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Science behind the cardio-metabolic benefits of omega-3 polyunsaturated fatty acids: biochemical effects vs clinical outcomes

Author Names:
Rohith N Thota, Jessica J.A. Ferguson, Kylie A Abbott, Cintia B Dias, Manohar L Garg

Author's Affiliation:
Nutraceuticals Research Program, School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan, NSW, Australia

Authors' last names:
Thota, Ferguson, Abbott, Dias, Garg

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Corresponding Author:
Professor Manohar Garg
305C Medical Sciences Building
University of Newcastle
Callaghan, NSW-2308
Australia
Phone: +61 2 49215647. Email:
manohar.garg@newcastle.edu.au

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Abstract

Lower incidence of cardiovascular disease (CVD) in the Greenland Inuit, Northern Canada and Japan has been attributed to their consumption of seafood rich in long chain omega-3 polyunsaturated fatty acids (LCn-3PUFA). While a large majority of pre-clinical and intervention trials have demonstrated heart health benefits of LCn-3PUFA, some studies have shown no effects or even negative effects. LCn-3PUFA have been shown to favourably modulate blood lipid levels, particularly a reduction in circulating levels of triglycerides. High density lipoprotein-cholesterol (HDL-C) levels are elevated following dietary supplementation with LCn-3PUFA. Although LCn-3PUFA have been shown to increase low-density lipoprotein-cholesterol (LDL-C) levels, the increase is primarily in the large-buoyant particles that are less atherogenic than small-dense LDL particles. The anti-inflammatory effects of LCn-3PUFA have been clearly outlined with inhibition of NFkB mediated cytokine production being the main mechanism. In addition, reduction in adhesion molecules (intercellular adhesion molecule, ICAM and vascular cell adhesion molecule 1,VCAM-1) and leukotriene production have also been demonstrated following LCn-3PUFA supplementation. Anti-aggregatory effects of LCn-3PUFA have been a subject of controversy, however, recent studies showing sex-specific effects on platelet aggregation have helped resolve the effects on hyperactive platelets. Improvements in endothelium function, blood flow and blood pressure after LCn-3PUFA supplementation add to the mechanistic explanation on their cardio-protective effects. Modulation of adipose tissue secretions including pro-inflammatory mediators and adipokines by LCn-3PUFA has re-ignited interest in their cardiovascular health benefits. The aim of this narrative review is to filter out the reasons for possible disparity between cohort, mechanistic, pre-clinical and clinical studies. The focus of the article is to provide possible explanation for the observed controversies surrounding heart health benefits of LCn-3PUFA.

Introduction

LCn-3PUFA (eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) have been shown to favourably modify CVD risk factors including blood lipids, inflammation, platelet aggregation and endothelial function. Systematic reviews and meta-analyses of observational studies and randomised controlled trials involving LCn-3PUFA supplementation confirmed their health benefits [1-3]. Dietary supplementation with LCn-3PUFA have also been shown to reduce the incidence of sudden cardiac death in patients with myocardial infarction [3]. Few meta-analyses have also shown no association of LCn-3PUFA supplementation with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke based on relative and absolute measures of association [4]. It is not surprising that while LCn-3PUFA exert beneficial effects in reducing deaths from cardiac causes, sudden cardiac death and death from all causes, dietary supplementation with LCn-3PUFA in patients with existing coronary heart disease (CHD) failed to demonstrate an association with a protective effect on major cardiovascular events [5-7]. Therefore, the value of LCn-3PUFAs in patients with CHD is still being debated [8, 9].

Mechanisms by which LCn-3PUFA exert their heart health benefits have been thoroughly investigated. Lipid-lowering, anti-inflammatory and anti-aggregatory effects account for most of the health benefits of LCn-3PUFA and molecular mechanisms involving a number of integrated signalling pathways have been described. This review considers the mechanisms by which the LCn-3PUFAs, DHA and EPA, may function to combat chronic diseases with a focus on CVD.

LCn-3PUFA and dyslipidaemia

It is well established that LCn-3PUFA supplementation lower plasma and hepatic triglyceride (TG) concentration [10-12] in a dose-dependent manner [13]. In addition, they also elicit mild increases in both LDL-C and HDL-C concentrations, however, yielding favourable particle size that are less atherogenic. Observational studies have demonstrated that circulating TG levels are negatively associated with dietary LCn-3PUFA[14-16] . Typical Western diets do not provide adequate dietary LCn-3PUFA (approximately 130 mg/day) [17], however, pharmacologic doses (i.e. >3g/day of EPA+DHA) manifest substantial TG-lowering effects [13, 18, 19]. Numerous trials have shown that dietary supplementation with EPA+DHA significantly lower plasma TG concentrations by about 25-30% (range 16-45%) when administered at doses 2 to 4g per day for at least 4 weeks [12, 19-23]. Clinically relevant doses (≥4g/day) are

recommended for those with substantially elevated circulating TG i.e. >5.65 mmol/L [24, 25]. Studies have shown that baseline TG concentrations >5.65 mmol/L are associated with larger reductions in plasma TG [11, 18, 20, 22, 26-28] and modest elevations of 5-10% in LDL-C and 1-3% in HDL-C [22].

It is widely reported that females have higher circulating DHA concentrations compared to males, independent of dietary intake [29-36]. It has been shown that gender is a significant confounding factor for the association between LCn-3PUFA status and circulating levels of TG [33]. Females aged ≥ 65 years had significantly higher erythrocyte LCn-3PUFA (i.e. omega-3 index; O3I) status when compared to male counterparts [37]. After controlling for confounders such as age, BMI and dietary intake the negative association between O3I and TG concentrations was only evident in females [37].

It has been repeatedly shown that when compared to EPA, DHA induces greater reduction in TG concentrations [38]. It is hypothesised that DHA leads to greater activation of lipoprotein lipase (LpL), therefore leading to increased TG clearance via the conversion of VLDL-C to LDL-C [39]. The modulation of lipid parameters by LCn-3PUFA could alleviate dyslipidaemia via several mechanisms discussed below.

VLDL-C production and non-esterified fatty acids

Raised concentration of TG is indicative of increased production of VLDL-TG or reduced clearance of TG [19]. Studies have demonstrated that regardless of the cause of hypertriglyceridemia (HTG), number of participants, tracer used in kinetic studies or the type of methodology used to model the effects; the root cause of decreased plasma TG by LCn-3PUFA is lowered hepatic VLDL-TG production [19]. Hepatic VLDL-TG synthesis is reduced following EPA+DHA supplementation in mild HTG individuals [19] and studies in cultured hepatocytes have demonstrated inhibition of VLDL-TG and assembly and secretion of apoB-100 [40, 41]. Peroxide derivatives of LCn-3PUFA have also been shown to stimulate the degradation of apoB-100, further facilitating the reduction of VLDL-TG secretion [42].

Independent of metabolic state, non-esterified fatty acids (NEFA) supplied to the liver are the primary source of fatty acids (FA) for the production of VLDL-TG. High levels of NEFA during HTG lead to increased VLDL apoC-III concentrations, a key inhibitor of LpL activity when bound to TG-rich lipoproteins [43]. Studies have observed that apoC-III levels positively correlate with plasma TG in HTG patients and this is primarily due to altered LpL function [43] and slowed TG hydrolysis [44]. LCn-3PUFA have been shown to block apoC-III accumulation

in VLDL particles, promoting LpL-mediated lipolysis, TG catabolism and subsequently enhance TG clearance [43, 45-47]. One study reported a significant reduction in apoC-III concentration following dietary DHA supplementation in HTG males [48]. Several studies including individuals with both normal and elevated TG levels have repeatedly shown concurrent reductions in NEFA and plasma TG after LCn-3PUFA supplementation [19, 49-55]. LCn-3PUFA reduce NEFA pools and minimize accumulation of FA by counteracting intracellular lipolysis in adipocytes via suppression of adipose tissue (AT) inflammation, enhanced extracellular lipolysis via LpL in tissues and by promoting β-oxidation in the liver and skeletal muscle [19]. Only one randomized controlled trial (RCT) to date has investigated the effects of LCn-3PUFA on NEFA as a primary endpoint and found that large amounts of LCn-3PUFA supplementation (9g/day for 7 days) in healthy, insulin sensitive normotriglyceridaemic individuals led to nearly 40% reduction in fasting NEFA and TG concentration [53]. Overall, LCn-3PUFA diminish NEFA accumulation, reduce FA delivery to the liver and therefore decrease the rate of FA incorporation into VLDL particles. As a result, VLDL production is reduced resulting in lower plasma TG concentrations.

Fatty acid trafficking between tissues

It has been demonstrated in cultured hepatocyte studies that LCn-3PUFA inhibit the assembly and secretion of VLDL by stimulating apoB degradation [42, 56] and minimize FA pool accumulation by up-regulating hepatocyte β-oxidation [40, 42]. Dietary LCn-3PUFA have been shown to up-regulate post-prandial LpL expression in the human AT, as well as enhance LpL activity in skeletal and heart muscle, the two major sites of FA utilisation and LpL expression [19]. In animals fed a high fat diet, dietary supplementation with LCn-3PUFA decreased adiposity [57-60], despite enhancing FA uptake due to raised expression of LpL and CD36 [61, 62]. Similarly, in human AT, dietary LCn-3PUFA upregulate the expression of postprandial LpL, indicating enhanced FA uptake by AT. However, no influence on body weight has been observed in several studies with an exception of only a few number of smaller trials [19, 63-65]. In addition, LCn-3PUFA up-regulate LpL lipolysis of plasma TG and β-oxidation thereby increasing uptake of FA into these tissues, resulting in a net reduction of FA available for hepatic lipogenesis and VLDL production. Furthermore, LCn-3PUFA are more potent than n-6PUFA as hypotriglyceridaemic agents in these processes of repartitioning FA by simultaneously downregulating genes that encode enzymes and proteins involved in lipogenesis as well as upregulate genes that encode proteins for stimulation of FA oxidation [47].

Modulation of nuclear receptors

Lipid metabolism is regulated by major nuclear receptors responsible for gene expression [19, 47, 66, 67]. Alterations in nuclear receptor transcription have been shown to mediate the TG-lowering properties of LCn-3PUFA [10, 68]. The net result of simultaneous stimulation of all nuclear factors by LCn-3PUFA is reflected by the reported reductions in plasma TG, with only minimal changes in LDL-C and HDL-C [47].

Activation and regulation of peroxisome proliferator-activated receptors (PPAR) is the most consistent TG-lowering mechanism driven by LCn-3PUFAs in animal studies [19]. PPAR, involving three subtypes (α, β and γ) regulate gene expression of proteins and enzymes involved in lipid and energy metabolism such as promotion of β-oxidation in the liver, AT, heart and skeletal muscle tissue (PPARα); storage of fatty acids as TG in AT (PPARγ); increased hepatic hydrolysis of TG-rich lipoproteins and decreased production of free fatty acids (FFA), VLDL and TG [19]. LCn-3PUFA such as EPA and DHA are also natural ligands to PPARs and farnesol X receptor (FXR) [69]. Moreover, eicosanoid metabolites 3-series and oxylipins originating from LCn-3PUFA are potent activators of PPARs compared to 2-series formed from n-6PUFA [19]. Thus, tissues abundant in EPA and DHA serve as potent PPAR activators and result in increased fatty acid catabolism [70]. Moreover, very high affinity binding of DHA to PPARs and retinoid X receptor RXR has been shown. Therefore, LCn-3PUFA can activate PPARα which increases the expression of FA oxidation genes resulting in a reduction in hepatic and plasma TG [71, 72].

Furthermore, activation of PPARα and FXR reduces apoC-III expression, therefore promoting LpL activity, enhancing catabolism/clearance of TG and [19, 28, 45, 47, 69, 73, 74] postprandial chylomicrons [75, 76]. In addition, FXR induces apoC-II and VLDL receptor gene expression. Since EPA and DHA are ligands for both PPARα and FXR, the changes in apoC-III, apoC-II and VLDL-receptor induced by activation of these nuclear factors may be playing a major role in the modulation of LpL activity and TG-lowering effects of LCn-3PUFA [69, 74, 77].

In addition, efficacy of LCn-3PUFA may be optimised through the interplay between oestrogen and PPAR. Oestrogen has been shown to potentiate the effects of PPAR so as to raise PPARα activity via interaction with PPARα gene transcription, leading to enhanced conversion of α-linolenic acid (ALA) to DHA [30]. The higher endogenous conversion of ALA to DHA seen in females compared to males [29, 31, 33] appears to be related to oestrogen levels, rather than a lack of testosterone [30]. Upregulation of endogenous conversion of ALA to DHA following

oestrogen treatment was shown in postmenopausal women receiving hormone replacement therapy, women receiving the contraceptive pill and male-to-female transsexuals receiving oral oestrogen therapy [31, 78].

SREBP-1c is the key regulator of hepatic lipogenesis, which is mediated by the binding of Liver X receptor (LXRα) to retinoid X receptors (RXRα) for its activation. DHA and EPA have been shown to inhibit LXRα/RXRα binding to downregulate the activity of SREBP-1c [79]. This prevents the expression and protein maturation of SREBP-1c, resulting in a net decrease in *de novo* lipogenesis and VLDL-C secretion [19].

In addition, PUFA appear to elicit inhibitory effects on hepatocyte nuclear factor-4α (HNF-4α) a major regulator of carbohydrate, cholesterol and fatty acid metabolism, which may explain some of the TG-lowering properties of LCn-3PUFA [47].

With respect to nuclear receptors, LCn-3PUFA lower TG by: suppressing hepatic lipogenesis via inhibition of SREBP-1c; upregulating hepatic and skeletal muscle fatty acid oxidation via PPAR activation; enhancing TG catabolism and clearance via both PPAR and FXR activation; and increasing glucose to glycogen flux via down-regulation of HNF-4α. This leads to an overall redirection of FA away from TG storage and towards oxidation, ensuing less substrates available for VLDL synthesis.

Intrahepatic lipids

Non-alcoholic fatty liver disease (NAFLD) is characterised by hepatic accumulation of TG in individuals not consuming excessive alcohol and presents as a range of liver diseases such as 'simple' steatosis to non-alcoholic steatohepatitis [80]. In addition, NAFLD is a classic comorbidity of HTG and is independently associated with chronic cardiometabolic conditions [80]. As high as 20% of the general western population have NAFLD [81] with the prevalence rising to 88% in the obese population [82]. We have recently demonstrated a sex-dependent inverse relationship between NAFLD and erythrocyte LCn-3PUFA concentrations in older adults, with women, compared to men, being more likely to have a lower risk of NAFLD when LCn-3PUFA status was higher [83]. In support of these findings, a recent systematic review and meta-analysis of human RCTs suggests that LCn-3PUFA supplementation significantly reduces liver fat, however, an optimal dose-response is yet to be established [80]. This same review demonstrated non-significant trends towards benefits in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following LCn-3PUFA therapy, however, there was significant heterogeneity between studies [80]. In humans [84, 85] and mice models of hepatic

steatosis [86, 87], supplementation with LCn-3PUFA ameliorated NAFLD accompanied by increased β-oxidation and lowered intracellular pools of FFA. The dose of LCn-3PUFA (3g/day vs 5g/day) does not appear to significantly influence steatosis grade when administered for six months [80]. A study in western diet-induced non-alcoholic steatohepatitis (NASH) showed dietary DHA was superior to EPA in attenuating the induced changes in plasma lipids and hepatic injury [88]. Hepatic metabolism, oxidative stress and fibrosis were reversed following dietary DHA treatment indicating a preventative role in NASH and reduced risk of hepatocellular carcinoma.

