



n-3 PUFA and Endothelial Dysfunction: Exploring the Link between Cardiovascular Disease and Risk Reduction

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Abstract

The loss of normal vascular function has an etiologic role in hypertension, atherogenesis, and vascular reactivity which lead to cardiovascular disease (CVD). Several societies across the world have established dietary recommendations for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), either from the intake of oily fish or EPA- and DHA-containing supplements that are principally for CVD risk reduction. There is growing evidence that dietary n-3 PUFA may reduce the development of CVD. Because of this, animal and human trials have been investigated for biochemical and metabolic parameters. This article provides information on the benefits of n-3 PUFA by showing the evidence from animal and clinical human studies which suggest that increasing intakes n-3 PUFA may reduce the risk of CVD by improving biomarkers, endothelial function and vascular reactivity.

Keywords: product, number that every digit as 1

Introduction

CVD is the group of diseases, which affect the cardiac muscle and/or blood vessels, and include Coronary Heart Disease (CHD) stroke, and peripheral vessel disease. CVD is one of the principal causes of death world-wide; more than 17.3 million people across the globe die from the disease each year. Mortality rates differ significantly between countries; rates are much lower in Japan and the Mediterranean countries when compared with those of Eastern Europe (Mendis, Puska, & Norrving, 2011). Worldwide, 80% of deaths from CVD occur in low and medium income countries. In 2010, CVD was the main cause of death in developing countries. In Thailand, 17% of non-communicable diseases (NCDs) are from CVD and the death rate is continuously rising (Mendis et al., 2011).

Although several theories have been proposed for the pathogenesis of CVD, the current trend is that a series of important risk factors, such as age, smoking, diet, obesity and vascular function, are

responsible for the pathology of the disease, all of which contribute to mechanisms that contribute to endothelial dysfunction, vascular reactivity (tone) and atherosclerosis (Cappuccio, Cook, Atkinson, & Strazzullo, 1997; Félétou & Vanhoutte, 2006). Risk factors associated with CVD can be grouped into three, separate categories. The first category is comprised of physiological risk factors, e.g. blood lipaemia, obesity, blood pressure and the vascular function. These are influenced by lifestyle choices, which are both modifiable and non-modifiable. Secondly, modifiable factors such as smoking, alcohol, exercise and dietary patterns and thirdly, non-modifiable factors, such as age, gender, genetics and ethnicity. Many CVD risk factors, such as smoking, hypertension, diabetes and hypercholesterolemia have been found to cause endothelial dysfunction (Brown & Hu, 2001).

There is accruing evidence to suggest that the quantity and composition of fat in the diet is a principal factor that influences endothelial cell



function and vascular tone. In particular, omega-3 fatty acids appear to play a significant role in the immune and inflammatory responses, the progression of arteriosclerosis, vascular reactivity and blood pressure control (Jung, Torrejon, Tighe, & Deckelbaum, 2008; Gogus & Smith, 2010). Since the first cross-cultural epidemiologic n-3 PUFA studies conducted in the 1970s, we have known that fish oil can prevent and reduce risk of cardiovascular disease. Most evidence from observational, clinical, animal, and in vitro studies suggests that increasing intakes n-3 PUFA may reduce the risk of CVD by improving endothelial function and vascular reactivity (Wang et al., 2006).

n-3 PUFA in oily fish or fish oil supplements which contain mostly EPA and DHA. Epidemiological and clinical studies have established that an increase of fish oil consumption can advance the vascular function by augmenting nitric oxide (NO) bioavailability (Schmitt & Dirsch, 2009). Most studies found the benefit of fish oil in long term consumption. Currently, there is some evidence to suggest that dietary fat impacts on vascular reactivity (Kris-Etherton, Harris, Appel, & Nutrition Committee, 2002; Massaro, Scoditti, Carluccio, & De Caterina, 2008; Matsumoto, Nakayama, Ishida, Kobayashi, & Kamata, 2009). The current article will provide some information and also the evidence to conclude the impact of n-3 PUFA on endothelial dysfunction related to CVD.

