis of EPA and DHA. All of them – AA, EPA and DHA, are the precursors of eicosanoids (Denniston et al, 2011, p 570).

N-6 AA derived eicosanoids stimulate vasoconstriction, aggregation of the platelets (blood clotting) and inflammation. (Denniston et al, 2011, p 571), which could result in increased risk of CVD.

N-3 derived eicosanoids, however, reduce blood clot formation, blood triglyceride levels, growth of atherosclerotic plaque, and reduce inflammation. Thereby, n-3 PUFA could lower the CVD risk. Further, n-3 PUFA derived eicosanoids also reduce the synthesis of n-6 AA derived eicosanoids.

Figure 1- Synthesizing pathways and physiological effects of eicosanoids (own figure)
(Mozaffarian & Wu, 2011), which is the reasoning behind recommendations for the ratio between dietary n-6 and n-3 PUFA intake.

The reduction in synthesis of n-6 AA derived eicosanoids, used to be considered sufficient for explaining the role of n-3 PUFA in CVD prevention. However, more recently some cardio-protective effects of the n-6 AA derived eicosanoids have also been noted (Ibid).

In addition to the anti-inflammatory and triglyceride lowering effects of n-3 PUFA derived eicosanoids, anti-arrhythmic properties of n-3 PUFA derived eicosanoids have been proposed (Mozaffarian & Wu, 2011).

As mentioned earlier in this chapter, arrhythmias can occur due to reduced blood flow to heart cells, which causes them to lack oxygen and become overloaded with calcium. In a recent study, some of the n-3 PUFA derived eicosanoids showed a nearly 1000-fold greater potency in reducing Calcium overload in rat ventricular myositis than its precursors EPA and DHA. N-6 AA derived epoxideicosatrienoic (EET) acid antagonized this effect (Arnold et al., 2010).

The proposed anti-arrhythmic effects have been observed in many human studies, however, the pathways behind this effect are not known (Mozaffarian & Wu, 2011). The effects could occur due to n-3 PUFA direct influence on the heart’s electrophysiology. This, in turn, could result in reduced myocyte (heart cell) excitability and systolic calcium fluctuations, especially in damaged or ischemic cells, which are susceptible to depolarization and triggered arrhythmia (Ibid). The anti-arrhythmic effects could also occur via indirect effects such as improved autonomic tone, myocardial efficiency or local inflammatory responses (Ibid).

As seen, there are multiple ways how the effects of n-3 PUFA consumption could interfere with the development and progression of CVD: n-3 PUFA lowers risk factors for CAD and MI such as inflammation, triglyceride levels, blood clot formation, and growth of the atherosclerotic plaque. Via its anti-arrhythmic properties, n-3 PUFA could prevent ventricular fibrillation, thereby lowering the risk of MI resulting in sudden cardiac death.

The role of n-3 PUFA in prevention of CVD is therefore a complex issue with multiple factors, and we could hypothesize, that n-3 PUFA can have different effects on different types and stages of CVD.
3. Method

This chapter describes the literature study which was carried out to answer the research questions.

In autumn of 2013, a literature study was conducted in Pubmed database, including Medline database (http://www.ncbi.nlm.nih.gov/pubmed/). The first search was a background search, aiming to gain a general overview of the scope of the literature. The search for “omega-3 OR n-3 OR EPA OR DHA AND cardiovascular disease OR cardiovascular OR cardiac OR heart disease,” in title or abstract field, with additional filters of free full text available, and English language, resulted in 373 303 articles.

Then, the search was narrowed to only “omega-3 AND cardiovascular disease”, including these terms in their titles. The same filters were applied. This search resulted in 20 results. The abstracts of these articles were examined, and included for use in this paper with the following aims:

- To include articles on both primary and secondary CVD prevention
- To include different types of studies – Randomized controlled trials (RCTs), cohorts and meta-analyses
- To include studies about both fish and n-3 PUFA supplementation consumption
- To include studies with large enough population to have statistical power
- To include the most recent evidence

The articles included in the study, were further analysed, and additional articles were identified from the reference lists.

To make sure that no key studies were left out due to using only these databases, an additional search was conducted on VIA University Libraries webpage (www.bibliotekerne.viauc.dk). This website allows to search all the journals available for VIA University College and other co-operating libraries (e.g. Statsbiblioteket).

The same search words “omega – 3” and “cardiovascular disease” were used. Most, but not all of the results were overlapping with those found on Pubmed. Based on the same criteria, some more studies were included.

All in all, 25 articles were included, from which 13 are presented and discussed in this paper, whereas others are used as background material.
4. Results

In this chapter, the results of the literature search are presented and discussed in order to answer the research question.

The chapter is divided into three main parts. Section 4.1. looks at the effects of n-3 PUFA in prevention of CVD in general. In order to organize the evidence, section 4.1. is further divided into three parts: section 4.1.1. presents the proof for n-3 PUFA effectiveness, 4.1.2. presents the proof of no effect, and in section 4.1.3. the factors, which possibly influence the study findings, will be described, with examples from the studies.