In the absence of weight loss, Parker et al also observed a clinically significant reduction in steatosis following LCn-3PUFA supplementation in five of the nine studies reviewed including amelioration of liver steatosis in 27% of the patients across the pooled studies [80]. It is known that individuals with NAFLD have low dietary intake of LCn-3PUFAs compared to healthy controls [89]. Preclinical data have shown that this profile is associated with a pro-inflammatory state and raised lipogenesis, promoting steatosis. In contrast, LCn-3PUFA have been shown to down-regulate SREBP-1c and upregulate of PPAR-α, thus favouring fatty acid oxidation and lowering steatosis [80, 90]. High intra-variability in liver tests hinder the ability to detect significant changes in liver function parameters [80], however, further research into the therapeutic effects of LCn-3PUFA supplementation in NAFLD is warranted to quantify reductions in liver fat via magnetic resonance imaging, the gold standard for measuring and quantifying liver fat.

Blood cholesterol

The cholesterolaemic effects of LCn-3PUFA are inconclusive as both hypo- and hypercholesterolaemic effects have been reported following dietary LCn-3PUFA supplementation in humans [10, 20, 91, 92]. However, intervention studies and systematic reviews have concluded that LCn-3PUFA supplementation poses no substantial effects on total cholesterol levels [18, 20, 26, 93, 94], with only mild increases attributed to small rises in LDL-C and HDL-C.

Low-density lipoprotein cholesterol (LDL-C) concentration and particle size

Dietary intervention studies suggest LCn-3PUFA supplementation increase LDL-C in a dose-dependent manner [95-97]. Our group has previously reported a significant rise in plasma TC and LDL-C following co-administration of LCn-3PUFA supplementation and a diet enriched with SFA when compared to a diet enriched with n-6PUFA [98], suggesting that background dietary

fat should be considered when assessing the LDL-modulating capacity of LCn-3PUFA. It has been shown that DHA increases LDL-C significantly more than EPA in people with or without HTG [39, 94, 99]. Elevations in LDL-C is indicative of enhanced LDL production and/or reduced fractional catabolic rate of LDL particles [69, 100]. Preclinical studies suggest that DHA-induced elevation in cholesterol ester transfer protein (CETP) activity [92] may be one of the factors contributing to raised LDL-C concentration. In this context, clinical studies have demonstrated a reduction in LDL-C following treatment with CETP inhibitors [101]. A significant increase in CETP activity and gene expression in DHA-treated hamsters compared to placebo and EPA-treated hamsters was also reported. [92]

Although elevations in LDL-C after dietary LCn-3PUFA supplementation in HTG individuals is believed to be unfavourable, it is often overlooked as indicating a potential improvement in LDL particle size that in fact is less atherogenic [102]. While circulating TG and cholesterol concentrations are well established risk factors for CVD, certain lipoprotein particle characteristics may be more accurate estimate of CVD risk such as mean LDL particle diameter and the number of small, dense LDL particles for the prediction of LDL-associated risk of CVD [103].

LDL particle size

Small VLDL species are readily converted to fast-floating LDL particles and are a likely consequence of hepatic overproduction of apoB [100] resulting into elevated plasma TG [45, 67]. It is well known that in circulation small, dense LDL are considered pro-atherogenic [102] and larger, buoyant LDL are favourable and cardio-protective [94, 102]. LCn-3PUFA supplementation has been shown to reduce VLDL particle size and concentration of large VLDL particles [48, 104, 105]. The effects of dietary LCn-3PUFA on LDL particles demonstrate a favourable shift from small to large LDL particles [48, 106], however some studies have reported no effect [105, 107]. One study observed significant increases in LDL-C without concomitant alterations in LDL particle size [108]. Discrepancies may be indicative of differential effects pertaining to DHA/EPA ratio, dose of LCn-3PUFA, duration of supplementation and the influence of background dietary fat.

DHA supplementation for 90 days led to significant reduction in fasting plasma TG, large VLDL-C and VLDL particle size with concurrent elevation in LDL-C, small VLDL, large LDL particles and mean LDL particle diameter in middle-aged HTG men [48]. Moreover, this was accompanied by reduced postprandial (0-6hr) plasma area-under-the-curve for TG, intermediate-dense lipoprotein (IDL), small dense LDL particles, large VLDL and small HDL

particles. Concurrent elevation in the area-under-the-curve for large LDL, large HDL and small VLDL particles was also reported [48]. In support of these findings, our research group showed that 4 weeks of fish oil supplementation in healthy adults led to significantly lower concentration of total, large, medium and small VLDL particles and a significant increase in medium-large LDL particle concentration [109]. As discussed in previous sections, the stimulation of LpL expression by LCn-3PUFA leads to the formation of lipoprotein remnants such as small VLDL and IDL. Since lipoprotein remnants have a greater affinity to the LDL-receptor (LDL-R) [110, 111], they compete with LDL particles for clearance [112]. Since LCn-3PUFA reduce hepatic expression of LDL-R [112]; this might explain the observed rise in large LDL particle concentration. It is possible that the discrepancies reported regarding the influence of LCn-3PUFA on change in LDL particle size is clouded by the type of background dietary fat. It is important that the background dietary fat is considered when investigating the modulatory effects of LCn-3PUFA on particle size shift. Furthermore, DHA supplementation does not increase the concentration of total LDL particles, but rather significantly reduces the concentration of small dense LDL particles [48]. This should lessen any concerns about potential increased CVD risk that might be inferred from raised plasma LDL-C concentration commonly observed following LCn-3PUFA supplementation.

LDL-receptor

Preclinical findings support the role of LCn-3PUFA as a regulator of LDL-R activity, however, findings remain inconsistent as to whether these modulatory effects are a result of DHA or EPA. Some studies suggest that the downregulation of LDL-R expression [79, 113] and protein and mRNA expression [92, 114] following DHA treatment is the key role of LCn-3PUFA in raising plasma LDL-C. Preclinical studies suggest DHA modulates the LDL-R directly by mediating hepatic LDL-R protein and mRNA expression, and indirectly via inhibition of SREBP-2 mRNA expression [92, 114]. A study in high fat diet-fed hamsters reported DHA treatment significantly decreased LDL-R protein and mRNA expression in the liver after 3 weeks [92]. Moreover, inhibition of mRNA expression of SREBP-2 was also observed with DHA-treated hamsters only, and both EPA and DHA significantly lowered SREBP-1. In human fibroblast and HepG2 cells, LDL-R protein abundance was significantly increased by 1- to 3-fold after EPA and DHA treatment [115]. These effects were independent of changes in SREBP-1 protein, however, this study along with another human HepG2 cellular study using DHA only found no significant changes in LDL-R mRNA levels [79]. Other studies in HepG2 cells have reported that EPA suppressed the binding of LDL to cells compared with oleic acid [116] and reduced LDL-R

activity and mRNA level when cells were incubated with EPA-enriched LDL from humans supplemented with dietary fish oil [117]. A study in cultured human liver HepG2 cells investigated the effects of different FA on the LDL-R in terms of three different stages of LDL expression: functional cellular LDL binding activity, amount of LDL-R protein and LDL-R mRNA level [114]. All three measurements of LDL-R binding activity decreased as the degree of FA unsaturation increased (palmitic ≥ oleic acid > linoleic acid > EPA). LDL-R activity, protein and mRNA levels were suppressed by a total of 40 and 70% in the presence of linoleic acid and EPA, respectively. Overall, the authors conclude decrease in LDL-R activity appeared to be due to reduction in the LDL-R numbers via downregulation of the LDL-R gene transcription. However, a recent study by Zhou et al has reported upregulation of LDL-R gene expression [79]. Notably, majority of the studies using human cell lines are limited to measurement of LDL-R binding activity [114], therefore, the effects of LCn-3PUFA on LDL-R activity warrants coupling with gene expression in future studies.

High-density lipoprotein cholesterol

Several clinical studies report supplementation with dietary LCn-3PUFA induce modest elevations in HDL-C [18, 20, 28, 94]. An average of 4 g/day of EPA and/or DHA increase HDL-C on average by 10-13% in individuals with hyperlipidaemia [11, 21]. The key mechanism behind the HDL-raising effect of LCn-3PUFA is the reduction in CETP activity [118, 119]. In vitro studies have reported significantly greater reduction in CETP activity with DHA compared to EPA [120]. Given the important role of CETP in lipid transfer between lipoproteins and the reduced activity of CETP coupled with lowered TG concentration following LCn-3PUFA therapy; the exchange of lipids is further lessened thus promoting the formation of larger HDL particles (HDL_2) that are rich in cholesterol and more cardio-protective, as opposed to smaller, triacylglycerol-rich HDL (HDL_3) prone to degradation [118, 119]. In addition, fish oils raise both HDL size and the number of large HDL particles, providing greater cardio- protection since HDL particle number and size is inversely associated with cardiovascular events [121-124]. In normotriglyceridaemic individuals, 4.5 g/day of fish oil supplementation led to a significant shift in HDL particle size from HDL3 to HDL2 subclass [122]. A similar observation was reported in healthy males whereby those receiving LCn-3PUFA from either a fish diet, fish oils (EPA+DHA) or pure DHA had over 50% elevation in HDL_2/HDL_3-C ratio after 15 weeks [123]. Consistent with these findings, our colleagues reported a reduction in very large HDL particle concentration and elevation in medium-large HDL particle concentration, average HDL size and HDL-C [109]. The mechanism behind this is likely due to enhanced enrichment of larger

HDL particles as fish oil treatment has been previously reported to increase HDL:apo A1 ratio as well as lower CETP activity [125]. Some studies have reported greater elevations in HDL-C after DHA supplementation compared to EPA and increase in HDL_2-C as high as 29% [94] and 50% increase in HDL_2/HDL_3-C ratio [123]. Elevation in not only HDL-C concentration but shift in particle size that are more cardio-protective could have noticeable clinical implications in addition to TG-lowering for the management of hyperlipidaemia and prevention of CVD.

ApoE polymorphism

Apolipoprotein E (apoE) is a structural and functional protein component of lipoproteins and plays an important role in their metabolism and clearance in the liver [126]. The apoE gene comprises three different alleles: E2, E3 and E4 which are responsible for variations in lipoprotein metabolism [127]. The majority of individuals possess the E3 allele [128, 129]. Studies have shown that compared to E2 carriers, E4 carriers are at a higher risk of not only CHD but also late-onset Alzheimer's disease, with variability attributed to modifiable factors such as dietary intake of SFA and cholesterol and smoking status [130, 131]. Moreover, E4 carriers are more responsive to dietary changes. Findings remain inconsistent surrounding the influence of apoE genotype on plasma lipid response to LCn-3PUFA supplementation, with indications of improved responsiveness in E4 carriers [130]. Conversely, Harris et al recently found no evidence for a harmful relationship between lipid markers (LDL-C, LDL particle number, apoB, TG or HDL-C) and the O3I by apoE genotype [132]. One study reported apoE genotype had a prominent impact on lipid response to daily supplementation of LCn-3PUFA in dyslipidaemic males [93]. E2 carriers experienced a significant reduction in postprandial TG as well as a trend towards elevation in LpL activity in non-E2 carriers. The influence of EPA and DHA-specific roles in modulation of apoE remains unclear, however, a study in males administered a high dose of DHA (3.7g/day) reported greater elevation in LDL-C in E4 carriers compared to E3 carriers in the DHA group only [112]. EPA had no effect on LDL-C in either group, but DHA significantly raised LDL-C by 10% from baseline in the E4 carriers compared to a non-significant 4% reduction in E3 individuals. Although the underlying mechanisms are unknown, LDL competitive uptake studies in HepG2 cells indicate that it is possibly attributed to either raised levels of apoE (ligand) contained in $VLDL_2$ of E4 carriers or altered apoB conformation and/or a shift in the orientation of the apoE protein leading to raised ability to interact/influence hepatic LDL-R; all of which are magnified following DHA supplementation [112]. The difference in lipid response to LCn-3PUFA across apoE variants suggests a potential impedance on the efficacy of LCn-3PUFA intervention for the management and prevention of dyslipidaemias. A

more comprehensive understanding and consistent findings are warranted to elicit the precise influence of EPA and DHA on lipid metabolism in the context of apoE polymorphism.

LCn-3PUFA and adipose tissue function:

The last three decades have seen an epidemic increase in the global obesity rates. Broadly characterised by having an excess accumulation of AT obesity is defined by the World Health Organisation as having a BMI $\geq$ 30kg/m^2. Obesity, particularly abdominal obesity, is strongly associated with development of insulin resistance (IR) and predisposes individuals to the development of many non-communicable diseases (NCDs) such as metabolic syndrome (MetS), NAFLD, type 2 diabetes (T2D) and cardiovascular disease (CVD). NCDs contribute significantly to the disease burden in both developed and developing nations, with global costs of diabetes-related healthcare more than tripling between 2003 and 2013 [133], making obesity-related metabolic dysfunction a key global health concern. There is an emerging body of evidence that sub-clinical inflammation of the AT is a key etiological factor linking obesity with IR and its metabolic sequalae [134]. Due to their well-characterised anti-inflammatory properties, there is interest in the role of the LCn-3PUFA, particularly EPA and DHA, in resolving AT inflammation, preventing the development of metabolic dysfunction and IR.

Adipose tissue as an endocrine organ

The primary function of AT has long been regarded as energy storage organ, however, in recent decades it has been increasingly recognised for its significant endocrine and immunological function [135]. AT consists primarily of adipocytes, but also contains a dense network of stromavascular tissue, which includes pre-adipocytes, vascular tissue, and immune cells.

In order to maintain energy balance adipocytes will either store FFA in the form of TG, or release FFA to be oxidised as fuel. To facilitate this, adipocytes display high levels of plasticity and adaptability, capable of expanding to many times its size. Expanding adipocytes facilitates TG storage, removing FFA from circulation and preventing lipid deposition in ectopic tissue (e.g. skeletal muscle, liver), preventing lipotoxicity [136]. Inability to adequately expand will result in higher levels of circulating NEFA and circulating TG, both associated with metabolic dysfunction. Since the discovery of Leptin in the 1990s, it is now known that AT is a highly dynamic tissue which secretes signalling molecules (known as adipokines) and cytokines (collectively referred to as adipocytokines) that are essential in maintaining whole-body glucose homeostasis [137]. The well-characterised adipokines are leptin, adiponectin resistin, vistafin, ormentin[138]. Dysregulation of adipokines and adipose-derived cytokines (e.g. TNF-α,

IL-6 and IL-1β) often occur in obesity and are associated with the development of IR and metabolic dysfunction [139]. Of these, adiponectin is of particular interest – it is a powerful insulin-sensitiser and anti-inflammatory small peptide hormone secreted by adipocytes that is downregulated with increasing adiposity, and is inversely correlated with IR [140]. Studies comparing insulin-resistant with insulin-sensitive obese individuals show that insulin-sensitive obese people have higher levels of adiponectin [141, 142]. Low levels of adiponectin have been observed in people with T2D, MetS and CVD. Importantly, adiponectin is modifiable, and increasing secretion of adiponectin also improves IR [143].