1. Cardiovascular disease

CVD is the group of heart and blood vessel disorders which comprises arteriosclerosis, stroke, coronary artery disease, heart valve disease, arrhythmia, hypertension, orthostatic hypotension, diseases of the aorta, disorders of the peripheral vascular system and congenital heart disease (Mendis et al., 2011).

1.1 Development of cardiovascular disease

Whilst it is true that cardiovascular disease can represent varying types of heart or blood vessel issues, it is used more often to indicate damage caused to heart or blood vessels by atherosclerosis and blood clotting (Klabunde, 2011). Atherosclerosis is a chronic inflammatory disease of the arterial wall that involves accumulation of low-density lipoprotein (LDL) cholesterol. LDL is a heterogeneous class of lipoprotein particles and LDL has 2 distinct parts; a hydrophobic core, which contain triglycerides and cholesterol esters, and, a hydrophilic shell which consists of phospholipids, free cholesterol and apolipoprotein B-100 (ApoB-100) (Gleissner, Leitinger, & Ley, 2007). When endothelial cell injury or dysfunction occurs by a loss of NO bioactivity in the vessel wall or excessive production of reactive oxygen species (ROS), LDL is deposited into the sub-endothelial space occurring in the main within the macrophages and which eventually causes the development of well-defined lesions in the arterial intima. Macrophages take up deposited LDL lipid to form foam cells. When foam cells accumulate in the arterial wall they lead to the first stage in atherosclerosis. Atherosclerosis shows as arterial lesions, or plaques, and these have been categorised as six principal categories of lesions which reflect the early, developing, and mature stages of the cardiovascular disease. For the first lesion, arterial sites was adapt thickening of the intima which is among the earliest changes. Type II lesions form as nodular areas of lipid deposition which represent lipid-filled macrophages such as foam cells. Type III lesions; continue with foam cell formation and macrophage necrosis contain small extracellular pools of lipid. Type IV lesions are explained by a comparatively thin tissue separation of the lipid core from the arterial lumen. Type V lesions show fibrous thickening of the vascular structure. Mature type VI lesions appear more complicated and are

characterized by calcified fibrous areas with visible ulceration. Atherosclerosis may cause the walls to thicken, to become stiff and restrict the blood flow to the organs and tissues (Figure 1) (Stocker & Keaney, 2004). Furthermore, blood clotting begins when endothelial cells in the blood vessel are damaged. This can happen by the atherosclerotic plaque site. Blood platelets are drawn to the site of injury, forming a thrombus. The thrombus may become loose and carried to other plaque sites. They can obstruct the blood flow and lead to CVD (Stanner, 2008).

1.2 Risk factors of cardiovascular disease

Cardiovascular risk factors describe characteristics that can be measured, and have been associated in prospective studies, to be predictive of the cardiovascular disease (Stanner, 2008). Modification of the risk factor may alter risk, depending on the kind of risk factor. Some risk factors are beyond control, whilst others can be. There is a summary of CVD risk factors shown in Table 1.1. In short, risk factors are all inter-connected. Genetics almost certainly play their part in promoting the risk of CVD, demonstrated clearly in the male propensity for obesity in later life. ‘Modifiable’ risk factors are those risk factors which do not appear to be associated with other recognized risk factors. It is today patently obvious to us that the preferences people exhibit about their diets and other lifestyle choices such as physical activity, choosing to smoke or to drink alcohol to excess all clearly impact several of the main risk factors of CVD. And of these modifiable factors, the most significant is undoubtedly diet. Higher consumption of Trans fatty acids invariably increases the risks of CVD, whilst conversely it is widely reported that an average intake of long chain (LC) n-3 PUFA significantly reduces the risk of CVD. Physiological risk factors are also those of the individual concerned. Diabetes, obesity, high blood lipid levels (especially LDL and TG) all in their turn will contribute to the risks of that individual going on to develop CVD (Stanner, 2008).