Section 4.2. aims to see if there is a difference between the preventive effects from n-3 PUFA supplements and dietary fish consumption.

Section 4.3. is the discussion section.

4.1. N-3 PUFA in prevention of CVD

Epidemiological, observational and clinical trials have led to official recommendations about n-3 PUFA or dietary fish consumption in order to prevent CVD (Kris-Etherton, Harris, Appel, & American Heart Association, 2002). As explained in chapter 2, n-3 PUFA have several functions which could result in reduction of CVD risk. However, not all the underlying mechanisms of n-3 PUFA are known. The research in this area is very active, and the amount of studies published about n-3 PUFA in CVD prevention has more than doubled from 2008 to 2011 (Delgado-Lista, Perez-Martinez, Lopez-Miranda, & Perez-Jimenez, 2012).

The literature search resulted in meta-analyses reaching different conclusions (Delgado-Lista et al., 2012; Trikalinos et al., 2012; Kwak, Myung, Lee, Seo & Korean Meta-analysis Study Group, 2012; Rizos, Ntzani, Bika, Kostapanos, Elisaf, 2012).

In order to shed light to the issue, individual studies from results of the literature search, are further investigated.
4.1.1. Proof of effectiveness

The results from the literature study which showed beneficial effects of n-3 PUFA intake on CVD risk are presented in the matrix below (Table 1) and described below the matrix.

Table 1 - Results of the literature search (n-3 PUFA effectiveness)

<table>
<thead>
<tr>
<th>Author, Year of publication</th>
<th>Study design, Duration</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings for n-3 PUFA</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>GISSI-Prevenzione Investigators, 1999</td>
<td>Open-label randomized controlled trial 3.5 years</td>
<td>N=11 342 14.7% women Post-MI (&lt; 3 months)</td>
<td>1) 850-882mg EPA+DHA/day 2) 300 mg vit E/day 3) 1 and 2 4) control</td>
<td>Total mortality: 20 % ↓ CV mortality: 30% ↓ Sudden death 45% ↓ No effect on non-fatal events</td>
<td>N-3 PUFA treatment leads to statistically significant and clinically important CV benefit in post-MI patients.</td>
</tr>
<tr>
<td>GISSI-HF Investigators, 2008 (a)</td>
<td>Randomized controlled trial 3.9 years</td>
<td>N=6975 22% women Heart failure</td>
<td>1) 850-882mg EPA+DHA/day 2) placebo Additional randomization to 10mg rosuvastatin or placebo</td>
<td>Total mortality risk: 1.8% ↓ CV mortality or hospitalization for CV reasons: 2.3% ↓ mostly via reduced arrhythmic effects</td>
<td>N-3 PUFA treatment provides a small beneficial effect on mortality and mortality or admission to hospital for CV reasons in HF patients.</td>
</tr>
<tr>
<td>Yokoyama, 2007</td>
<td>Open-label randomized controlled trial 4.6 years</td>
<td>N= 18 645 69% women Elevated cholesterol, independent of CHD history</td>
<td>1) 1800mg EPA + Statins 2) Statins only (Statin – 10mg pravastatin or 5mg simvastatin/day)</td>
<td>Frequency of major coronary events 19% ↓ Risk of angina 24% ↓ Risk of non-fatal coronary events 19% ↓ No difference in coronary death and sudden cardiac death Similar effects in primary and secondary prevention groups.</td>
<td>EPA is promising for prevention of major coronary events, especially non-fatal events in Japanese, hypercholesterolaemic patients.</td>
</tr>
</tbody>
</table>
The two largest European trials on n-3 PUFA and CVD prevention are conducted by an Italian GISSI Investigators group. In order to different between them, the trial from 1999, will be referred to as GISSI-P, and the trial from 2008, GISSI-HF.

In the GISSI-P trial among Italian recent post MI (within 3 months) patients, a dosage of 850-882mg combined EPA and DHA per day significantly reduced the risk of all-cause and cardiovascular death. Decrease in the rates of sudden death accounted for 59% of the reduction in total mortality. It was the first large clinical trial to propose the antiarrhythmic properties of n-3 PUFA.

In GISSI-HF trial, supplementation with the same dosage of EPA and DHA, in patients with heart failure, reduced both total mortality and admissions to hospital due to cardiovascular reasons. These reductions, too, were mainly due to decreases in the rate of arrhythmic events.

In the prospective study by Albert et al., low/moderate fish intake (1-2 servings of fish per week; circa 200mg of EPA and DHA per day) compared to intake interval of less than monthly, reduced the risk of sudden death by 52%. However, no additional significant reduction in risk occurred with increasing intake. This finding was consistent in all the fish categories, besides dark-meat fish group.