Adipose tissue inflammation and insulin resistance

Growth of AT occurs either by hypertrophy (i.e. expansion of adipocytes) or hyperplasia (e.g. recruitment of pre-adipocytes). Healthy expansion of AT provides a depot for circulating FFA, removing them from circulation and preventing ectopic lipid deposition in skeletal muscle and/or hepatic tissue. Healthy expanding AT requires growth of vascular tissue (i.e. angiogenesis) to enable proper oxygenation and function. Growth of AT creates pockets of hypoxia, which triggers an inflammatory response and releases monocyte chemotactic protein-1 (MCP-1) from adipocytes, which encourages migration of monocytes into AT [144]. There is also a phenotype switch in the macrophages, with monocytes differentiating into the "classically activated" pro-inflammatory M1 macrophages, as opposed to the "alternatively activated" M2-type macrophages normally resident in healthy AT. M2 macrophages secrete anti-inflammatory compounds such as IL-10 and are associated with insulin-sensitive tissue. In contrast, M1-type macrophages promote a pro-inflammatory environment, with an increase the release of inflammatory markers such as TNF-α and IL-6. This inflammatory response promotes the release of Vascular Endothelial Growth Factor (VEGF) which is the primary driver of angiogenesis in AT [145]. However, while inflammatory mediators are essential to the remodelling process, chronic sub-clinical inflammation also impairs insulin signalling and is a key etiological factor in the development of IR. Chronic inflammation associated with IR is characterised by higher circulating levels of C-reactive protein (CRP), IL-6, TNF-α and IL1-β, and low levels of adiponectin. Chronic inflammation can occur as a result of an insufficient vascular response, or inability to adequately resolve the inflammatory response. Prolonged hypoxia in adipocytes leads to apoptosis, macrophage infiltration and fibrosis of AT, limiting the further expandability of AT, increasing circulating FFA and ectopic lipid deposition, and further contributing to the development of IR.

Role of omega-3 PUFAs in adipose tissue function

Whilst research interest into the LCn-3PUFA were initially due to their apparent benefits to cardiovascular health, they are now well established as being important immune regulators via the eicosanoid pathway. The biologically active EPA (c20:5n-3) and DHA (c22:6n-3) are incorporated into nearly all cells and tissues in the body, including AT. Levels of EPA and DHA in AT are reflective of both long-term dietary intake and endogenous synthesis from ALA precursor [146], and supplementation with EPA and DHA increases AT levels in a dose-dependent manner [147]. Dietary recommendations to decrease intake of saturated fats has led to dietary patterns changing across recent decades, with an increased intake of omega-6 polyunsaturated fatty acids through a greater reliance on seed oils, resulting in an imbalance of n-6:n-3 intake, from 1-4:1 ratio thought to be associated with good health, to 20:1 in a typical western diet. Research suggests that this has become reflected in changes to the FA composition of AT, with a 136% increase in AT linoleic acid (c18:2n-6) seen over the past half century in the United States [148], a finding which is likely paralleled in other western nations. Due to the opposing role of n-6 and n-3 fatty acids via the lipoxygenase (LOX) and cyclooxygenase (COX) pathways, this shift in the fatty acid profile could potentiate a pro-inflammatory environment in the AT, characterised by elevated levels of pro-inflammatory markers and low adiponectin, thus pre-disposing overweight individuals to the development of IR, NAFLD, MetS and T2D. Restoring n-3PUFA in AT could ameliorate AT dysfunction via several well-described mechanisms, outlined below **(Figure 1).**

Specialised pro-resolving lipid mediators

Metabolites of EPA and DHA form protectins, resolvins, and maresins – specialised pro-resolving lipid mediators (SPM) responsible for the active resolution of an acute inflammatory response [149]. SPMs are synthesised enzymatically via the LOX pathway from either n-3PUFA (EPA, DHA) or n-6PUFA (Arachidonic Acid; ARA) precursors. In AT lacking the required substrates to form SPMs, an acute inflammatory process could fail to resolve, creating a chronic pro-inflammatory environment. SPMs have been identified in human AT depots, including D-series SPMs (derived from DHA), resolvins D1 (RvD1) and D2 (RvD2); protectin D1 (PD1); Maresin 1 (MaR1) and E-series SPM (derived from EPA) including resolvin E1 (RvE1), and Lipoxin A_4 (derived from arachidonic acid), as well as classic eicosanoids such as prostaglandin and leukotrienes [150]. LCn-3PUFA play an essential role in the resolution of AT inflammation via their metabolites [151]. Pre-clinical studies have shown that SPMs exert anti-

inflammatory effects via actions on both adipocytes and macrophages. Administration of RvD1 (2μg/kg/day via peritoneal injection) decreases macrophage accumulation and restores insulin sensitivity in *db/db* mice [152] Both MaR1 and DHA have been shown to decrease levels of IL-1β and TNF-α in diet-induced obese mice; however DHA comparatively had the greater anti-inflammatory effect. Supplementation with DHA was associated with a significant increase in 17-HDHA levels in AT. Furthermore, RvD1 and RvD2 have been shown to polarize resident macrophages (M1-type) to the anti-inflammatory M2-type macrophages [150]. RvD1 has also been shown to potentiate the anti-inflammatory actions of IL-10, decreasing expression of IL-6, IL-1β, IL-8 and TNF-α ex vivo in AT explants from obese individuals [153].

To date the majority of evidence for SPMs are pre-clinical, and whether levels of SPM in AT can be boosted via dietary or supplemental LCn-3PUFA interventions is unclear. There is some evidence that supplementation with LCn-3PUFA increases the levels of E- and D-series metabolites in AT. Five weeks of fish oil supplementation showed an increase in PD1 and RvD1 in AT of *ob/ob* mice, resulting in improved insulin sensitivity. Itariu et al [154] showed that 8 weeks of LCn-3PUFA intervention (1840mg EPA + 1520mg DHA/day) decreased expression of inflammatory genes in AT, increased production of adipose-derived cytokines and a concomitant increase in RvE1, 17-HDHA, PD1, RvD1 in AT, and reduced circulating levels of IL-6 in severely obese (BMI ≥40kg/m^2) people without diabetes. Consistent with the M2/M1 macrophage polarization observed in pre-clinical studies, Itariu et al also noted a significant decrease in *CD40*, a marker of M1 macrophage infiltration, with no changes to *MRC1* or *CD163* (markers of M2 macrophage). Further, 6-month supplementation with DHA-rich fish oil prevented the decline in RvD1 in peripheral blood mononuclear cells normally seen in Alzheimer's disease [155]. Supplementation of pregnant women with EPA and DHA during pregnancy increased levels of SPM 18-hydroxyeicosapentanoic acid and 17-hydroxydocosahexacoic acid (17-HDHA) in placental tissue by two- to three-fold, and 17-HDHA was positively correlated with placental DHA levels [156].

GPR-120

There are several receptors that bind with free fatty acids of varying chain lengths and affect biological functions within the body. G-coupled protein receptor 120 (GPR120; also known as Free Fatty Acid Receptor 4) binds with medium to long-chain (>18C) fatty acids, displaying the highest affinity for EPA and DHA [157]. Stimulation of GPR-120 has anti-inflammatory effects and has been proposed to have therapeutic potential in the treatment of T2D [158]. GPR-

120 is readily expressed in AT, with smaller amounts found in intestine, pancreas, spleen and macrophage. In intestine GPR120 acts as a 'fat sensor' promoting secretion of glucagon-like peptide-1, an incretin hormone that enhances glucose-dependent secretion of insulin from the pancreatic beta-cells [159]. In AT GPR120 is expressed primarily within the adipocyte, with smaller amounts found in the stromavascular network [157]. Stimulation of GPR120 in T3T-L1 adipocytes blocks the nuclear translocation of the nuclear-factor kappa p65 subunit, attenuating the lipopolysaccharide-induced production of IL-6 [160], and EPA ameliorates palmitate-induced inflammation in adipocytes, an effect abolished by silencing of the GPR-120 gene [161]. DHA has also been shown pre-clinically to inhibit activation of the NLRP3 inflammasome, which was partially inhibited by blocking the GPR-120 receptor [162].

GPR120 has also been proposed to play a significant role in adipogenesis in AT, with some evidence that stimulation of GPR-120 promotes maturation of pre-adipocytes, as well as increasing release of VEGF, decreasing inflammation and increasing insulin sensitivity [163]. Further, there is some evidence that DHA stimulates glut-4 translocation to the plasma membrane in skeletal muscle via a GPR120 dependent mechanism, however this has only been demonstrated *in vitro* [164]. There is some limited evidence from animal models that LCn-3PUFAs may promote the metabolism of brown AT and 'beiging' of white AT [165], though to date this hasn't been shown in humans and the clinical application is questionable, given the small amount of brown AT present in human adults.

PPAR-γ

EPA and DHA both act as natural ligands for peroxisome proliferator-activated receptor gamma (PPAR-γ). Activation of PPAR-γ increases transcription of adiponectin [166]. Adiponectin was correlated with erythrocyte membrane phospholipid EPA and DHA content in healthy and glucose intolerant individuals [167]. Four weeks of a DHA-enriched canola oil (delivering 1.1g/day of DHA) in adults with abdominal obesity showed a modest but significant increase in adiponectin compared with control (corn/safflower oil blend) [168] group. Consistent with this, a recent meta-analysis including 14 RCTs and 1323 participants also reported a modest increase in adiponectin in response to LCn-3PUFA intervention. However, significant heterogeneity was present which was not explained by differences in dose, duration, or delivery of EPA/DHA. Whilst *in vitro* studies find EPA and DHA both promote transcription of adiponectin [169, 170], one clinical study found DHA was more effective than EPA at increasing adiponectin in men and women at risk of CVD [171]. However six months of n-3PUFA

supplementation (320mg EPA + 200mg DHA/day) in adults with T2D failed to show any effect on adiponectin levels [172]. Further research is required to elaborate on whether DHA or EPA is the more effective n-3PUFA for modulation of adiponectin levels, and whether disease state (e.g. T2D) affects this process.

Increased Angiogenesis

In addition to their potential role in the resolution of adipose tissue AT inflammation, LCn-3PUFA may induce angiogenesis in AT, thus improving vascularisation and promoting healthy AT function. Fish oil supplementation increased the number of capillaries in adipocytes in obese IR individuals [173], and EPA increases VEGF in mature adipocytes *in vitro*, via both GRP-120 and PPAR-γ mediated pathways [145]. Whether DHA affects AT angiogenesis is unknown. This effect appears to be limited to AT, as EPA and DHA suppress VEGF in microvascular endothelial cells [174].

LCn-3PUFA and Metabolic Disorders

Despite strong and convincing evidence from pre-clinical and in-vitro studies that LCn-3 PUFA have insulin-sensitising effects, evidence that LCn-3PUFA can prevent T2D development remains limited. Amongst countries with a higher prevalence of obesity, the countries with a higher intake of fish and seafood have a lower prevalence of T2D [175], and some longitudinal studies have found that a higher plasma or erythrocyte LCn-3PUFA levels are associated with a lower incidence of T2D over ~11 years follow-up [176, 177]. However, no relationship was seen between either EPA, DHA or the O3I and incident T2D over 11 years follow up in 6379 post-menopausal women, with the exception of an inverse relationship noted between O3I and incident T2D in women under 70 years of age [178]. Multiple systematic reviews have resulted in equivocal findings. There appears to be a geographical variation to the relationship between LCn-3PUFA intake and T2D, with an inverse association found in Asian and Australasian countries, no relationship found in European countries, and a direct relationship in studies out of America [179]. Reasons for this variation are unknown, but may be due to differences in the consumption patterns or cooking methods used, or genetic variation or background diet. Randomized controlled trials have also failed to demonstrate a clear benefit of LCn-3PUFA on measures of IR and/or insulin sensitivity, either in those with [180] or without [181] T2D, though the latter did note a significant improvement in HOMA-IR.

It isn't clear why the strong mechanistic and evidence gathered from rodent models has not translated well into clinical end-point studies. There are obvious differences between human

and rodent metabolism, however *ex vivo* studies using human adipocytes and/or muscle biopsies also provide supporting evidence for beneficial effects in humans. Many of the animal studies find fish oil fed during development protects against high-fat diet induced metabolic dysfunction, whereas many of the human studies look to rescue established IR in adults. Furthermore, given that humans only turn over 10% of adipocytes each year, the capacity for incorporation of EPA and DHA into adult AT may be limited. However, supplementation studies have shown that EPA and DHA do become incorporated into adipocytes in a dose-dependent manner and AT has a good correlation with medium-to-long term LCn-3PUFA intake [182] in adults, suggesting that there is a good level of conservation of LCn-3PUFA. There is some evidence, however, that the age of adipocytes could impact on results, as *in vitro* evidence has shown EPA and DHA to reduce inflammation and increase transcription in young T3T-L1 adipocytes to a significantly greater effect than in aged adipocytes [183]. Taken together, these suggest that the greater potential for LCn-3PUFAs is in the prevention of AT inflammation, rather than the restoration of insulin sensitivity, particularly once IR and/or hyperglycaemia are present.

Dose, duration, and mix of EPA vs DHA could also be a confounding factor affecting outcomes. Given that it takes around 4-6 weeks of supplementation before the levels begin to increase in AT [184], studies would need at least this duration in order to detect an effect. There also exists the possibility that EPA and DHA have differential effects on glucose metabolism, however most *in vivo* studies compare a combination of these two fatty acids, and few have done a direct comparison. Alliare et al [171] found that DHA increased adiponectin compared with EPA, however did not note any significant differences for CRP, IL-6 or TNF-α. Baseline inflammatory status could also potentially confound results. A RCT stratified participants by baseline inflammation status, and found that DHA reduced the area-under-the-curve for insulin, but only in the subgroup with the raised inflammation status at baseline [185].

Our research group has recently reported a significant inverse association between O3I and T2D in overweight older females which was not apparent amongst overweight males [186], and hypothesised that there is a sex-dependent response to omega-3 fatty acids. An inverse relationship has also been noted in females between O3I and body weight [183, 187] and NAFLD [83]. There are also sex-dependent differences in response to LCn-3PUFA supplementation in terms of muscle response to exercise training [188] and platelet aggregation [189]. Further to this, we conducted a systematic review and meta-analysis of RCTs which provided support for a sex-dependent effect, with studies longer that 8 weeks showing an improvement in IR in

females [186], an effect which was not evident in males. Sex-dependent differences in LCn-3PUFA metabolism are well known, with females displaying a higher metabolism from ALA to EPA and particularly DHA than age-matched males [190]. In addition, premenopausal women are somewhat protected against development of IR and T2D compared with men of similar age. Whilst this has been largely attributed to the protection by oestrogens, the precise mechanism of how oestrogens protect from diabetes is largely unknown. Females have been shown to have an enhanced resolution of inflammation compared with males, mediated in particular by an increased level of D-series resolvins [191], which could explain the discrepancy between the sexes. Taken together, these findings suggest that the SPMs derived from DHA in AT may be a contributing factor to the protection that pre-menopausal women have from the development of IR. Furthermore, a sex-specific benefit of LCn-3PUFA on IR e in women might indicate a biological purpose relevant to pregnancy, a condition characterised by increased DHA requirements, AT growth and progressive IR. Indeed, pregnancies complicated by gestational diabetes show a decline in the DHA content of erythrocyte membranes [192] which can be ameliorated with DHA supplementation [193]. More research is required in this area to determine whether a low level of DHA pre-pregnancy is associated with an increased risk of developing gestational diabetes.