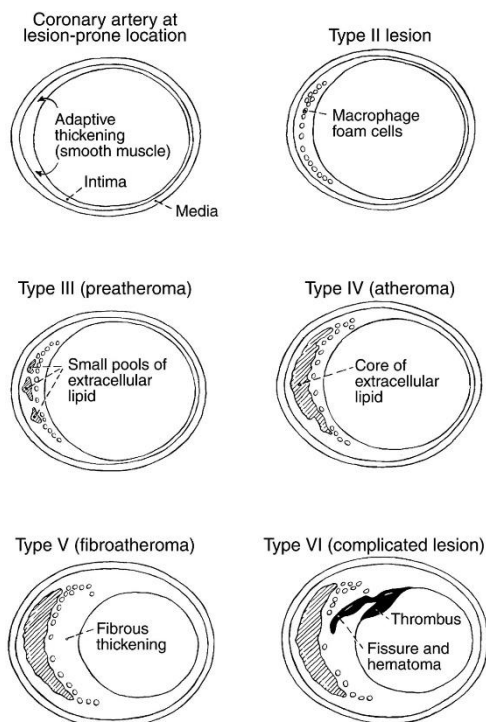


Figure 1 Varying stages of atherosclerosis
(Stocker & Keaney, 2004)

**Table 1** Summary of risk factors for cardiovascular disease*

Risk factors	Direction of association	Modifiable by dietary factors	Relevance to CVD
Non modifiable risk factor			
Age	Increases with age	No	Increased prevalence as population ages
Gender	Males are at higher risk than female	No	Increases in obese men and postmenopausal women
Genetic	Some gene variants have an influence on CVD (i.e. ApoE4, ApoE2 apoAI/CIII/AIV region)	No	Genetic factors may significantly increase or decrease CVD risk
Ethnic group	South Asians, Caribbean, and West Africans from south London are at particular high risk of CVD*	No	May reflect hypertension and insulin resistance
Modifiable risk factor			
Smoking	Smoking increase risk	Yes	Increases oxidative stress and impairs endothelial function
Alcohol consumption	Alcohol consumption increase risk but it depends on dose and type e.g. one glass of red wine a day reduces risk	Yes	Increases oxidative stress and LDL oxidation and impairs endothelial function
Exercise	Being active decreases risk	Yes	Decrease excess body fat, LDL cholesterol, triglyceride levels blood glucose and blood pressure. Increases HDL cholesterol level and insulin sensitivity
Diet	Some diets reduce risk	Yes	<i>Trans</i> fatty acids increase risk. Omega-3 fatty acid and phenolic compounds decrease risk.
Physiological risk factor			
Blood lipid	Higher blood cholesterol level, higher LDL-cholesterol, higher triglyceride and lower HDL cholesterol increase risk	Yes	Increase atherosclerotic, atherosclerosis, blood clotting and hypertension.
Diabetes	Diabetes increases risk	Yes	Decrease insulin sensitivity
Obesity	Overweight and obesity increase risk via other CVD risk factors	Yes	Increase excess body fat, LDL cholesterol, triglyceride levels blood glucose and blood pressure. Decreases HDL cholesterol level and insulin sensitivity
Oxidative stress	Oxidative stress increases risk	Yes	Increase ox-LDL
Inflammatory	Inflammation increase risk	Yes	Increase ICAMs, VCAMs and atherosclerosis
Vascular function	Vascular function	Yes	Decrease NO. Increase ET-1 and TXA ₂

*Adapted from Cappuccio et al., 1997; Assman, Cullen, Jossa, Barry, & Mancini, 1999; Stanner, 2008.

Apo: Apolipoprotein, ET-1: Endothelin-1, ICAMs: Intercellular adhesion molecule, HDL: Low density lipoprotein, LDL: Low density lipoprotein, NO: Nitric oxide, TXA₂: Thromboxane A₂, and VCAMs: Vascular cell adhesion molecule



2. Mechanisms by which long chain n-3 polyunsaturated fatty acids exert their cardioprotective actions

Fat is a major source of the fuel for the body and supports the fat-soluble vitamins absorption. Whilst saturated fatty acid are synthesised by the body and provide sufficient level needs for physical and chemical function of the body, some fatty acid such as n-3 PUFA are not produced by the body and are only obtainable through the diet. n-3 PUFAs play a vital role as structural membrane lipids, in particular in the nerve tissue and in the retina, and are precursors to eicosanoids. The n-3 PUFA comprise more than one double bond and have a tail of 16-carbon atoms or more. n-3 PUFA is also known in terms of ω -3 fatty acid. The term "n-3" describes the fact that the third carboxyl group starts with a double bond from the methyl end of carbon chain. The two main fatty acids of LC n-3 PUFAs are EPA, all-cis-5,8,11,14,17-eicosapentaenoic acid, and DHA, all-cis-docosa-4,7,10,13,16,19-hexaenoic, which have a double bond between the 5 and 6 carbon atoms and the 20 and 22 carbons, respectively. All double bonds are in the cis- form (Lee & Lip, 2003; Simopoulos, 2008).