Controversially to these studies, mainly finding reductions in fatal, but not in non-fatal cardiovascular (CV) events, Yokoyama et al. observed a beneficial effect of n-3 PUFA (1800mg/day) in addition to statin-treatment in non-fatal, but not in fatal CV events in Japanese hypercholesterolaemic patients.
4.1.2. Proof of no effect

However, not nearly all studies reported the effectiveness of n-3 PUFA consumption in CVD prevention. The main results from the literature search, finding no effect, will be presented Table 2 and described below.

**Table 2 - Results of the literature search (no effect of n-3 PUFA)**

<table>
<thead>
<tr>
<th>Author, year of publication</th>
<th>Study design, Duration</th>
<th>Population</th>
<th>Intervention</th>
<th>Main findings for n-3 PUFA</th>
<th>Conclusion</th>
</tr>
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<tbody>
<tr>
<td>Kromhout et al., 2010</td>
<td>Double blinded randomized controlled trial</td>
<td>n=4837 (21.8% women) Post MI (&lt;10 years) Diabetes n=1014</td>
<td>Margarines: 1) EPA+DHA 2) ALA 3) EPA+DHA+ALA 4) Placebo Average intake: 226mg EPA/day; 150 mg DHA/day;</td>
<td>Plasma EPA levels 53.3% and DHA levels 28.6% increased, but no significant reduction in the rate of major CV events.</td>
<td>Treatment with n-3 PUFA did not significantly reduce the rates of major CV events.</td>
</tr>
<tr>
<td>Kromhout et al., 2011</td>
<td>Post-study analysis of Kromhout 2010</td>
<td>n=1014 Post MI (&lt;10 years) with diabetes</td>
<td></td>
<td>Significantly lower rates of ventricular arrhythmias.</td>
<td>Low dose supplementation with n-3 PUFA exerts a preventive effect against ventricular-arrhythmia related events in post-MI patients with diabetes.</td>
</tr>
<tr>
<td>The ORIGIN Trial Investigators, 2012</td>
<td>Double blinded randomized controlled trial</td>
<td>N=12536 32% women Impaired glucose metabolism or diabetes</td>
<td>1) 465mg EPA + 375mg DHA/day (=840mg n-3 PUFA/day) 2) Placebo (olive oil)</td>
<td>No significant reduction of CV death, major non-fatal CV events, total mortality, death from arrhythmia</td>
<td>Treatment with n-3 PUFA did not significantly reduce the risk of major CV events at a high risk population.</td>
</tr>
<tr>
<td>Rauch et al, 2010</td>
<td>Double blinded randomized controlled trial</td>
<td>N= 3804 25.6% women Post-MI (within 3-14 days)</td>
<td>1)1 gram highly purified EPA/day 2)Placebo (olive oil)</td>
<td>No significant reductions in sudden cardiac death, total mortality, or major CV and cerebrovascular events.</td>
<td>Guideline-adjusted post-MI treatment results in low rates of sudden cardiac death and other clinical events during 1 year after MI, which is not further reduced by intake of n-3 PUFA</td>
</tr>
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Galan et al., 2010

Double blinded randomized controlled trial
4,7 years
N=2501
20.8% women
Post-MI or stroke (<12 months) or unstable angina
1) 600mg EPA+DHA/day
2) Placebo
and 1) 560 μg 5-methyltetrahydrofolate, 3 mg vitamin B-6, 20 μg vitamin B-12/day
2) Placebo
Plasma n-3 PUFA levels 37% increased, no effect on non-fatal MI, stroke, CV mortality, total mortality
Does not support the use of n-3 PUFA supplements in patients with history of MI or ischemic stroke, at least if started after the acute phase of the initial event.

Burr et al., 2003

Randomized controlled trial
9 years (follow-up ranging 3-9 years)
3114 men with angina
Dietary advice:
1) at least 2 portions of fatty fish/week or <3g supplements/day
2) ↑ fruit and vegetable intake
3) to do 1 and 2
4)“sensible eating” advice – non-specific advice including neither 1 or 2
No effect on total mortality; Increased risk of cardiac mortality in fish group, especially in those taking n-3 PUFA supplements
Men advised to eat fatty fish, especially those taking supplements, experienced an unexplained increase in risk of cardiac death.

FFQ – food frequency questionnaire

The trial by Kromhout et al., (2010), found no effect supplementation with their trial margarines (increasing daily EPA and DHA intake by ca 400mg) over 40 months on major cardiovascular events among post MI patients (Kromhout, Giltav, Geleijnse, 2010). A non-significant reduction of 10% was observed in the ventricular-arrhythmia-related events among the EPA plus DHA group. The reduction was found to be significant only among the diabetic population, in a post-study analysis (Kromhout et al., 2011).

An extensive RCT was carried out by ORIGIN (Outcome Reduction with an Initial Glargine Intervention) Trial Investigators among 12 536 patients from 40 different countries, suffering from diabetes or dysfunctional glucose metabolism. Patients selected had a history of MI or heart failure and were given n-3 PUFA dosage of 900mg per day. Despite the supplementation, no reduction in death from CV causes, major CV events (non-fatal MI or stroke), death from any cause, or death from arrhythmia was observed over the 6.2 year follow up period.