LCn-3PUFA and systemic inflammation

Persistent inflammation in an uncontrolled manner for prolonged periods in tissue can cause excessive damage, that is often involved in the pathogenesis of chronic inflammatory diseases. Usually inflammation is confined to a tissue or local site, but depending on severity and magnitude of response in these uncontrolled conditions, it spreads to other tissues in the periphery. Low grade chronic inflammatory responses are implicated in the pathobiology of diabetes mellitus, atherosclerosis, neurodegenerative diseases and tumor growth [194]; whereas high grade chronic inflammatory responses involving hyper expression of pro-inflammatory cytokines is implicated in the development of autoimmune diseases, rheumatoid arthritis, sepsis, cancer [195].

Eicosanoid production

Prostaglandins (PGs), Thromboxane (TXs) and Leukotrienes (LTs) collectively termed as eicosanoids are one of the key regulators of inflammation, primarily involved in modulating intensity and duration of inflammation in tissues [196]. These are produced from 20 carbon chain PUFAs, mainly ARA and EPA. Since the inflammatory cells predominantly contain higher proportions of ARA, it serves as a major substrate for eicosanoids [197]. ARA is metabolised in

the presence of COX to produce a series of PGs and TXs (2 series). The other metabolic pathway of ARA is catalysed by LOX pathway that gives rise to hydroxy, hydroperoxy derivatives and LTs (4 series) [196]. Antagonising the metabolic pathways of ARA by LCn-3PUFA is regarded as one of the primary anti-inflammatory mechanism [198]. Increased intake of either EPA or DHA reduces the availability of ARA through competitive substrate binding in the phospholipids of cell membranes that are involved in the process of inflammation [199]. This is substantiated from the animals fed diets rich in LCn-3PUFA that demonstrated the reduction in ARA derived PGE_2, LTB_4, and pro-inflammatory cytokines expression [199, 200]; which is consistent with the results obtained from clinical trials with supplementation of LCn-3PUFA. However, this physiological activity involving modulation of inflammation by LCn-3PUFA is dose-dependent [201]; suggesting a minimum dose of 1.3g of EPA per day [202]. EPA can also act as substrate for COX and LOX enzymes, but the metabolites such as LTB_5 and LTE_5 are 10 to 100-fold less potent chemotactic agents, thus reducing the production of highly potential pro-inflammatory mediators of ARA [203].

Resolution of inflammation – specialised pro-resolution lipid mediators (SPM)

Although it is well known that inflammation is the primary defence mechanism for host tissue repair in response to injury or infection, several research reports reveal prolonged activation or failure in resolution as a central pathogenic component in several diseases [195]. For several years, the treatment of chronic inflammation is aimed at reducing the pro-inflammatory cytokine expressions (infliximab, adalimumab), antagonising enzymes that are involved in the pro-inflammatory process (nonsteroidal anti-inflammatory drugs that block COX enzymes), antihistamines to reduce the allergic inflammatory responses and glucocorticoids to supress the pro-inflammatory gene expression. These are effective in the short term, and are often used for long-term chronic inflammatory disease management [204, 205].

In addition to the well-established anti-inflammatory effect of LCn-3PUFAs, unbiased lipidomic research on exudates of resolution of acute inflammation identified a set of LCn-3PUFA derived SPMs [206]. These are with distinct chemical structures, termed as resolvins from EPA (E-series) and DHA derived resolvins (D-series), protectins and maresins [206]. The synthesis of these SPMs involves COX and LOX pathways, with different stereospecific epimers produced in cells with/without aspirin administration [207]. E-series resolvins (E1 and E2) are produced in healthy individuals or in those supplementated with EPA. Oxygenation of EPA mediated by a cytochrome P450 pathway generates 18R-(hydroperoxy) eicosapentaenoic acid (18R-HpEPE) which is reduced via a peroxidase to unstable 18R-HEPE. A second

lipoxygenation catalyzed by 5-LOX forms hydroperoxide, which is then transformed to epoxide that undergoes enzymatic hydrolysis to produce RvE1 and RvE2 [208]. RvE1 acts as a resolution agonist through blocking neutrophil transendothelial migration, thereby reducing the inflammation [209]. RvE2 demonstrated potent anti-inflammatory effects by reducing the zymosan-induced neutrophil infiltration [210]. Release of 5-LOX in leukocytes that are involved in inflammation is pivotal for these beneficial effects of resolvins. Two G-protein coupled receptors, Chem23 and leukotriene B_4 receptor (BLT1) were identified on cell types that are involved in the anti-inflammatory and pro-resolution activities of these resolvins [211, 212]. Binding of RvE1 to Chem23 on monocytes and dendritic cells leads to downregulation of TNF-stimulated NF-κB activation, TNF being a key regulator of acute inflammation [212]. Activation of BLT1 on neutrophils leads to attenuation of pro-inflammatory signals that are linked with leukotriene-B4 [211]. Administration of RvE1 and RvE2 demonstrated equipotent pro-resolution effects such as reduction in neutrophil infiltration in murine models of acute inflammation at dose level of 100ng per mouse [210]. This provides evidence at the molecular level for the effects of LCn-3PUFA on chemotaxis of human granulocytes, which is observed in several LCn-3PUFA supplementation studies [213, 214]. However, discrepancies exist between the study reports regarding the dose levels due to the differences between the EPA/DHA composition variance in different formulations.

In addition to EPA, DHA can also serve as a substrate for two bio-active SPMs termed as 17S and 17R D-series resolvins [214]. The anti-inflammatory activities of these resolvins are of particularly interest in tissues such as the brain, synapses and retina that are enriched with DHA. These compounds exhibited potent anti-inflammatory activity by limiting transendothelial migration of neutrophils [215]. Additionally, macrophages incubated with RvD2 showed enhanced phagocytosis of both apoptotic neutrophils and endocytosis [215]. Overall, resolvins through inhibition of neutrophil infiltration, reduction in defesin (neutrophil secretion peptide) release, decrease adhesion receptor surface and by blocking neutrophil transepithelial migration displayed potent pro-resolution activity. DHA also produces another set of SPMs named protectins. These are distinctly different from the other set by having a conjugated triene double bond and biological activity. Protectin D1 (also termed as neuroprotection D1 depending on biological origin) is produced by peripheral mononuclear cells in LOX dependent manner via 16(17)-epoxide intermediate [216]. It exhibits potent anti-inflammatory activity through attenuation of NF-κB and COX-2 expression, reduction in T-cell migration, promotion of T-cell apoptosis, increase in expression of CCR5 and decreased toll like receptor mediated macrophage activation [217, 218]. Given the fact that enrichment of tissues with EPA and DHA

(through supplementation or diet) increase these resolvins concentration [219], these molecular effects substantiate evidence for beneficial effects of LCn-3PUFA.

Transcription factors

There are several transcription factors that are involved in the initiation and inhibition of inflammation. Among these factors NF-κB is a key transcription factors that induces the expression of several cytokines, enzymes (COX) and adhesion molecules that are implicated in the process of inflammation [220, 221]. Endotoxins such as lipopolysaccharides or external inflammatory stimuli triggers the activation of this transcription factor through toll like receptor (TLR4) [221]. LCn-3PUFA supplementation has been shown to decrease the activation of NF-κB in human monocytes by reducing IκB phosphorylation [222]. In vitro cell culture studies reported reductions in the activation of this transcription factor on incubating macrophages, dendritic cells and monocytes with both EPA and DHA [223]. Another possible mechanism for effects of LCn-3PUFA on NF-κB is PPAR mediated inhibition of NFκB DNA binding activity [224]. LCn-3PUFA has been shown to activate and increase the expression of PPARγ in the endotoxin stimulated dendritic cells which is closely associated with the reduction of NFκB and pro-inflammatory cytokines, suggesting a potential anti-inflammatory mechanism of LCn-3PUFA [225]. Recent research on GPCRs provided another plausible explanation for LCn-3PUFA mediated modulation of NFκB. LCn-3PUFA, particularly DHA, has been recently identified as a GPR120 agonist that is involved in the anti-inflammatory signalling pathways. This provides deeper insights into cellular level receptor based pro-inflammatory inhibition effects of LCn-3PUFA [226].

Cytokines

Cytokines are small protein molecules that are released from cells such as monocytes, macrophages and adipocytes that are primarily involved in inflammation. These protein molecules such as TNF-α, IL-6 and IL-1β are involved in the pathogenesis of many inflammatory driven diseases [227]. Although the key mechanism of LCn-3PUFA is replacement of AA through substrate competition, several cell culture studies and clinical studies reported beneficial effect of LCn-3PUFA supplementation on cytokine production [202]. Animals fed with diets enriched with LCn-3PUFA demonstrated decreased production of pro-inflammatory cytokines from the macrophages as well as the circulation levels in endotoxin induced inflammation models [228]. Incubation of human peripheral endothelial cells with either EPA or DHA in *in-vitro* studies have shown to inhibit the release of TNF-α and other pro-inflammatory interleukins [229, 230]. Supplementation of LCn-3PUFA in the form of fish oil has shown to reduce the systemic circulation levels of pro-inflammatory cytokines such as TNF-α (20-35%↓) and

IL-6 (35%↓) [230-233] in healthy as well patients with inflammatory and metabolic diseases [234]. However, discrepancies exist regarding the effects of LCn-3PUFA on CRP, with few studies showing negative and few positive effects, that are summarised in previously published systematic reviews and meta-analyses [235-237]. These discrepancies are attributed to the different dose levels, duration and variation in EPA/DHA ratio of fish oil formulations. Overall observations from the RCTs conclude that supplementation of LCn-3PUFA above 2g/day for more than 12 weeks in both patients and healthy people may be of clinical benefit on inflammatory outcomes.

LCn-3PUFA, endothelial function and blood pressure:

Endothelium is an important regulator of the vascular homeostasis, being not only a barrier but also a key signal transducer. It controls the vascular tone by releasing vasoactive molecules that trigger vasoconstriction and relaxation. Vessel relaxation is controlled via generation of nitric oxide (NO), which diffuses to the vascular smooth muscle cells signalling vasodilation. NO is produced from L-arginine by the action of endothelial nitric oxide synthase (eNOS), which is activated by shear stress or signalling molecules, such as adenosine, bradykinin, VEGF and serotonin [238]. Vasodilation can also be triggered by endothelium derived hyperpolarising factors, which depolarise vascular smooth muscle cells by increasing potassium conductance [238, 239]. Alternatively, vasoconstriction is regulated via the production of endothelin and vasoconstrictor prostanoids, and via the conversion of angiotensin I to angiotensin II. The motions of the vessels in turn regulate the supply of oxygen to the tissue, changes in vascular structure, metabolic demand and organ perfusion [238].

Damage to the endothelial wall causes endothelial dysfunction, characterised by increased expression of inflammatory cytokines and adhesion molecules. E-selectin is the adhesion molecule most specific to endothelial dysfunction, being positively associated with atherosclerosis and CVD risk factors. However, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and P-selectin levels are also associated with endothelial dysfunction. Damage to the endothelial wall can also be determined by an increase in the circulating levels of asymmetric dimethylarginine, an inhibitor of eNOS, indicating a reduction in nitric oxide production, by a change in the balance between plasminogen activator and plasminogen activator inhibitor 1 or by the presence of mature endothelial cells and endothelial progenitor cells in the circulation [238].

Dietary LCn-3PUFA incorporated into the phospholipids of the endothelial cell membrane, modulate cell membrane fluidity and composition. The endothelial cell membrane contains

receptors and signalling molecules responsible for regulating important pathways in the cell's function [240], thus incorporation of LCn-3PUFA to the endothelial cell membrane can also affect the transcription of bioactive molecules and the functionality of membrane receptors.

In vitro studies have assessed the effects of LCn-3PUFA on endothelial function and demonstrated that LCn-3PUFA stimulates NO production by inducing eNOS expression and activation, and by reducing the levels of asymmetric dimethylarginine, an inhibitor of eNOS [240, 241]. EPA has been shown to activate eNOS by inducing its dissociation from caveolin, an eNOS activity regulator, and its translocation from the cell membrane to the cytosol [242]. DHA has been shown to modulate membrane composition and to displace eNOS and caveolin-1 from the caveolae fraction, activating eNOS. Caveolae is a signalling microdomain in the cell membrane [243].

Human studies using flow mediated dilatation (FMD), a direct measure of NO bioavailability, as end point have reported either no association of LCn-3PUFA intake with improvement in FMD or a small improvement [241, 244, 245]. However, those studies vary in LCn-3PUFA dose (0.45 to 4.7g/day) and form (ALA, EPA, DHA or EPA and DHA combined), population studied (age, sex, health status, genetic background), sample size (18 to 310), study duration (2 to 52 weeks), occlusion position for FMD and study design. Furthermore, these studies do not consider the effect of the subject's background diet, as highlighted by Wang and colleagues [244]. According to Wang et al [244], LCn-3PUFA improves FMD in subjects with poor cardiovascular health, but not in healthy subjects. Indeed, a more recent study has demonstrated an improvement in FMD of subjects with metabolic syndrome after 2g LCn-3PUFA (46% EPA: 36% DHA) daily supplementation for 12 weeks [246].

LCn-3PUFA supplementation has also been associated with an improvement in the circulating levels of adhesion molecules. A meta-analysis of RCTs has identified that LCn-3PUFA supplementation reduces soluble ICAM-1 (sICAM-1) in healthy subjects and in subjects with dyslipidaemia, despite no effect to soluble VCAM-1 (sVCAM-1), soluble P-selectin (sP-selectin) and soluble E-selectin (sE-selectin) [247]. Study assessing changes in dietary LCn-3PUFA of hyperlipidaemic, hypertensive and diabetic patients demonstrated that an increase in LCn-3PUFA over a one year period decreased sICAM and sVCAM, and was associated with an improvement in peripheral small artery endothelial function [248]. Another study demonstrated that the effect of LCn-3PUFA on adhesion molecules is dose and sex dependent. After consuming 6.6g of EPA+DHA for 12 weeks, heathy males and females had a decrease in sP-selectin and only females had a decrease in sVCAM-1, while change in sICAM-1 was not significant [249]. Differences in adhesion molecules' outcomes may be due to the variability in

study design and duration, population, dose and form of LCn-3PUFA consumed. Furthermore, Eschen et al [249] findings suggest that relatively high doses of LCn-3PUFA may be necessary to cause a decrease in sVCAM-1 and sP-selectin. It has also been suggested that LCn-3PUFA may reduce oxidative stress. Despite controversies on the pro-oxidative activity of LCn-3PUFA, in vitro studies have demonstrated that LCn-3PUFA reduces the production of reactive oxygen species by modulating inducible nitric oxide synthase and NADPH oxidase [240]. An animal study has demonstrated that diets enriched with linolenic acid resulted in lower oxidation of LDL and NADPH compared to diets richer in n-6PUFA [250]. In another study LCn-3PUFA supplementation mitigated oxidative stress in menopause induced animals by down-regulating NADPH oxidase activity [251].