Rich sources of LC n-3 PUFAs, include fish and other marine products, particularly cold water fish, sea fish and oily fish, or in supplements produced from fish oils. In the UK, the daily recommendation is 300–500 mg of EPA+DHA per day; the World Health Organisation (WHO) and many countries have a similar recommendation [9]. The American Heart Association recommends eating oily fish on two occasions each week, or roughly 500 mg daily of EPA+DHA. They recommend for coronary heart disease patients a consumption of 1 g per day of fish oil (Harris, 2004). There is no guideline for n-PUFA in the Thai dietary guideline. However, the US Food and Drug Administration advises that if the

average daily consumption of fish oil is more than 3 g from capsules there is a risk of side effects such as gastrointestinal upset, clinical bleeding and an elevation of LDL cholesterol in hypertriglyceridemia patients (Kris-Etherton et al., 2002).

Inflammation of the blood vessel walls at each stage, from initiation to progression, and eventually plaque deposition, is a key process occurring in atherosclerosis. The mechanisms underlying the inflammation and atherosclerosis are the result of leukocytes. Endothelium does not generally support binding with white blood cells in normal situation. After initiation of an atherogenic diet, patches of arterial endothelial cells start to express on their surface selective adhesion molecules that bind to leukocytes (Libby, 2012).

Both animal and human studies show that the intake of large amounts of fish and of n-3 PUFA can reduce the risk of CVD (Simopoulos, 2008). Hu and colleague in 2002 studied 84,688 females, monitoring them over a sixteen year period. A higher consumption of fish or n-3 PUFA was connected with a lower risk for CHD after adjusting for standard risk factors. In secondary prevention trials, increased fatty fish consumption or fish oil supplementation decreased coronary risks. Weiner and colleague in 1986 studied the effect of cod-liver oil on the development and advance of coronary artery disease in 18 coronary balloon abrasion pigs and fed a cod-liver-oil supplement diet for eight month compared with control normal diet. They found significantly less disease in those animals fed cod-liver oil compared with the normal group (Hu et al., 2002)

The possible mechanism underlying the action of long chain n-3 polyunsaturated fatty acid against the development of CVD is that they may compete with n-6 fatty acids for prostaglandin and leukotriene synthesis at the cyclooxygenase pathway and lipoxygenase level to produce weak, inflammatory



substances. n-3 PUFA in term of DHA and EPA lead to decreased Prostaglandin E_2 (PGE_2) production, decreased in Thromboxan A_2 (TXA_2) and decreased in Leukotriene B_4 (LTB_4) formation which are a strong vasoconstrictor, a strong inflammation and a strong inducer of leukocyte adherence. In addition, they are a weak vasoconstrictor and a less powerful inducer of leukocyte adherence, a weak platelet aggregator such as PGE_3 production, decreased in TXA_3 and decreased in LTB_5 (Simopoulos, 2008). Furthermore, the cardioprotective effect of n-3 PUFA may result from reduction of endothelial activation or expression of cell adhesion molecules (Libby, 2012).

3. Review of the effect of n-3 PUFA on endothelial dysfunction

The endothelium is the monolayer of epithelial cells which line each blood vessel. They consists of approximately 1×10^{13} cells forming an approximate 1 kg organ. The structural roles of the endothelium are the regulation of vascular tone, the regulation of immune and inflammatory responses, the production of various agents such as vasodilator (i.e. NO and Prostacyclin (PGI_2)) and inflammatory mediators (i.e. cell adhesion molecule (CAMs) and LTs) and the maintenance of homeostasis and vascular permeability (Sumpio, Riley, & Dardik, 2002). The endothelium is responsible for the production of NO, the most important mediator of endothelial vasodilation and a critical molecule for the control of inflammatory and antithrombotic activities (Libby, 2012).