The trial by Rauch et al., evaluated the efficiency of EPA supplementation in addition to guideline adjusted post-MI treatment. It was found that guideline adjusted treatment results in low rates of
sudden death or other CV events in the time period of 1 year after MI; and supplementation with 1g EPA per day provides no further benefits.

The findings of the trial by Galan, et al., are in line with the suggestion that n-3 PUFA has no additional benefits over medicine. The study found that even though supplementation with 600mg EPA and DHA per day increased the plasma n-3 PUFA levels by 37%, it had no effect on the rates of non-fatal MI and stroke, all-cause, and CV mortality.

Surprisingly, the trial by Burr and colleagues, found no effect of fish intake on total mortality; but rather, an increase in cardiac death, especially sudden death, was observed in the fish-advice group, among the subpopulation using fish oil supplements.

4.1.3 Factors influencing study findings

The two previous sections clearly demonstrate that studies reach different conclusions about the role of n-3 PUFA in CVD prevention. Analysing different factors which may influence the findings of different studies, might bring some clearness to the issue.

Medication

The most popular explanation for controversial findings for n-3 PUFA efficiency in prevention of CVD, states that the largest preventive effects are seen in older studies, when cardiovascular treatment was not as efficient as contemporary medicine (Barringer, 2012; Mozaffarian & Wu 2011; Rizos et al., 2012). Participants in more recent trials benefit from more advanced medical therapies and cardiovascular drugs, which can “hide” the benefits of n-3 PUFA. (Ibid)

The most efficient class of drugs for lowering (recurrent) CVD risk is statins (GISSI-HF Investigators, 2008b). Statins mainly act via reducing lipid levels, but have also been postulated to have other effects like anti-inflammatory, antihypertrophic and anti-arrhythmic (Ibid). Since 1998, statins have been routinely recommended to reduce the risk of (recurrent) CVD. (Rizos, et al., 2012)

GISSI-P trial was carried out in the so-called pre-statin-era, and found significant reductions in total and cardiac mortality, especially in sudden death, among post-MI patients. (Table 1)
On the other hand, the trial by Galan and colleagues, also among post-MI patients, found no effect of n-3 PUFA supplementation (Table 2). In that trial, 94% of participants in n-3 PUFA group used lipid lowering agents (including statin) at baseline (Galan et al., 2010).

These differences between the pre- and post-statin-era could, in themselves, involve an influence on the results of studies.

Further, there are also differences in intakes of other drugs, between the studies. For example, whereas the use of aspirin in the intervention groups was similar between GISSI-P and the trial by Galan, – 92 and 94%, respectively, the intake of beta-blockers (anti-arrhythmic) and ACE inhibitors (reducing hypertension), was much higher in Galan study – 43,9 vs. 68,1 % using beta blockers, and 38,5 vs. 52,3 % ACE inhibitors, respectively.

On top of that, the intervention group in the trial by Galan et al., was also consuming other anti-hypertensive drugs like calcium channel blockers (16,3 %) and angiotensin II receptor blockers (7%). These intakes were not reported in GISSI-P.

Differences in medication use could possibly explain the different findings.

Further, drugs with same effects can yield different efficiency. Even if the proportions of participants using a medicine are the same, the benefit gained from the medicine, could vary from person to person. Taken that the pharmaceutics industry, like any other, is improving and developing with time, it is even more likely the case with trials dating from different times.

Further, possible interactions between n-3 PUFA and medicine cannot be ruled out either.

As seen, the associations between n-3 PUFA and specific CV drugs are not completely clear. The differences in medicine intake might explain the controversial conclusions between studies.

*Baseline n-3 PUFA intake and study dosage of n-3 PUFA*

An apparent plateau level of risk reduction of sudden death, cardiac death and CHD has been observed at n-3 PUFA intakes of 200-250 mg EPA and DHA per day, or 1-2 portions of fatty fish per week (Albert, et al.1998; Mozzafarian & Rimm, 2006; et al.; Trikalinos et al., 2012).
This means that populations with baseline intake below this level could experience a risk reduction with increasing their initial n-3 PUFA intakes, whereas an increased intake above this level would not result in a lower risk. Therefore, it is important to consider the baseline intake levels and the study dosages of n-3 PUFA.

For example, reduced amount of ventricular arrhythmia related events among diabetic population were observed where baseline intake levels of n-3 PUFA were 120-130mg/day (Kromhout et al., 2010). On the other hand, among patients with dysfunctional glucose metabolism or diabetes, with average baseline n-3 PUFA intake of 210mg per day, no benefits of n-3 PUFA supplementation were found (The ORIGIN Trial Investigators, 2010).