The LCn-3PUFA effect on endothelial dysfunction seems to be dependent on LCn-3PUFA dose and form, intervention design and length, and population characteristics. Thus, different studies fail to agree on the effects LCn-3PUFA to endothelial dysfunction. However, evidence suggests that LCn-3PUFA can improve endothelial function via modulating endothelial cell fluidity and composition, improving relaxation and constriction of the vessels and inhibiting the secretion of adhesion molecules and inflammatory cytokines by the endothelial tissue.

The beneficial effects of LCn-3PUFA on endothelial function and inflammation provides the primary basis for these studies to evaluate its effects on blood pressure (BP). The effects of LCn-3PUFA on blood pressure (BP) were extensively evaluated in both observational and interventional studies [252]. Observational studies have shown an inverse correlation between erythrocyte membrane content of LCn-3PUFA and 24 h, day and night time BP [253]. Regression analysis indicated a decrease in 4.4 mm Hg of mean arterial pressure with an increase in plasma EPA by 69.6 mg per litre [252]. LCn-3PUFA supplemented in form of fish oils have shown to lower the BP in normotensive [254] and hypertensive subjects [255], but not in all the intervention trials [256]. Meta-analyses indicated LCn-3PUFA supplementation resulted in small but significant (2.1-5.5 mm Hg for systolic BP, and 1.5-3.5 mm Hg for diastolic BP) decrease in blood pressure only in hypertensive subjects [257] [258]. This blood pressure effect of LCn-3PUFA was not observed in the normotensive subjects [259]. Recently published meta-analyses considering both dietary sources and supplements has shown a significant decrease in both hypertensive and normotensive subjects [260]. The beneficial effects of LCn-3PUFA on blood pressure were observed at an average dose, 3.8g/day with average supplementation duration of 3 months [260]. However, these meta-analyses concluded the effects of LCn-3PUFA on BP were not dose dependent. Though there is an ambiguity in these dose dependent blood pressure

lowering effects of LCn-3PUFA, available evidence strongly suggest that increase in plasma and erythrocyte content of LCn-3PUFA could lower the BP to a clinically significant extent.

LCn-3PUFA and Platelet Aggregation

Increased platelet activity has been shown to predict a prothrombotic state and is a major risk factor for the development of heart attacks, strokes, and venous thromboembolism [261]. Platelet activation and aggregation play an important role in determining a prothrombotic state. Although pharmaceutical agents such as aspirin, heparin, and warfarin can reduce prothrombotic tendency, long-term drug treatment may produce a variety of serious side effects, including bleeding. Diet is generally recognized to be significantly involved in modifying the individual risk for the development of thrombotic diseases, although its influence during the treatment of these disorders is probably less important. The most common method of measuring platelet aggregation involves in vitro tests of blood (platelet rich plasma fraction or whole blood) samples. Aggregating agents such as ARA, adenosine diphosphate (ADP) and collagen are added to the blood samples, or spontaneously occurring aggregation is measured. The resulting platelet aggregation is used as a measurement of the potential for platelets to aggregate in the human body [262].

Dietary supplementation with LCn-3PUFAs, EPA and DHA has been shown to inhibit platelet aggregation, however, the published literature has yielded equivocal results. A recent meta-analysis demonstrated that LCn-3PUFA supplementation is associated with a significant reduction in platelet aggregation in response to various agonists when the participants were at poor health status, but not in healthy individuals [263]. High-risk patients with CVD and even diabetics may potentially benefit from LCn-3PUFA therapy but it may not be effective in primary prevention [263]. Agren et al. compared 3 sources of EPA and/or DHA [264]. Collagen aggregation was reduced in subjects on both fish oil supplementation and fish diet, but not in those consuming pure DHA oil [264]. From this, they concluded that while LCn-3PUFA impair platelet aggregation, DHA is less potent than fish oil or dietary fish at moderate doses [264]. The GISSI prevention study provides compelling evidence that cardioprotective effects of LCn-3PUFA are independent of the standard cardiovascular pharmacotherapy, including statins and aspirin [265]. The data from randomized placebo controlled studies suggested that the addition of 1-2 g of prescription LCn-3PUFAs to conventional statins and aspirin in patients with

documented coronary artery disease and high TG significantly reduced ADP-induced aggregation [266].

The mechanism by which supplementation with LCn-3PUFA decreases platelet aggregation remains unknown. Previous studies have suggested that LCn-3PUFA incorporate into platelet membrane phospholipids, leading to a concomitant reduction of n-6PUFA (including ARA), along with an increase in EPA. EPA can compete with ARA and inhibit the COX-1 pathway. The mechanism by which supplementation with LCn-3PUFA decreases platelet aggregation, has also been attributed to a decrease in thromboxane A2 (a potent pro-aggregatory eicosanoid) with a concomitant increase in prostaglandins and thromboxane of 3-series [267]. It has also been suggested that LCn-3PUFA could reduce the aggregation of platelets by increasing the synthesis of nitric oxide in endothelial cells [268].

The effects of fish oil on ex vivo platelet aggregation have been inconsistent and remains controversial, confounded by differences in the proportion of the various LCn-3PUFA (EPA versus DPA or DHA) in the supplements, dose, and duration of intervention, selection criteria for participants (healthy, non-obese/obese individuals and younger/elderly individuals, hypercholesterolemic subjects, diabetic or CVD patients, smoking status and alcohol consumption), methodology (platelet rich plasma or whole blood) and agonists used to assess platelet aggregation. Recognizing that male and female sex hormones have different effects on platelet function, sex differences in response to LCn-3PUFA supplementation have also been reported. Carefully planned RCTs involving LCn-3PUFA supplementation keeping all covariates in consideration are warranted to delineate the exact role of LCn-3PUFA in prevention of heart attacks, stroke and deep vein thrombosis.

LCn-3PUFA and Cardiac Arrhythmias:

Despite a fall in mortality from coronary heart disease, sudden cardiac deaths associated with fatal arrhythmia remains the cause of most deaths. Mortality statistics from several countries indicate that up to 80% of sudden deaths are due to ventricular fibrillation. Cardiac arrhythmia suppression trial failed to show any significant decrease in mortality due to coronary artery disease when antiarrhythmic drugs were administered. As a result of ischemia, the electrical properties of the myocytes change, leading to arrhythmias. Ventricular fibrillation, the most common fatal arrhythmia occurs when electrical impulses from damaged cardiac muscle results in breakdown of the normal synchronicity of heart contractions. Cardiac arrhythmia occurs during the early and potentially reversible phase of ischemia and after reperfusion [269].

In the Diet and Reinfarction Trial, consumption of about 300g fatty fish per week (corresponding to 2.5g of EPA) was found to be successful in reducing mortality by 29% in men during the first two years following myocardial infarct [270]. Another study found that compared to no intake, dietary EPA and DHA (equivalent to one fatty fish meal per week) was associated with a 50% reduction in the risk of primary cardiac arrest [271]. The Lyon Diet Heart Study reported that patients assigned to the Mediterranean diet rich in ALA had a reduced rate of recurrence of cardiac events [272]. The antiarrhythmic effect of LCn-3PUFA in humans has been confirmed by another recent study [273]. Another placebo-controlled, double blind study showed that ventricular premature complexes decreased by 48% in the fish oil group and by 25% in the placebo group. The antiarrhythmic properties of LCn-3PUFA have been studied in vivo by measuring the ventricular fibrillation threshold (VFT) of animals whose diets were supplemented with fish oils. VFT is the amount of current required to induce VF during myocardial ischemia. The antiarrhythmic actions of LCn-3PUFA have also been studied in vitro using cultured neonatal cardiac myocytes. Modification of the fatty acid composition of membrane phospholipids and formation of eicosanoids by dietary LCn-3PUFA, direct effect of NEFAs on the myocardium, effects of LCn-3PUFA on the inositol lipid cycle and cell signalling and on Ca^{2+} channels are some of the suggested mechanisms for antiarrhythmic potential of LCn-3PUFA [274].

The GISSI Prevenzione trial involving 11,324 patients showed that LCn-3PUFA supplementation resulted in a significant reduction in deaths from cardiovascular causes over 3.5 years of follow-up driven by a reduction in sudden cardiac death [275]. However, three randomised controlled trials involving patients with implantable cardiac defibrillators failed to confirm effects of fish oil in the prevention of sudden cardiac death [276,277, 278]. In a systematic review and meta-analysis including more than 30,000 patients, LCn-3PUFA supplementation was associated with a significant reduction in cardiac deaths but showed no effect on arrhythmias [279]. Ongoing trials and future studies with LCn-3PUFA might help to clarify whether the reduction in deaths from cardiac causes results from a reduction of arrhythmias or from a delay in the progression of coronary artery disease.

Conclusion

Scientific studies published in the last 4 decades have established that LCn-3PUFA favourably modulate risk factors for chronic diseases. LCn-3PUFA reduce blood lipid levels, promote anti-atherogenic lipoprotein profile, reduce inflammation, lower BP, promote resolution of

inflammation, improve endothelial function, reduce platelet aggregation and reduce the risk of fatal arrhythmias **(Figure 2)**. LCn-3PUFA also improve AT function by promoting angiogenesis and enhancing secretion of healthy peptides (cytokines, hormones and adipokines etc). Epidemiological studies examining the relationship between LCn-3PUFA intake and/or blood levels of LCn-3PUFA also support their role in promoting health benefits. However, randomised controlled trials and systematic reviews have failed to provide unequivocal evidence for the role of LCn-3PUFA in prevention of cardio-metabolic diseases. It is clearly evident from this review that dose, duration, and background diet composition may play an important role in determination of the health benefits of LCn-3PUFA. Fish oils are not just fish oils and EPA/DHA ratio of the supplement is an important determinant of the health benefits of LCn-3PUFA. LCn-3PUFA appears to affect the risk factors differentially in men and women. Additionally, there is evidence of gene polymorphism in determination of the health benefits of LCn-3PUFA. Long-term, dose dependent, randomised clinical trials stratified by sex, using defined EPA/DHA ratio and considering gene polymorphism as a confounding factor are warranted to resolve the existing controversies. Practicality of such trials involving large number of participants and therefore requiring access to sizeable funding commitments, although not out of question, may not be within easy reach.

Conflict of interest

There are no conflicts to declare.

References:

1. R. Chowdhury, S. Warnakula, S. Kunutsor and et al., *Annals of Internal Medicine*, 2014, **160**, 398-406.
2. C. Wang, W. S. Harris, M. Chung, A. H. Lichtenstein, E. M. Balk, B. Kupelnick, H. S. Jordan and J. Lau, *The American journal of clinical nutrition*, 2006, **84**, 5-17.
3. P. E. Marik and J. Varon, *Clinical cardiology*, 2009, **32**, 365-372.
4. E. C. Rizos, E. E. Ntzani, E. Bika, M. S. Kostapanos and M. S. Elisaf, *Jama*, 2012, **308**, 1024-1033.
5. Y. T. Wen, J. H. Dai and Q. Gao, *Nutrition, metabolism, and cardiovascular diseases : NMCD*, 2014, **24**, 470-475.
6. M. Casula, D. Soranna, A. L. Catapano and G. Corrao, *Atherosclerosis. Supplements*, 2013, **14**, 243-251.
7. H. C. Bucher, P. Hengstler, C. Schindler and G. Meier, *The American journal of medicine*, 2002, **112**, 298-304.
8. T. Aung, J. Halsey, D. Kromhout, H. C. Gerstein, R. Marchioli, L. Tavazzi, J. M. Geleijnse, B. Rauch, A. Ness, P. Galan, E. Y. Chew, J. Bosch, R. Collins, S. Lewington, J. Armitage and R. Clarke, *JAMA cardiology*, 2018, **3**, 225-234.
9. D. S. Siscovick, T. A. Barringer, A. M. Fretts, J. H. Wu, A. H. Lichtenstein, R. B. Costello, P. M. Kris-Etherton, T. A. Jacobson, M. B. Engler, H. M. Alger, L. J. Appel and D. Mozaffarian, *Circulation*, 2017, **135**, e867-e884.
10. M. L. Fernandez and K. L. West, *J. Nutr.*, 2005, **135**, 2075-2078.
11. A. Pirillo and A. L. Catapano, *Atheroscler. Suppl.*, 2013, **14**, 237-242.
12. K. Musa-Veloso, M. A. Binns, A. C. Kocenas, T. Poon, J. A. Elliot, H. Rice, H. Oppedal-Olsen, H. Lloyd and S. Lemke, *Nutr. Rev.*, 2010, **68**, 155-167.
13. Y. Park and W. S. Harris, *J. Med. Food.*, 2009, **12**, 803-808.
14. J. C. Lopez-Alvarenga, S. O. E. Ebbesson, L. O. E. Ebbesson, M. E. Tejero, V. S. Voruganti and A. G. Comuzzie, *Metabolism: clinical and experimental*, 2010, **59**, 86-92.
15. G. Pounis, M. de Lorgeril, P. Salen, F. Laporte, V. Krogh, A. Siani, J. Arnout, F. P. Cappuccio, M. van Dongen, M. B. Donati, G. de Gaetano and L. Iacoviello, *Nutrition, metabolism, and cardiovascular diseases : NMCD*, 2014, **24**, 883-890.
16. I. J. Hansen-Krone, K. F. Enga, J. M. Sudduth-Klinger, E. B. Mathiesen, I. Njolstad, T. Wilsgaard, S. Watkins, S. K. Braekkan and J. B. Hansen, *The Journal of nutrition*, 2014, **144**, 861-867.
17. A. C. Skulas-Ray, P. M. Kris-Etherton, W. S. Harris, J. P. Vanden Heuvel, P. R. Wagner and S. G. West, *The American journal of clinical nutrition*, 2011, **93**, 243-252.
18. E. M. Balk, A. H. Lichtenstein, M. Chung, B. Kupelnick, P. Chew and J. Lau, *Atherosclerosis*, 2006, **189**, 19-30.
19. G. C. Shearer, O. V. Savinova and W. S. Harris, *Biochimica et biophysica acta*, 2012, **1821**, 843-851.
20. G. D. Eslick, P. R. Howe, C. Smith, R. Priest and A. Bensoussan, *International journal of cardiology*, 2009, **136**, 4-16.
21. A. Lewis, S. Lookinland, R. L. Beckstrand and M. E. Tiedman, *J. Am. Acad. Nurse. Prac.*, 2004, **16**, 384-395.
22. W. S. Harris, *Am. J. Clin. Nutr.*, 1997, **65**, 164S-154S.
23. W. S. Harris, M. Miller, A. P. Tighe, M. H. Davidson and E. J. Schaefer, *Atherosclerosis*, 2008, **197**, 12-24.
24. D. Weitz, H. Weintraub, E. Fisher and A. Z. Schwartzbard, *Cardiol Rev*, 2010, **18**, 258-263.
25. S. M. Grundy, J. I. Cleeman, S. R. Daniels, K. A. Donato, R. H. Eckel, B. A. Franklin, D. J. Gordon, R. M. Krauss, P. J. Savage, S. C. Smith, Jr., J. A. Spertus, F. Costa, A. American Heart, L. National Heart and I. Blood, *Circulation*, 2005, **112**, 2735-2752.