Endothelial activation/dysfunction occurs when the homeostatic balance described is upset by an imbalance between vasodilation and vasoconstriction, inhibition and stimulation of smooth muscle cell proliferation and migration, and thrombogenesis and fibrinolysis, leading to various adverse effects

(Davignon & Ganz, 2004). Markers of endothelial dysfunction have been proposed and include impaired endothelium-dependent vasodilation, indicative of a decreased production of vasodilators including NO and PGI_2 , increased vasoconstrictor production, e.g. Endothelin-1 (ET-1) and TXA_2 production, increased pro-thrombotic and pro-coagulant activity, linked to increased soluble cell adhesion molecule (sCAMs), and increased cellular permeability (Stanner, 2008). However, there are many other markers that increase endothelial dysfunction, including cell adhesion molecules (i.e. ICAMs and VCAMs), Tumor necrosis factors (TNF) and LTB_4 (Patey et al., 1996). These cellular adhesion molecules (ICAMs and VCAMs) are active in several inflammatory stimuli which play a role in atherosclerosis (Blankenberg, Barbaux, & Tiret, 2003).

Endothelial dysfunction is deemed critical in the development of atherosclerosis (Christon, 2003). As a consequence of its location, the endothelium is exposed to high concentrations of lipoproteins and fatty acids. As high fat meals have been associated with atherosclerosis, it is not surprising that there is increasing scientific interest in the link between endothelial dysfunction, the progression of atherosclerosis and dietary fat intake.

Many studies have been performed into the role of individual fatty acids in endothelial function. They will be described in this section.

3.1 n-3 PUFA intake and endothelial function/dysfunction: evidence from in vivo studies

Many studies have investigated the relationship between n-3 PUFA and CVD, a limited amount of these studies focused on the relationship between dietary n-3 PUFA amount and endothelial function which may relate to CVD.

There is proof from animal work suggesting that dietary n-3 PUFA may influence endothelial



function. Davis and colleague in 1987 investigated the effect of feeding fish oil on the progression of rhesus monkey atherosclerosis ($n=8/\text{group}$) by feeding them diets containing 2% cholesterol and either 25% coconut oil, 25% fish oil/coconut oil (1:1), or 25% fish oil/coconut oil (3:1) for 12 months. They indicated that both fish oil- containing diets can reduce serum cholesterol levels and inhibit atherosclerosis compared to a pure coconut oil/cholesterol diet in rhesus monkeys (Davis et al., 1987).

Zhu, Smith, Sievers, Isenberg, and Parmley (1988) demonstrated the effects of dietary fish oil on cholesterol-induced atherosclerosis by using 36 New Zealand rabbits in (9 rabbits/group) which were fed a 0.3% cholesterol diet for ten weeks for group I. The rest were fed 1, 2 and 3 ml/day, of fish oil containing 180 mg/ml EPA and 120 mg/ml DHA, respectively. They found dietary fish oil may reduce the development of aortic and pulmonary atherosclerosis in cholesterol-fed rabbits. However, fish oil did not show to significantly alter the total serum cholesterol and high density lipoprotein (Zhu et al., 1988).

Engler, Engler, Pierson, Molteni, and Molteni (2003) found that spontaneously hypertensive rats (SHR) fed DHA enriched oil had significantly decreased blood pressure and vascular wall thicknesses in the coronary, thoracic, and abdominal aorta compared with corn/soybean oil. However, contractile responses to agonists mediated by receptor stimulation and potassium depolarization was not altered in DHA-fed SHR. Interestingly, endothelial-dependent relaxation induced by acetylcholine did not change in DHA fed animals, suggesting that endothelial-derived nitric oxide production/release is not affected by dietary DHA (Engler et al., 2003).

Furthermore, a mostly common effect of $n-3$ fatty acids in animals is a significant reduction in risk

of CVD, which have seen with fish-oil supplementation in humans also. However, there are variances between animals and humans arising not only from underlying species differences in fatty acid metabolism, but also from differences in the design of experiments. Additionally, the most prominent is the tendency to feed animals much larger amounts of $n-3$ fatty acids than supplements available to humans.