Sufficient baseline levels for prevention of fatal CV events in both control and intervention groups at baseline, might explain why no effects of n-3 PUFA supplementation were found in the latter trial, even though larger intervention dosages were used than in the trial by Kromhout et al. (840mg per day vs. circa 400 mg per day) (Table 2).

Further, EPA and DHA have also been suggested to incorporate different benefits (Mozaffarian & Wu, 2006) and therefore the “ratio” between their intake, could influence the effects on CVD. However, information regarding the subject is rather insufficient (Ibid), making it impossible to analyse or discuss these differences further in this paper.

**Study characteristics**

Evidence from studies with different design also varies. Observational studies usually report more beneficial effects of n-3 PUFA than RCTs (Trikalinos et al., 2012). Difference is understandable, as the first takes people with no or rare fish (n-3 PUFA) consumption and compares them with population groups with higher intakes. Controlled trials, however, recruit a random sample of people with various dietary intakes and randomize them to either control- or intervention group. So various background diets providing various plasma n-3 PUFA levels are present in both groups.

Another problem with observational studies is, that often only baseline estimation of the diet is included, and analyses of the associations between dietary intake and long-term CV outcomes are made under the assumption that the diet of participants remains the same over long time periods.
Duration

It only took three months in GISSI-P trial to see significant reductions in mortality in the EPA and DHA group (Marchioli et al., 2008), while the survival curves in GISSI-HF started to diverge only after two years. (GISSI-HF Investigators, 2008 a)

In the trial by Kromhout et al., (2011), a reduction in the risk of fatal CHD was observed after 2,5 years of n-3 PUFA supplementation, but by the end of the trial, 40 months from baseline, the effect had disappeared.

These examples perfectly illustrate the influence of trials’ duration on their conclusions.

Placebo

Another important parameter in trials is whether or not a placebo is used, and what is provided as placebo. Using olive oil or margarines as placebo, like done in most of the studies presented in section 4.1.2., can influence the ratio of n-3/n-6 PUFA. Thereby, the placebo material could influence the CV outcomes in placebo group.

Including no placebo in the control groups, like done in open-label studies, could also influence the results: as participants are not blinded, the intervention group could experience placebo effects of the intervention.

Source of n-3 PUFA

Trials have mostly been carried out using fish oil supplements. (Table 1; Table 2) However, the idea of their role in CVD prevention originates from observing low rates of CVD in Greenland Eskimos, who in general have a high dietary intake of seafood (Bang et al. 1980). While all the pathways of the effects of fish consumption or n-3 PUFA intake are not clear, fish consumption having different effects than n-3 PUFA supplementation is possible. This would be especially relevant for best health promotion, and therefore deserves further investigation.
4.2 Fish vs. supplement consumption

Albert et al., observed an association between fish consumption and reduction of sudden cardiac death, which was significant among intakes of various types of fish, but not consistent with intakes of dark meat fish (Albert et al. 1989). As this is the type of fish with highest contents of n-3 PUFA (Ibid), the finding seems to suggest that some other nutrient, capable of influencing CVD risk, is present in fish.

Different effects of n-3 PUFA supplementation and fish consumption have also been observed in human trials (Burr et al., 2003) and in-vitro studies (Hoshi et al., 2013).

We well now look into the results of literature search, which analysed the effects of fish consumption on CVD.

<table>
<thead>
<tr>
<th>Author, year of publication</th>
<th>Study design, Duration</th>
<th>Population</th>
<th>Intervention</th>
<th>Results for n-3 PUFA</th>
<th>Conclusion</th>
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<tbody>
<tr>
<td>Hu et al., 2002</td>
<td>Prospective cohort</td>
<td>84688 women free from CVD and cancer</td>
<td>5 FFQ from 1980-1994</td>
<td>Compared to intake of fish &lt;1 per month, women who ate fish more often, had a lower risk of CHD, with multivariable relative risks reducing with increasing intake up to 5 or more portions per month. Also higher n-3 PUFA intake was associated with lower CHD risk. The association was stronger for fatal than for non-fatal cases. For non-fatal, it was only significant among non-aspirin users.</td>
<td>Among women, higher consumption of fish and n-3 PUFA is associated with lower CHD risk, especially for CHD death.</td>
</tr>
</tbody>
</table>
Prospective cohort
9.3 years
N=3910 age 65+
how many women free from CVD
Consumption of fried fish, vs. tuna or other fish, estimated in FFQ
Consumption of 3 or more portions/week of tuna or other fish reduced the risk of IHD death by 49% and risk of antiarrhythmic IHD death by 58%, but had no effect on non-fatal MI risk. Fried fish was not associated with IHD risk, but showed trends towards increased risk
Among people aged 65+, modest consumption of tuna or other broiled or baked fish, is associated with lower risk of IHD death, especially arrhythmic IHD death. The cardiac benefits of fish consumption might depend on the type of fish meal consumed.