26. S. Egert, F. Kannenberg, V. Somoza, H. F. Erbersdobler and U. Wahrburg, *J. Nutr.*, 2009, **139**, 861-868.
27. Y. Park and W. S. Harris, *Journal of lipid research*, 2003, **44**, 455-463.
28. T. A. Jacobson, *American Journal of Clinical Nutrition*, 2008, **87**, 1981-1990.
29. L. Bakewell, G. C. Burdge and P. C. Calder, *Br. J. Nutr.*, 2007, **96**, 93.
30. A. P. Kitson, C. K. Stroud and K. D. Stark, *Lipids*, 2010, **45**, 209-224.
31. C. E. Childs, M. Romeu-Nadal, G. C. Burdge and P. C. Calder, *The Proceedings of the Nutrition Society*, 2008, **67**, 19-27.
32. F. L. Crowe, C. M. Skeaff, T. J. Green and A. R. Gray, *The British journal of nutrition*, 2008, **99**, 168-174.
33. S. Lohner, K. Fekete, T. Marosvolgyi and T. Decsi, *Ann. Nuri. Metab.*, 2013, **62**, 98-112.
34. A. Sala-Vila, W. S. Harris, M. Cofan, A. M. Perez-Heras, X. Pinto, R. M. Lamuela-Raventos, M. I. Covas, R. Estruch and E. Ros, *The British journal of nutrition*, 2011, **106**, 425-431.
35. R. C. Block, W. S. Harris and J. V. Pottala, *The open biomarkers journal*, 2008, **1**, 1-6.
36. W. S. Harris, K. J. Reid, S. A. Sands and J. A. Spertus, *The American journal of cardiology*, 2007, **99**, 154-158.
37. J. J. Ferguson, M. Veysey, M. Lucock, S. Niblett, K. King, L. MacDonald-Wicks and M. L. Garg, *J. Nutr. Biochem.*, 2016, **27**, 233-240.
38. M. Y. Wei and T. A. Jacobson, *Current atherosclerosis reports*, 2011, **13**, 474-483.
39. M. Y. Wei and T. A. Jacobson, *Current atherosclerosis reports*, 2011, **13**, 474-483.
40. C. A. Lang and R. A. Davis, *J. Lipid. Res.*, 1990, **31**, 2079-2086.
41. H. Wang, X. Chen and E. A. Fisher, *J. Clin. Invest.*, 1993, **91**, 1380-1389.
42. E. A. Fisher, M. Pan, X. Chen, X. Wu, H. Wang, H. Jamil, J. D. Sparks and K. J. Williams, *J Biol Chem*, 2001, **276**, 27855-27863.
43. M. Larsson, E. Vorrsjo, P. Talmud, A. Lookene and G. Olivecrona, *J Biol Chem*, 2013, **288**, 33997-34008.
44. R. L. Dunbar, S. J. Nicholls, K. C. Maki, E. M. Roth, D. G. Orloff, D. Curcio, J. Johnson, D. Kling and M. H. Davidson, *Lipids. Health. Dis.*, 2015, **14**.
45. M. H. Davidson, K. C. Maki, H. Bays, R. Carter and C. M. Ballantyne, *Journal of clinical lipidology*, 2009, **3**, 332-340.
46. E. Swahn, H. von Schenck and A. G. Olsson, *Clin. Drug. Invest.*, 1998, **15**, 473-482.
47. M. H. Davidson, *Am. J. Cardiol.*, 2006, **98**, 27i-33i.
48. D. S. Kelley, D. Siegel, M. Vemuri and B. E. Mackey, *Am. J. Clin. Nutr.*, 2007, **86**, 324-333.
49. D. C. Chan, G. F. Watts, T. A. Mori, P. H. R. Barrett, T. G. Redgrave and L. J. Beilin, *American Journal of Clinical Nutrition*, 2003, **77**, 300-307.
50. D. C. Chan, G. F. Watts, P. H. Barrett, L. J. Beilim, T. G. Redgrave and T. A. Mori, *Diabetes*, 2002, **51**, 2377-2386.
51. D. C. Chan, G. F. Watts, T. A. Mori, P. H. Barrett, T. G. Redgrave and L. J. Beilim, *Am. J. Clin. Nutr.*, 2003, **77**, 300-307.
52. P. Bordin, O. A. Bodamer, S. Venkatesan, R. M. Gray, P. A. Bannister and D. Halliday, *Eur. J. Clin. Nutr.*, 1998, **52**, 104-109.
53. P. C. Dagnelie, T. Rietveld, G. R. Swart, T. Stijnen and J. W. van den Berg, *Lipids.*, 1994, **29**, 41-45.
54. N. Abate, M. Chandalia, P. G. Snell and S. M. Grundy, *J. Clin. Endocrinol. Metab.*, 2004, **89**, 2750-2755.
55. M. Pavlic, R. Valero, H. Duez, C. Xiao, L. Szeto, B. W. Patterson and G. F. Lewis, *Arterioscler. Thromb. Vasc. Biol.*, 2008, **28**, 1660-1665.
56. X. Zheng, M. Avella and K. M. Botham, *Biochem. J.*, 2001, **357**, 481-487.
57. F. Belzung, T. Raclot and R. Groscolas, *Am. J. Physiol.*, 1993, **264**, R1111-R1118.

58. P. Flachs, O. Horakova, P. Brauner, M. Rossmeisl, P. Pecina, N. Franssen-van Hal, J. Ruzickova, J. Sponarova, Z. Drahota, C. Vlcek, J. Keijer, J. Houstek and J. Kopecky, *Diabetologia.*, 2005, **48**, 2365-2375.
59. T. Arai, H. J. Kim, H. Chiba and A. Matsumoto, *J. Atheroscler. Thromb.*, 2009, **16**, 674-683.
60. M. H. Rokling-Andersen, A. C. Rustan, A. J. Wensaas, O. Kaalhus, H. Wergedahl, T. H. Rost, J. Jensen, B. A. Graff, R. Caesar and C. A. Drevon, *B. J. Nutr.*, 2009, **102**, 995-1006.
61. I. Ikeda, J. Kumamaru, N. Nakatani, M. Sakono, I. Murota and K. Imaizumi, *J. Nutr.*, 2001, **131**, 1159-1164.
62. G. H. D. A. Alexander Aguilera, M. Lara Barcelata, O. Angulo Guerrero, and R.M. Oliart Ros, *J. Nutr. Biochem.*, 2006, **17**, 760-765.
63. C. Couet, J. Delarue, P. Ritz, J. M. Antoine and F. Lamisse, *Int. J. Obes. Relat. Metab. Disord.*, 1997, **21**, 637-643.
64. T. A. Mori, D. Q. Bao, V. Burke, I. B. Puddey, G. F. Watts and L. J. Beilin, *Am. J. Clin. Nutr.*, 1999, **70**, 817-825.
65. M. Kunesova, R. Braunerova, P. Hlavaty, E. Tvrzicka, B. Stankova, J. Skrha, J. Hilgertova, M. Hill, J. Kopecky, M. Wagenknecht, V. Hainer, M. Matoulek, J. Parizkova, A. Zak and S. Svacina, *Physiol. Res.*, 2006, **55**, 63-72.
66. H. Pritchard, J. J. P. Kastelein, A. Stalenhoef and J. Jonker, *Journal*, 2000, **151**, 118.
67. M. A. Micallef and M. L. Garg, *J. Nutr. Biochem.*, 2009, **20**, 927-939.
68. D. B. Jump, *Curr. Opin. Lipidol.*, 2002, **13**, 155-164.
69. H. E. Bays, A. P. Tighe, R. Sadovsky and M. H. Davidson, *Expet. Rev. Cardiovasc. Ther.*, 2008, **6**, 391-409.
70. Y. Zhang, C. L. Oltman, T. Lu, H. Lee, K. C. Dellsperger and M. VanRollins, *Am. J. Physiol. Heart. Circ. Physiol.*, 2001, **280**, H2430-H2440.
71. Y. Adkins and D. S. Kelley, *The Journal of nutritional biochemistry*, 2010, **21**, 781-792.
72. O. A. Gani and I. Sylte, *J. Mol. Graph. Model.* , 2008, **27**, 217-224.
73. G. Krey, O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M. G. Parker and W. Wahli, *Mol. Endrocrinol.*, 1997, **11**, 779-791.
74. T. Claudel, Y. Inoue, O. Barbier, D. Duran-Sandoval, V. Kosykh, J. Fruchart, J.-C. Fruchart, F. J. Gonzalez and B. Staels, *Gastroenterol.*, 2003, **125**, 544-555.
75. A. Bobik, *Circulation*, 2008, **118**, 702-704.
76. N. Maedan, H. Li, D. Lee, P. Oliver, S. H. Quarfordt and J. Osada, *J. Biol. Chem.*, 1994, **269**, 23610-23616.
77. A. Zhao, J. Yu, J. L. Lew, L. L. Huang, S. D. Wright and J. Cui, *DNA. Cell. Biol.*, 2004, **23**.
78. E. J. Giltay, L. J. Gooren, A. W. Toorians, M. B. Katan and P. L. Zock, *American Journal of Clinical Nutrition*, 2004, **80**, 1167-1174.
79. Y. Zhou, Y. Guo, X. Zhuang and Z. Du, *Mol Med Rep*, 2015, **11**, 2329-2333.
80. H. M. Parker, N. A. Johnson, C. A. Burdon, J. S. Cohn, H. T. O'Connor and J. George, *Journal of hepatology*, 2012, **56**, 944-951.
81. J. D. Browning, L. S. Szczepaniak, R. Dobbins, P. Nuremberg, J. D. Horton, J. C. Cohen, S. M. Grundy and H. H. Hobbs, *Hepatology*, 2004, **40**, 1387-1395.
82. P. Angulo, *Nutr. Rev.*, 2007, **65**, S57-63.
83. M. Rose, M. Veysey, M. Lucock, S. Niblett, K. King, S. Baines and M. L. Garg, *J. Nutr. Intermed. Metab.*, 2016, **5**, 78-85.
84. A. J. Cussons, G. F. Watts, T. A. Mori and B. G. Stuckey, *J. Clin. Endocrinol. Metab.*, 2009, **94**, 3842-3848.
85. V. Nobili, G. Bedogni, A. Alisi, A. Pietrobattista, P. Rise, C. Galli and C. Agostoni, *Arch. Dis. Child.*, 2011, **96**, 350-353.
86. A. Gonzalez-Periz, R. Horrillo, N. Ferre, K. Gronert, B. Dong, E. Moran-Salvador, E. Titos, M. Martinez-Clemente, M. Lopez-Parra, V. Arroyo and J. Claria, *FASEB J.*, 2009, **23**, 1946-1957.

87. I. P. Alwayn, K. Gura, V. Nose, B. Zausche, P. Javid, J. Garza, J. Verbesey, S. Voss, M. Ollero, C. Andersson, B. Bistrian, J. Folkman and M. Puder, *Pediatr. Res.*, 2005, **57**, 445-452.
88. D. B. Jump, C. M. Depner, S. Tripathy and K. A. Lytle, *Adv Nutr*, 2015, **6**, 694-702.
89. H. Cortez-Pinto, L. Jesus, H. Barros, C. Lopes, M. C. Moura and M. E. Camilo, *Clin. Nutr.*, 2006, **25**, 816-823.
90. P. Pettinelli , T. del Pozo, J. Araya, R. Rodrigo, A. V. Araya, G. Smok, A. Csendes, L. Gutierrez, J. Rojas, O. Korn, F. Maluenda, J. C. Diaz, G. Rencoret, I. Braghetto, J. Castillo, J. Poniachik and L. A. Videla, *Biochim. Biophys. Acta–Mol. Basis. Dis.*, 2009, **1792**, 1080-1086.
91. X. Zheng, M. Avella and K. M. Botham, *Biochem. J.*, 2001, **357**, 481-487.
92. T. Ishida, M. Ohta, M. Nakakuki, H. Kami, R. Uchiyama, H. Kawano, T. Notsu, K. Imada and H. Shimano, *Prostaglandins, leukotrienes, and essential fatty acids*, 2013, **88**, 281-288.
93. A. M. Minihane, S. Khan, E. C. Leigh-Firbank, P. Talmud, J. W. Wright, M. C. Murphy, B. A. Griffin and C. M. Williams, *Arteriosclerosis, Thrombosis, and Vascular Biology*, 2000, **20**, 1990-1997.
94. T. A. Mori, V. Burke, I. B. Puddey, G. F. Watts, D. N. O'Neal, J. D. Best and L. J. Beilin, *American Journal of Clinical Nutrition*, 2000, **71**, 1085-1094.
95. E. Dewailly, C. Blanchet, S. Lemieux, L. Sauve, S. Gingras, P. Ayotte and B. J. Holub, *The American journal of clinical nutrition*, 2001, **74**, 464-473.
96. P. Angerer and C. von Schacky, *Current opinion in clinical nutrition and metabolic care*, 2000, **3**, 439-445.
97. C. von Schacky, *The American journal of clinical nutrition*, 2000, **71**, 224s-227s.
98. C. B. Dias, L. G. Wood and M. L. Garg, *European journal of clinical nutrition*, 2016, **70**, 812-818.
99. T. A. Jacobson, S. B. Glickstein, J. D. Rowe and P. N. Soni, *J Clin Lipidol*, 2012, **6**, 5-18.
100. L. Calabresi, D. Donati, F. Pazzucconi, C. R. Sirtori and G. Franceschini, *Atherosclerosis*, 2000, **148**, 387-396.
101. S. E. Nissen, J. Tardif, S. J. Nicholls, J. K. Revkin, C. L. Shear, W. T. Duggan, W. Ruzyllo, W. B. Bachinsky, G. P. Lasala and E. M. Tuzcu, *New. Engl. J. Med.*, 2007, **356**, 1304-1316.
102. C. J. Packard, *Biochem. Soc. Trans.*, 2003, **31**, 1066-1069.
103. M. Rizzo and K. Berneis, *QJM*, 2006, **99**, 1-14.
104. D. M. Jacobs, V. V. Mihaleva, D. B. van Schalkwijk, A. A. de Graaf, J. Vervoort, F. A. van Dorsten, R. T. Ras, I. Demonty, E. A. Trautwein and J. van Duynhoven, *Mol Nutr Food Res*, 2015, **59**, 1745-1757.
105. I. L. Mostad, K. S. Bjerve, S. Lydersen and V. Grill, *Eur. J. Clin. Nutr.*, 2008, **62**, 419-429.
106. M. D. Griffin, T. A. Sanders, I. G. Davies, L. M. Morgan, M. D. J., F. Lewis, S. Slaughter, J. A. Cooper, G. J. Miller and B. A. Griffin, *Am. J. Clin. Nutr.*, 2006, **84**, 1290–1298.
107. S. Khandelwal, I. Demonty, P. Jeemon, R. Lakshmy, R. Mukherjee, R. Gupta, U. Snehi, D. Niveditha, Y. Singh, H. C. van der Knaap, S. J. Passi, D. Prabhakaran and K. S. Reddy, *The British journal of nutrition*, 2009, **102**, 722-732.
108. A. A. Rivellese, A. Maffettone, B. Vessby, M. Uusitupa, K. Hermansen, L. Berglund, A. Louheranta, B. J. Meyer and G. Riccardi, *Atherosclerosis*, 2003, **167**, 149-158.
109. C. B. Dias, N. Amigo, L. G. Wood, R. Mallol, X. Correig and M. L. Garg, *Metabol.*, 2017, **68**, 11-19.
110. T. A. Mori, R. Vandongen, L. J. Beilin, V. Burke, J. Morris and J. Ritchie, *Am. J. Clin. Nutr.*, 1994, **59**, 1060-1068.
111. B. A. Griffin, *Lipids.*, 2001, **36**, S91-S97.
112. E. Olano-Martin, E. Anil, M. J. Caslake, C. J. Packard, D. Bedford, G. Stewart, D. Peiris, C. M. Williams and A. M. Minihane, *Atherosclerosis*, 2010, **209**, 104-110.
113. M. Notarnicola, C. Messa, M. G. Refolo, V. Tutino, A. Miccolis and M. G. Caruso, *Lipids. Health. Dis.*, 2010, **9**, 135.
114. S. Pal, A. M. Thomson, C. D. K. Bottema and P. D. Roach, *J. Nutr. Biochem.*, 2002, **13**, 55-63.