3.2 Evidence from human studies

$n-3$ PUFA in the diet is known to influence vascular reactivity (Kris-Etherton et al., 2002; Shaw, Hall, Jeffs, & Williams, 2007; Armah et al., 2008). There are many studies that have reported the effect of chronic changes to dietary fat intake on vascular reactivity. Mori et al. (2004) studied 59 overweight mildly hyperlipidaemic men. They were randomised to receive 4 g per day of purified EPA, DHA, or olive oil (placebo) capsules while continuing their regular diets for six weeks. They found that only DHA supplementation significantly improved vasodilator mechanisms and attenuated constrictor responses measured on forearm blood flow (Mori et al., 2004). In a similar investigation, West et al. (2005) examined the acute postprandial effects of unsaturated fatty acids meals containing EPA vs. DHA and ALA on flow-mediated dilation in 18 type 2 diabetes patients. They found that flow-mediated dilation was significantly increased in EPA vs DHA fat meals and ALA fat meals but no significant effects in only monounsaturated fatty acids meals (West et al., 2005). Armah et al. (2008) showed that a fish oil meal improved acute postprandial vascular reactivity in 25 healthy subjects compared with standard test meal. After 4 hours the subjects' vascular reactivity, when measured primarily by laser Doppler iontophoresis, was significantly decreased when they consumed the fish oil meal (Armah et al., 2008). Many studies suggest that $n-3$ PUFA can



have acute and chronic effects on other markers of vascular function. A study of Fah et al. in 2010 on the acute effect of fish oil on vascular function found that a high-fat meal (HFM) which contained 10 g of mixed fat causes acute impairments in endothelial function, whereas a moderate consumption of EPA and DHA (1 g of fish used in this study) helped to protect endothelial dysfunction which induced by an HFM (Fah et al., 2010).

Johansen, Seljeflot, Høstmark, and Arnesen (1999) found that CHD patients who received a daily supplementation of 0.45 g of EPA and 0.39 g of DHA for 6 months experienced decreases in haemostatic markers of atherosclerosis such as thrombomodulin (TM) and 4,5 vWF, whereas markers of inflammation (sE-selectin, sP-selectin and sVCAM-1) were increased (Johansen et al., 1999). Furthermore, Lopez-Garcia et al. (2004) found that the consumption of a long chain n-3 fatty acids rich meal containing 2 concentrations and 3 fatty acids of 1.25 and 1.45 g/d of ALA, DPA and DHA, respectively led to reductions in sVCAM-1, sICAM-1, E-selectin and IL-6 concentrations (Lopez-Garcia et al., 2004). van den Elsen and her group in 2014 also studied fish oil and endothelial dysfunction makers. They found the fish oil diet reduced the arterial blood pressure. It also improved endothelial function by reduced membrane arachidonic acid content and decreased thromboxane concentrations in plasma (van den Elsen et al., 2014).

Conclusion and Suggestion

Cardiovascular disease is the principal cause of death across the planet. Over the last two decades clinical intervention studies, epidemiologic observations and many investigations have been carried out on metabolism of polyunsaturated fatty

acids in general and n-3 PUFA in particular. It is now accepted that fish oil, in particular that of n-3 PUFA, is important for human growth and development and also that they play an essential role in inhibiting and reducing the incidence of coronary heart disease, hypertension, diabetes and inflammatory and immune disorder.

The link between diet and CVD has been conclusively demonstrated and there is some evidence to suggest that this is mediated by the effects of n-3 PUFA on endothelial function. The long chain fatty acids, EPA and DHA have been shown to have an efficacious effect upon the endothelial function in a range of subjects including healthy adults, smokers group and CHD patients following both acute and chronic administration. Observational data suggests that serum fatty acid composition, representative of dietary fat intake, is associated with various markers of endothelial function. However, there is very limited evidence from intervention studies that can help elucidate whether these are real relationships or whether they reflect confounding factors such as dietary n-3 PUFA mediated differences in vasodilators such as NO and ET-1. There is a need for future human intervention studies which will allow for a full comparison of the effects of fatty acid from fish oil on endothelial functions and associated mechanistic work in order to fully explain the mechanisms involved.

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