Streppel et al, 2008
Prospective cohort
40 years
N=1088+ additional 1939 men healthy?
Five dietary and physiological examinations, including cross-check dietary history interview with dieticians.
Long-term, but not recent fish intake (average 22g/day) was associated with reduced CHD risk.
The association decreased with increasing age. Long-term fatty fish (7g/day) consumption reduced risk for sudden death by 54%.
The inverse relation between long-term fish consumption and CHD death decreases with age. Fatty fish consumption lowered sudden coronary death risk. No dose-response effect was observed.

FFQ- food frequency questionnaire

Hu and colleagues investigated the associations between fish intake and CHD risk in women participating in Nurses’ Health Study. The study found an inverse association between fish intake and CHD risk (Hu et al., 2002). Compared to fish intake of less than 1 portion per month, the relative risks for CHD reduced with increasing intakes. The associations were found stronger for fatal CHD, whereas the association for non-fatal CHD risk was only significant among non-regular aspirin users (Ibid).

In another study, intakes of fried fish, which is usually lean, and consumption of tuna or other broiled or baked fish, usually with higher n-3 PUFA content, were estimated. Whereas the consumption of tuna or other baked or broiled fish significantly reduced the risk of IHD, especially of fatal IHD, consumption of fried fish was not associated with reduced IHD risk (Mozzafarian, et al. 2003).

The differences between recent and long-term fish consumption in CHD risk among men, were investigated in a study with 40 years of duration (Streppel, Ocke, Boshhuizen, Kok, & Kromhout, 2008). An inverse relation between long-term fish intake (22g n-3 PUFA per day on average), and CHD death was found, whereas recent fish intake was not correlated with the risk of fatal CHD. The risk of sudden CHD death was inversely associated only with the intake of fatty fish, at average amounts of 7g per day, but not with lean fish (Ibid).
4.3 Discussion

Taken the results of the trials, and the various aspects mentioned which influence the results, the role of n-3 PUFA in CVD prevention, is still not clearly defined.

As hypothesized, the effect of n-3 PUFA seem to be different for different kinds of CVD: Several trials reported n-3 PUFA efficiency in prevention of fatal, but not of non-fatal events (Burr et al., 1989; GISSI-Prevenzione Investigators, 1999). This has also been observed in cohort studies, both among men (Albert et al., 1998) and women (Hu et al., 2002).

Fatal events

All studies presented in this paper, which found n-3 PUFA to be effective in prevention of fatal CV events, found a reduction in sudden death. (Table 1 and Table 3) This reduction was large and highly significant in the studies - for example, in GISSI-P, the reduction in events of sudden death counted for 59% of the n-3 PUFA advantage on mortality (Marchioli et al., 2008).

As explained in chapter 2, sudden death occurs via ventricular arrhythmias caused by MI. The findings that n-3 PUFA does not prevent MI from occurring, but can prevent it from resulting in sudden death, is interesting, and suggest that n-3 PUFA prevent CVD via its anti-arrhythmic effects.

Indeed, n-3 PUFA supplementation reduced CV mortality and admissions to hospital due to CV reasons in patients with heart failure mainly via reduction of arrhythmic events. (GISSI-HF Investigators 2008a), and reduced only ventricular arrhythmia related events in the trial by Kromhout et al., 2010 (Kromhout et al., 2011).

Interestingly, the anti-arrhythmic effects seem to occur already at relatively low doses. Both GISSI trials used a dose under 1 gram EPA and DHA per day. In observational studies consuming as little as 1 portion of fish a week, was associated with reduced risk of sudden cardiac death. (Albert et al. 1998)

Also, in some cases, the reductions in sudden death seem to become significant relatively fast. For example, in GISSI-P trial, it took only 4 months of n-3 PUFA supplementation to significantly reduce
the rate of sudden deaths (Marchioli et al., 2008). In GISSI-HF trial, the survival curves started diverging after 2 years (GISSI-HF Investigators, 2008).

This evidence suggest that n-3 PUFA is effective in prevention of fatal CVD, especially sudden death, and that these effects can occur on relatively low doses during a relatively short period of time.

Non-fatal events

Moving on to non-fatal CV events, the evidence is less clear. Only one cohort and two trials presented in this paper, reported n-3 PUFA benefits on non-fatal CVD prevention. Further, even though the cohort found an association between n-3 PUFA intake and reduced risk of CHD, it was only significant among women not using aspirin on regular basis (Hu et al., 2012), again demonstrating the influence of medicine intake on n-3 PUFA efficiency.

Two trials also reported reductions in non-fatal CV events. One of them was GISSI-HF, in which only arrhythmia related events were reduced. The other trial was the large Japanese study by Yokoyama et al.