115. S. Yu-Poth, D. Yin, P. M. Kris-Etherton, G. Zhao and T. D. Etherton, *J. Nutr.*, 2005, **135**, 2541-2545.
116. H. B. Sanchez, L. Yeih and T. F. Osbourne, *J. Biol. Chem.*, 1995, **270**, 1161-1169.
117. S. Lindsey, A. Pronczuk and K. C. Hayes, *Journal of lipid research*, 1992, **33**, 647-658.
118. P. P. de Silva, A. Agarwal-Mawal, P. J. Davis and S. K. Cheema, *Nutr Metab (Lond)*, 2005, **2**, 8.
119. P. J. Nestel, *Am. J. Clin. Nutr.*, 2000, **71**, 228-231.
120. R. Hirano, O. Igarashi, K. Kondo, H. Itakura and A. Matsumoto, *Lipids*, 2001, **36**, 401-406.
121. R. H. Mackey, P. Greenland, D. C. Goff, Jr., D. Lloyd-Jones, C. T. Sibley and S. Mora, *J. Am. Coll. Cardiol.*, 2012, **60**, 508-516.
122. J. S. Wooten, K. D. Biggerstaff and V. Ben-Ezra, *J Appl Physiol (1985)*, 2009, **107**, 794-800.
123. J. J. Agren, O. Hanninen, A. Julkunen, L. Fogelholm, H. Vidgren, U. Schwab, O. Pynnönen and M. Uusitupa, *Eur. J. Clin. Nutr.*, 1996, **50**, 765-771.
124. M. Petersen, H. Pedersen, A. Major-Pedersen, T. Jensen and P. Marckmann, *Diab. Care.*, 2002, **25**, 1704-1708.
125. E. Burillo, P. Martin-Fuentes, R. Mateo-Gallego, L. Baila-Rueda, A. Cenarro, E. Ros and F. Civeira, *Curr. Vasc. Pharmacol.*, 2012, **10**, 432-441.
126. J. H. Havel and J. P. King, in *The Metabolic Basis of Inherited Disease*, eds. C. R. Scriver, A. L. Beaudet, V. S. Sly and Valle, McGraw Hill, New York, 6 edn., 1989, pp. 1215-1250.
127. G. Kolovou, D. Damaskos, K. Anagnostopoulou and D. V. Cokkinos, *Ann. Clin. Lab. Sci.*, 2009, **39**, 120-133.
128. R. W. Mahley, S. C. Rall and Jr, *Annu. Rev. Genomics. Hum. Genet.*, 2000, **1**, 507-537.
129. D. M. Hallman, E. Boerwinkle, N. Saha, C. Sandholzer, H. J. Menzel, A. Csazar and G. Utermann, *Am J Hum Genet*, 1991, **49**, 338-349.
130. A. M. Minihane, *Nutrients*, 2016, **8**, 123.
131. S. Liang, L. M. Steffen, B. T. Steffen, W. Guan, N. L. Weir, S. S. Rich, A. Manichaikul, J. D. Vargas and M. Y. Tsai, *Atherosclerosis*, 2013, **228**, 181-187.
132. W. S. Harris, J. V. Pottala, D. L. Thiselton, S. A. Varvel, A. M. Baedke, T. D. Dayspring, G. R. Warnick and J. P. McConnell, *J. Cardiovasc. Trans. Res.*, 2014, **7**, 526-532.
133. World Health Organisation, *Journal*, 2014.
134. G. R. Hajer, T. W. van Haeften and F. L. Visseren, *European heart journal*, 2008, **29**, 2959-2971.
135. E. E. Kershaw and J. S. Flier, *The Journal of clinical endocrinology and metabolism*, 2004, **89**, 2548-2556.
136. J. M. Rutkowski, J. H. Stern and P. E. Scherer, *The Journal of Cell Biology*, 2015, **208**, 501-512.
137. E. D. Rosen and B. M. Spiegelman, *Nature*, 2006, **444**, 847-853.
138. M. Qatanani and M. A. Lazar, *Genes & development*, 2007, **21**, 1443-1455.
139. S. Stojsavljevic, M. Gomercic Palcic, L. Virovic Jukic, L. Smircic Duvnjak and M. Duvnjak, *World J Gastroenterol*, 2014, **20**, 18070-18091.
140. T. Kadowaki, T. Yamauchi, N. Kubota, K. Hara, K. Ueki and K. Tobe, *J Clin Invest*, 2006, **116**, 1784-1792.
141. S. Ahl, M. Guenther, S. Zhao, R. James, J. Marks, A. Szabo and S. Kidambi, *The Journal of clinical endocrinology and metabolism*, 2015, **100**, 4172-4180.
142. C. A. Aguilar-Salinas, E. G. Garcia, L. Robles, D. Riano, D. G. Ruiz-Gomez, A. C. Garcia-Ulloa, M. A. Melgarejo, M. Zamora, L. E. Guillen-Pineda, R. Mehta, S. Canizales-Quinteros, M. T. Tusie Luna and F. J. Gomez-Perez, *The Journal of clinical endocrinology and metabolism*, 2008, **93**, 4075-4079.
143. T. Yamauchi, J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M. L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel and T. Kadowaki, *Nat Med*, 2001, **7**.
144. A. Y. Lemoine, S. Ledoux and E. Larger, *Thrombosis and haemostasis*, 2013, **110**, 661-668.

145. A. U. Hasan, K. Ohmori, K. Konishi, J. Igarashi, T. Hashimoto, K. Kamitori, F. Yamaguchi, I. Tsukamoto, T. Uyama, Y. Ishihara, T. Noma, M. Tokuda and M. Kohno, *Mol. Cell. Endocrinol.*, 2015, **406**, 10-18.
146. L. M. Arterburn, E. B. Hall and H. Oken, *The American journal of clinical nutrition*, 2006, **83**, 1467s-1476s.
147. J. I. Fenton, E. A. Gurzell, E. A. Davidson and W. S. Harris, *Prostaglandins Leukotrienes and Essential Fatty Acids*, 2016, **112**, 12-23.
148. S. J. Guyenet and S. E. Carlson, *Advances in Nutrition: An International Review Journal*, 2015, **6**, 660-664.
149. M. C. Basil and B. D. Levy, *Nat Rev Immunol*, 2016, **16**, 51-67.
150. E. Titos, B. Rius, A. Gonzalez-Periz, C. Lopez-Vicario, E. Moran-Salvador, M. Martinez-Clemente, V. Arroyo and J. Claria, *Journal of immunology (Baltimore, Md. : 1950)*, 2011, **187**, 5408-5418.
151. J. Claria, C. Lopez-Vicario, B. Rius and E. Titos, *Molecular aspects of medicine*, 2017, DOI: 10.1016/j.mam.2017.03.004.
152. J. Hellmann, Y. Tang, M. Kosuri, A. Bhatnagar and M. Spite, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 2011, **25**, 2399-2407.
153. E. Titos, B. Rius, C. López-Vicario, J. Alcaraz-Quiles, V. Garća-Alonso, A. Lopategi, J. Dalli, J. J. Lozano, V. Arroyo, S. Delgado, C. N. Serhan and J. Clària, *Journal of Immunology*, 2016, **197**, 3360-3370.
154. B. K. Itariu, M. Zeyda, E. E. Hochbrugger, A. Neuhofer, G. Prager, K. Schindler, A. Bohdjalian, D. Mascher, S. Vangala, M. Schranz, M. Krebs, M. G. Bischof and T. M. Stulnig, *The American journal of clinical nutrition*, 2012, **96**, 1137-1149.
155. X. Z. Wang, E. Hjorth, I. Vedin, M. Eriksdotter, Y. Freund-Levi, L. O. Wahlund, T. Cederholm, J. Palmblad and M. Schultzberg, *Journal of lipid research*, 2015, **56**, 674-681.
156. J. A. Keelan, E. Mas, N. D'Vaz, J. A. Dunstan, S. Li, A. E. Barden, P. J. Mark, B. J. Waddell, S. L. Prescott and T. A. Mori, *Reproduction*, 2015, **149**, 171-178.
157. B. M. Moran, P. R. Flatt and A. M. McKillop, *Acta Diabetol*, 2016, **53**, 177-188.
158. S. Talukdar, J. M. Olefsky and O. Osborn, *Trends Pharmacol Sci*, 2011, **32**, 543-550.
159. A. Hirasawa, K. Tsumaya, T. Awaji, S. Katsuma, T. Adachi, M. Yamada, Y. Sugimoto, S. Miyazaki and G. Tsujimoto, *Nat Med*, 2005, **11**, 90-94.
160. A. U. Hasan, K. Ohmori, T. Hashimoto, K. Kamitori, F. Yamaguchi, T. Noma, J. Igarashi, K. Tsuboi, M. Tokuda, A. Nishiyama and M. Kohno, *Biochem Biophys Res Commun*, 2017, **486**, 76-82.
161. H. Yamada, T. Umemoto, M. Kakei, S. I. Momomura, M. Kawakami, S. E. Ishikawa and K. Hara, *Nutrition and Metabolism*, 2017, **14**.
162. Y. Yan, W. Jiang, T. Spinetti, A. Tardivel, R. Castillo, C. Bourquin, G. Guarda, Z. Tian, J. Tschopp and R. Zhou, *Immunity*, 2013, **38**, 1154-1163.
163. T. Song, Y. Yang, Y. Zhou, H. Wei and J. Peng, *Cellular and Molecular Life Sciences*, 2017, DOI: 10.1007/s00018-017-2492-2, 1-11.
164. N. Kim, J. O. Lee, H. J. Lee, H. I. Kim, J. K. Kim, Y. W. Lee, S. K. Lee, S. J. Kim, S. H. Park and H. S. Kim, *J Biol Chem*, 2015, **290**, 20438-20447.
165. T. Quesada-Lopez, R. Cereijo, J. V. Turatsinze, A. Planavila, M. Cairo, A. Gavalda-Navarro, M. Peyrou, R. Moure, R. Iglesias, M. Giralt, D. L. Eizirik and F. Villarroya, *Nature communications*, 2016, **7**, 13479.
166. M. Iwaki, M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima and I. Shimomura, *Diabetes*, 2003, **52**, 1655-1663.
167. Y. Min, C. Lowy, S. Islam, F. S. Khan and R. Swaminathan, *European journal of clinical nutrition*, 2011, **65**, 690-695.
168. L. Baril-Gravel, M. E. Labonte, P. Couture, M. C. Vohl, A. Charest, V. Guay, D. A. Jenkins, P. W. Connelly, S. West, P. M. Kris-Etherton, P. J. Jones, J. A. Fleming and B. Lamarche, *Nutrition Metabolism & Cardiovascular Diseases*, 2015, **25**, 52-59.