As seen from Table 1, Yokoyama, et al. used higher dosages than other studies presented in this paper. The daily dosage was 1800mg of EPA. Further, it was carried out among Japanese population, which already has high consumption of fish – the daily n-3 PUFA consumption in Japan is about 15 times higher than in an average Western diet (Iso, et al. 2006). If, as discussed above, only low dosages are needed to prevent fatal CV events, the entire study population might have been protected from fatal CV events due to their high n-3 PUFA intake. This is further supported by the cardiac death rates per 1000 person years in their control group of, which was only 2,5 whereas in GISSI-P for example, it was 17 (Kromhout, Yasuda, Geleijnse & Shimokawa, 2012).

Also, the entire study population was already using statins, which should reduce both fatal and non-fatal CVD risk (GISSI-HF Investigators, 2008 b). The fact that n-3 PUFA supplementation had such a large impact – 19% risk reduction on non-fatal coronary events and 24% reduction in angina, on top of the effects of statins, is very interesting and suggests that some effects possibly occur only at very high intakes of n-3 PUFA.
Clearly, more trials would be needed to clarify the effects of n-3 PUFA intake on fatal and non-fatal CVD events, the pathways to these effects, and the dosages needed to reach the effects among various population groups.

Nevertheless, from the above, two suggestions arise:

1) N-3 PUFA intake helps to prevent fatal CVD events via its anti-arrhythmic effects, occurring relatively fast and at relatively low intakes

2) The preventive effects from fatal events require very high intakes of n-3 PUFA.

These two suggestions could hold true, and even explain the evidence finding no effect of n-3 PUFA consumption in CVD prevention.

Studies which found no effect on any major CV outcomes may include populations with high enough baseline n-3 PUFA intakes, already protecting them from fatal events, but not high enough intervention dosages to prevent non-fatal cases. Thereby resulting in possibly false conclusions that n-3 PUFA plays no role in CVD prevention.

Also, long-term, but not recent intake of n-3 PUFA has been associated with reduced CHD death risk. (Streppel et al., 2008) this can pose an important influence on study conclusions.

In Western countries, the traditional diet often does not include a lot of fish (Trikalinos et al., 2012). However, it is general knowledge, that fish and n-3 PUFA are healthy, especially for cardiovascular health. Therefore, it is likely that after experiencing a CV event, people might increase their n-3 PUFA intake. If a trial is carried out among patients with recent CV event, the duration of the study might not be long enough to observe the effects of long-term fish consumption.

On the other hand, the trial by Yokoyama and colleagues was carried out in a population with a long tradition of consuming high amounts of fish and seafood. This might be another reason why, quite controversially to other studies, n-3 PUFA was seen to prevent non-fatal CV events.
It seems that n-3 PUFA is effective in prevention of CVD. It prevents fatal CV events, and possibly, the effects on non-fatal CVD occurring at mega doses or via long-term consumption. The studies presented in this paper claiming no effect could still be consistent with these conclusions: they had high enough baseline n-3 PUFA intakes and too low dosages, too high intake of medicine, or too short duration to observe benefits of long-term fish/n-3 PUFA consumption.

It is important to note, however, that these results and discussion were based on trials carried out in secondary prevention or high risk populations. More studies are needed to see if the effects are the same in primary prevention.

The discussion then leads to the possible differences between the consumption of n-3 PUFA from supplements or from dietary fish.

Consumption of the type of fish with high n-3 PUFA content has been shown not to reduce CHD death risk, whereas other, leaner types did (Albert et al, 1998). On the same time, the exact opposite has also been observed – consumption of fatty, but not of lean fish reduced the risk of CHD death. (Streppel et al., 2008).

If only fatty fish reduces the risk of CVD, one could expect that n-3 PUFA supplementation and dietary fish consumption have similar cardio-protective effects. Which is supported by the fact that EPA and DHA supplementation, as well as dietary fish consumption have shown to reduce the rates of fatal CV events (Table 1 and Table 3). Nevertheless, in-vitro trials and studies on humans provide reasons to believe that n-3 PUFA from natural foods (e.g. fatty fish consumption) acts differently than n-3 PUFA consumed via dietary supplements (Hoshi et al., 2013; Burr 2003), or that something else is present in dietary fish, which can provide cardio-protective effects, independently of its n-3 PUFA content (Albert et al., 1998).

In the lack of adequate trials, it is challenging, if not impossible, to further analyse the similarities and differences between the consumption of n-3 PUFA supplementation and dietary fish intake. However, both have the potential capacity to reduce CVD risk, especially fatal CV events (Table 1 and Table 3).
5. In conclusion

5.1 Best Practice Considerations

The role of n-3 PUFA in prevention of CVD is a complex issue, which involves multiple aspects.

Health professionals need to base their decisions on empirical evidence. However, this might be challenging in the area of fish and n-3 PUFA consumption and risk of CVD. The precise pathways and effects are not known, and studies were presented, which reported indecisive results.

Nevertheless, observational studies uniformly reported that fish intake was associated with decreased risk of sudden death both in men and in women. Also, several trials reported reductions in all fatal CV events and total mortality in n-3 PUFA supplementation groups.

As most of the controlled evidence for n-3 PUFA effectiveness in CVD prevention results from secondary prevention populations, all people with a history of CV events should be recommended to consume fatty fish at least 1-2 portions per week, to reduce the risk of major (fatal) CV events.

For primary population clients, it would be advisable to explain, that it is not completely sure if fish oil per se reduces the CVD risk. Nevertheless, fish is a nutritious food item with various health benefits, which can easily fit into, and improve, a healthy diet.

It is important to avoid overemphasizing n-3 PUFA in itself, especially in a situation where not all the aspects are known - people’s belief that n-3 PUFA supplementation provides protection from development or progression of CVD, might increase their risk-taking behaviors in other lifestyle factors. Such risk compensation has been observed in other contexts (Richens, Imrie & Copas, 2000), and there is no reason to assume that this could not occur in prevention of CVD. Also, stressing the importance of one specific nutrient by health professionals, might lead the general population to a distorted view of what a healthy lifestyle is.

Consequently, supplementation should be left for those who do not like, or have intolerances to, fish and other seafood; or who are aiming for intakes not practical to be consumed via dietary fish intake, including plasma triglyceride or blood pressure reduction, occurring at dosages higher than 3g n-3PUFA per day. Patients with angina should probably be advised to consult with their doctor before starting using n-3 PUFA supplements.
It is a complex topic, currently investigated more than ever, so in order to provide best health promotion, it is important to stay posted with scientific evidence and look forward to further trials.

5.2 Conclusion

The role of n-3 PUFA from fish and fish oil supplements is a complicated issue with multiple aspects, and the empirical evidence stays mixed and confusing.

Whereas several physiological pathways exist, through which n-3 PUFA could prevent various types of CVD, not all of them are known.

The results of the studies were found to be influenced by various aspects related to the characteristics of the populations and study design. By pooling the results from such various trials together, it is no wonder, that meta-analyses find confusing results.

The proof of n-3 PUFA effectiveness in CVD prevention is clearer in secondary prevention and in high risk population groups, where n-3 PUFA intake seems to reduce fatal CV events. This is especially true for arrhythmia-related fatal events like sudden death, and for total mortality. The anti-arrhythmic properties of n-3 PUFA seem to arise already in quite low dosages and during a relatively short time.

The effects of n-3 PUFA on non-fatal CV events and effects of n-3 PUFA in primary prevention are less clear. However, there is reason to believe that relatively high intake of n-3 PUFA over a long period, also reduces the risk of non-fatal CV events.

N-3 PUFA from natural foods and from supplements might have different cardio-protective effects. Also, dietary fish consumption might have different effects on CVD than consumption of n-3 supplements, as fish contains nutrients besides n-3 PUFA. Differential effects of n-3 PUFA supplements and fish intake have been observed.

However, due to the lack of randomized controlled trials on dietary fish intake on humans, it is impossible to really explain the differences or make generalizations.

Still, fish and seafood consumption might be preferred over consumption of n-3 PUFA supplements, as it might nudge the whole diet in a more healthy direction. However, both of them seem to play a
role in CVD prevention, by reducing the risk of fatal CV events and total mortality, and possibly decreasing the rate of non-fatal CV events.

5.3 Perspectives

One major drawback in the area of the research is the lack of trials carried out on primary prevention population. While understanding the difficulties in conducting such a trial, for example with the duration, sufficient control of dietary intakes, including the background diet, and the costs of such study, it could provide enormous benefits for health promotion.

In addition, as dietary fish intake and the use of n-3 PUFA supplements might have different effects on CV health, trials on dietary fish intake are also necessary. These, however, might be even more challenging to conduct, especially among primary prevention populations.

Whereas a trial where all the meals are provided by the research staff could be one way of doing it, it is almost impossible to imagine such a trial in practical setting! Especially if it needs to be large enough to have sufficient statistical power, long enough in duration, and several studies would have to be conducted to be able to draw meaningful conclusions. Also, even if such studies would/could be conducted, another problem would be, that they would be open-label trials, posing their own biases.

Further, comparisons, especially in the form of controlled trials, between intakes of fish with different n-3 PUFA content and CV outcomes, could help to clarify, if n-3 PUFA is the most important nutrient in CVD risk reduction, or is there something else present in fish, which might play a role. It might be that n-3 PUFA is the most important, but that it has different effects based on whether it is taken via supplements or dietary fish consumption. Therefore, the trial should compare different levels of n-3 PUFA intake via fish and CV outcomes, versus different levels of n-3 PUFA intake via dietary supplements. In that way, both the questions – Is n-3 PUFA the most important component in fish, providing its cardio-protective effects, and is there any differences in the effects between n-3 PUFA intakes from fish or from supplements in CVD prevention; could be further analysed.
As demonstrated, a lot is known about the role of n-3 PUFA and dietary fish consumption in prevention of CVD, but much remains to be investigated. Therefore, it is expected to be an active research topic over the next years, hopefully providing further answers soon.
List of References