169. A. Banga, R. Unal, P. Tripathi, I. Pokrovskaya, R. J. Owens, P. A. Kern and G. Ranganathan, *American Journal of Physiology - Endocrinology And Metabolism*, 2009, **296**, E480-E489.
170. J. M. Tishinsky, D. W. L. Ma and L. E. Robinson, *Obesity*, 2011, **19**, 262-268.
171. J. Allaire, P. Couture, M. Leclerc, A. Charest, J. Marin, M.-C. Lépine, D. Talbot, A. Tchernof and B. Lamarche, *The American journal of clinical nutrition*, 2016, **104**, 280-287.
172. M. G. Jacobo-Cejudo, R. Valdés-Ramos, A. L. Guadarrama-López, R.-V. Pardo-Morales, B. E. Martínez-Carrillo and L. S. Harbige, *Nutrients*, 2017, **9**, 573.
173. M. Spencer, B. S. Finlin, R. Unal, B. Zhu, A. J. Morris, L. R. Shipp, J. Lee, R. G. Walton, A. Adu, R. Erfani, M. Campbell, R. E. McGehee, Jr., C. A. Peterson and P. A. Kern, *Diabetes*, 2013, **62**, 1709-1717.
174. W. Zhuang, G. Wang, L. Li, G. Lin and Z. Deng, *J Cardiovasc Transl Res*, 2013, **6**, 287-293.
175. A. Nkondjock and O. Receveur, *Diabetes Metab*, 2003, **29**, 635-642.
176. L. Djousse, M. L. Biggs, R. N. Lemaitre, I. B. King, X. Song, J. H. Ix, K. J. Mukamal, D. S. Siscovick and D. Mozaffarian, *American Journal of Clinical Nutrition*, 2011, **94**, 527-533.
177. M. J. Takkunen, U. S. Schwab, V. D. de Mello, J. G. Eriksson, J. Lindstrom, J. Tuomilehto and M. I. Uusitupa, *Eur J Nutr*, 2015, DOI: 10.1007/s00394-015-0911-4.
178. W. S. Harris, J. Luo, J. V. Pottala, K. L. Margolis, M. A. Espeland and J. G. Robinson, *PLoS One*, 2016, **11**, e0147894.
179. A. Wallin, D. Di Giuseppe, N. Orsini, P. S. Patel, N. G. Forouhi and A. Wolk, *Diabetes care*, 2012, **35**, 918-929.
180. J. Hartweg, R. Perera, V. Montori, S. Dinneen, H. A. Neil and A. Farmer, *Cochrane Database Syst Rev*, 2008, DOI: http://dx.doi.org/10.1002/14651858.CD003205.pub2, CD003205.
181. A. O. Akinkuolie, J. S. Ngwa, J. B. Meigs and L. Djousse, *Clinical nutrition (Edinburgh, Scotland)*, 2011, **30**, 702-707.
182. L. Hodson, C. M. Skeaff and B. A. Fielding, *Progress in lipid research*, 2008, **47**, 348-380.
183. A. Prostek, M. Gajewska, D. Kamola and B. Bałasińska, *Lipids in Health and Disease*, 2014, **13**, 3.
184. M. B. Katan, J. P. Deslypere, A. P. van Birgelen, M. Penders and M. Zegwaard, *Journal of lipid research*, 1997, **38**, 2012-2022.
185. L. M. Browning, J. D. Krebs, C. S. Moore, G. D. Mishra, M. A. O'Connell and S. A. Jebb, *Diabetes Obes Metab*, 2007, **9**, 70-80.
186. K. A. Abbott, T. L. Burrows, R. N. Thota, S. Acharya and M. L. Garg, *The American journal of clinical nutrition*, 2016, **104**, 1470-1484.
187. E. Mingay, M. Veysey, M. Lucock, S. Niblett, K. King, A. Patterson and M. Garg, *Journal of Nutrition & Intermediary Metabolism*, 2016, **5**, 70-77.
188. M. Da Boit, A. M. Hunter and S. R. Gray, *Metabolism*, 2017, **66**, 45-54.
189. M. Phang, L. Lincz, M. Seldon and M. L. Garg, *The Journal of nutritional biochemistry*, 2012, **23**, 1128-1133.
190. G. C. Burdge and P. C. Calder, *Reproduction, nutrition, development*, 2005, **45**, 581-597.
191. K. S. Rathod, V. Kapil, S. Velmurugan, R. S. Khambata, U. Siddique, S. Khan, S. Van Eijl, L. C. Gee, J. Bansal, K. Pitrola, C. Shaw, F. D'Acquisto, R. A. Colas, F. Marelli-Berg, J. Dalli and A. Ahluwalia, *The Journal of Clinical Investigation*, 2017, **127**, 169-182.
192. Y. Min, K. Ghebremeskel, C. Lowy, B. Thomas and M. A. Crawford, *Diabetologia*, 2004, **47**, 75-81.
193. Y. Min, O. Djahanbakhch, J. Hutchinson, S. Eram, A. S. Bhullar, I. Namugere and K. Ghebremeskel, *Clinical nutrition (Edinburgh, Scotland)*, 2016, **35**, 608-614.
194. A. M. Minihane, S. Vinoy, W. R. Russell, A. Baka, H. M. Roche, K. M. Tuohy, J. L. Teeling, E. E. Blaak, M. Fenech, D. Vauzour, H. J. McArdle, B. H. A. Kremer, L. Sterkman, K. Vafeiadou, M. M. Benedetti, C. M. Williams and P. C. Calder, *The British journal of nutrition*, 2015, **114**, 999-1012.
195. R. Medzhitov, *Nature*, 2008, **454**, 428-435.

196. C. D. Funk, *Science (New York, N.Y.)*, 2001, **294**, 1871-1875.
197. E. Ricciotti and G. A. FitzGerald, *Arteriosclerosis, thrombosis, and vascular biology*, 2011, **31**, 986-1000.
198. P. C. Calder, *British Journal of Clinical Pharmacology*, 2013, **75**, 645-662.
199. L. D. Peterson, N. M. Jeffery, F. Thies, P. Sanderson, E. A. Newsholme and P. C. Calder, *Lipids*, 1998, **33**, 171-180.
200. R. S. Chapkin, C. C. Akoh and C. C. Miller, *Journal of lipid research*, 1991, **32**, 1205-1213.
201. D. Rees, E. A. Miles, T. Banerjee, S. J. Wells, C. E. Roynette, K. W. Wahle and P. C. Calder, *The American journal of clinical nutrition*, 2006, **83**, 331-342.
202. P. C. Calder, *Biochim Biophys Acta*, 2015, **1851**, 469-484.
203. T. H. Lee, R. L. Hoover, J. D. Williams, R. I. Sperling, J. Ravalese, 3rd, B. W. Spur, D. R. Robinson, E. J. Corey, R. A. Lewis and K. F. Austen, *The New England journal of medicine*, 1985, **312**, 1217-1224.
204. C. M. Ulrich, J. Bigler and J. D. Potter, *Nat Rev Cancer*, 2006, **6**, 130-140.
205. J. R. Vane, in *Advances in Eicosanoid Research*, eds. C. N. Serhan and H. D. Perez, Springer Berlin Heidelberg, Berlin, Heidelberg, 2000, DOI: 10.1007/978-3-662-04047-8_1, pp. 1-23.
206. C. N. Serhan, C. B. Clish, J. Brannon, S. P. Colgan, N. Chiang and K. Gronert, *The Journal of experimental medicine*, 2000, **192**, 1197-1204.
207. C. N. Serhan, *The American Journal of Pathology*, 2010, **177**, 1576-1591.
208. C. N. Serhan, *Pharmacology & therapeutics*, 2005, **105**, 7-21.
209. J. M. Schwab, N. Chiang, M. Arita and C. N. Serhan, *Nature*, 2007, **447**, 869-874.
210. E. Tjonahen, S. F. Oh, J. Siegelman, S. Elangovan, K. B. Percarpio, S. Hong, M. Arita and C. N. Serhan, *Chemistry & biology*, 2006, **13**, 1193-1202.
211. M. Arita, T. Ohira, Y. P. Sun, S. Elangovan, N. Chiang and C. N. Serhan, *Journal of immunology (Baltimore, Md. : 1950)*, 2007, **178**, 3912-3917.
212. M. Arita, F. Bianchini, J. Aliberti, A. Sher, N. Chiang, S. Hong, R. Yang, N. A. Petasis and C. N. Serhan, *The Journal of experimental medicine*, 2005, **201**, 713.
213. E. B. Schmidt, J. O. Pedersen, K. Varming, E. Ernst, C. Jersild, N. Grunnet and J. Dyerberg, *Arteriosclerosis and thrombosis : a journal of vascular biology*, 1991, **11**, 429-435.
214. P. C. Calder, *The American journal of clinical nutrition*, 2006, **83**, 1505s-1519s.
215. M. Spite, L. V. Norling, L. Summers, R. Yang, D. Cooper, N. A. Petasis, R. J. Flower, M. Perretti and C. N. Serhan, *Nature*, 2009, **461**, 1287-1291.
216. N. G. Bazan, D. L. Birkle and T. S. Reddy, *Biochem Biophys Res Commun*, 1984, **125**, 741-747.
217. V. L. Marcheselli, S. Hong, W. J. Lukiw, X. H. Tian, K. Gronert, A. Musto, M. Hardy, J. M. Gimenez, N. Chiang, C. N. Serhan and N. G. Bazan, *J Biol Chem*, 2003, **278**, 43807-43817.
218. V. L. Marcheselli, P. K. Mukherjee, M. Arita, S. Hong, R. Antony, K. Sheets, J. W. Winkler, N. A. Petasis, C. N. Serhan and N. G. Bazan, *Prostaglandins Leukot Essent Fatty Acids*, 2010, **82**, 27-34.
219. S. M. Grenon, C. D. Owens, E. V. Nosova, M. Hughes-Fulford, H. F. Alley, K. Chong, S. Perez, P. K. Yen, J. Boscardin, J. Hellmann, M. Spite and M. S. Conte, *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease*, 2015, **4**, e002034.
220. T. Lawrence, *Cold Spring Harbor Perspectives in Biology*, 2009, **1**, a001651.
221. P. P. Tak and G. S. Firestein, *J Clin Invest*, 2001, **107**, 7-11.
222. Y. Zhao, S. Joshi-Barve, S. Barve and L. H. Chen, *Journal of the American College of Nutrition*, 2004, **23**, 71-78.
223. J. Y. Lee, K. H. Sohn, S. H. Rhee and D. Hwang, *J Biol Chem*, 2001, **276**, 16683-16689.
224. H. E. Xu, M. H. Lambert, V. G. Montana, D. J. Parks, S. G. Blanchard, P. J. Brown, D. D. Sternbach, J. M. Lehmann, G. B. Wisely, T. M. Willson, S. A. Kliewer and M. V. Milburn, *Molecular cell*, 1999, **3**, 397-403.
225. F. Zapata-Gonzalez, F. Rueda, J. Petriz, P. Domingo, F. Villarroya, J. Diaz-Delfin, M. A. de Madariaga and J. C. Domingo, *Journal of leukocyte biology*, 2008, **84**, 1172-1182.

226. D. Y. Oh, S. Talukdar, E. J. Bae, T. Imamura, H. Morinaga, W. Fan, P. Li, W. J. Lu, S. M. Watkins and J. M. Olefsky, *Cell*, 2010, **142**, 687-698.
227. M. D. Turner, B. Nedjai, T. Hurst and D. J. Pennington, *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 2014, **1843**, 2563-2582.
228. G. L. Bannenberg, N. Chiang, A. Ariel, M. Arita, E. Tjonahen, K. H. Gotlinger, S. Hong and C. N. Serhan, *Journal of immunology (Baltimore, Md. : 1950)*, 2005, **174**, 4345-4355.
229. S. Endres, R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonnemann, J. W. M. van der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempner, P. C. Weber, E. J. Schaefer, S. M. Wolff and C. A. Dinarello, *New England Journal of Medicine*, 1989, **320**, 265-271.
230. G. E. Caughey, E. Mantzioris, R. A. Gibson, L. G. Cleland and M. J. James, *The American journal of clinical nutrition*, 1996, **63**, 116-122.
231. H. Shahbakhti, R. E. Watson, R. M. Azurdia, C. Z. Ferreira, M. Garmyn and L. E. Rhodes, *Photochemistry and photobiology*, 2004, **80**, 231-235.
232. B. Tartibian, B. H. Maleki and A. Abbasi, *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine*, 2011, **21**, 131-137.
233. M. Haghiac, X.-h. Yang, L. Presley, S. Smith, S. Dettelback, J. Minium, M. A. Belury, P. M. Catalano and S. Hauguel-de Mouzon, *PLoS ONE*, 2015, **10**, e0137309.
234. O. D. Rangel-Huerta, C. M. Aguilera, M. D. Mesa and A. Gil, *The British journal of nutrition*, 2012, **107 Suppl 2**, S159-170.
235. W. Xin, W. Wei and X. Li, *BMC Cardiovascular Disorders*, 2012, **12**, 77.
236. M. C. W. Myhrstad, K. Retterstøl, V. H. Telle-Hansen, I. Ottestad, B. Halvorsen, K. B. Holven and S. M. Ulven, *Inflammation Research*, 2011, **60**, 309-319.
237. M. C. Mocellin, C. Q. Camargo, E. A. Nunes, G. M. Fiates and E. B. Trindade, *Clinical nutrition (Edinburgh, Scotland)*, 2016, **35**, 359-369.
238. J. E. Deanfield, J. P. Halcox and T. J. Rabelink, *Circulation*, 2007, **115**, 1285-1295.
239. J. P. J. Halcox, S. Narayanan, L. Cramer-Joyce, R. Mincemoyer and A. A. Quyyumi, *American Journal of Physiology - Heart and Circulatory Physiology*, 2001, **280**, H2470-H2477.
240. M. Zanetti, A. Grillo, P. Losurdo, E. Panizon, F. Mearelli, L. Cattin, R. Barazzoni and R. Carretta, *Biomed Res Int*, 2015, **2015**, 791978.
241. G. Colussi, C. Catena, M. Novello, N. Bertin and L. A. Sechi, *Nutrition, Metabolism and Cardiovascular Diseases*, 2017, **27**, 191-200.
242. M. Omura, S. Kobayashi, Y. Mizukami, K. Mogami, N. Todoroki-Ikeda, T. Miyake and M. Matsuzaki, *FEBS letters*, 2001, **487**, 361-366.
243. Q. Li, Q. Zhang, M. Wang, F. Liu, S. Zhao, J. Ma, N. Luo, N. Li, Y. Li, G. Xu and J. Li, *Archives of Biochemistry and Biophysics*, 2007, **466**, 250-259.
244. Q. Wang, X. Liang, L. Wang, X. Lu, J. Huang, J. Cao, H. Li and D. Gu, *Atherosclerosis*, 2012, **221**, 536-543.
245. W. Xin, W. Wei and X. Li, *PLOS ONE*, 2012, **7**, e46028.
246. D. Tousoulis, A. Plastiras, G. Siasos, E. Oikonomou, A. Verveniotis, E. Kokkou, K. Maniatis, N. Gouliopoulos, A. Miliou, T. Paraskevopoulos and C. Stefanadis, *Atherosclerosis*, 2014, **232**, 10-16.
247. Y. Yang, N. Lu, D. Chen, L. Meng, Y. Zheng and R. Hui, *The American journal of clinical nutrition*, 2012, **95**, 972-980.
248. J. Merino, A. Sala-Vila, R. Kones, R. Ferre, N. Plana, J. Girona, D. Ibarretxe, M. Heras, E. Ros and L. Masana, *The Journal of nutritional biochemistry*, 2014, **25**, 642-646.
249. O. Eschen, J. H. Christensen, R. De Caterina and E. B. Schmidt, *Nutrition, Metabolism and Cardiovascular Diseases*, 2004, **14**, 180-185.
250. L. G. Yang, Z. X. Song, H. Yin, Y. Y. Wang, G. F. Shu, H. X. Lu, S. K. Wang and G. J. Sun, *Lipids*, 2016, **51**, 49-59.

251. G. Gortan Cappellari, P. Losurdo, S. Mazzucco, E. Panizon, M. Jevnicar, L. Macaluso, B. Fabris, R. Barazzoni, G. Biolo, R. Carretta and M. Zanetti, *The Journal of nutritional biochemistry*, 2013, **24**, 371-379.
252. K. H. Bønaa , K. S. Bjerve , B. Straume , I. T. Gram and D. Thelle *New England Journal of Medicine*, 1990, **322**, 795-801.
253. G. Colussi, C. Catena, V. Dialti, F. Pezzutto, L. Mos and L. A. Sechi, *American journal of hypertension*, 2014, **27**, 471-481.
254. S. Rogers, K. S. James, B. K. Butland, M. D. Etherington, J. R. O'Brien and J. G. Jones, *Atherosclerosis*, 1987, **63**, 137-143.
255. H. R. Knapp and G. A. FitzGerald, *The New England journal of medicine*, 1989, **320**, 1037-1043.
256. K. BØNAA, *Journal of Internal Medicine*, 1989, **225**, 105-110.
257. M. C. Morris, F. Sacks and B. Rosner, *Circulation*, 1993, **88**, 523-533.
258. L. J. Appel, E. R. Miller, III, A. J. Seidler and P. K. Whelton, *Archives of Internal Medicine*, 1993, **153**, 1429-1438.
259. F. Campbell, H. O. Dickinson, J. A. Critchley, G. A. Ford and M. Bradburn, *European journal of preventive cardiology*, 2013, **20**, 107-120.
260. P. E. Miller, M. Van Elswyk and D. D. Alexander, *American journal of hypertension*, 2014, **27**, 885-896.
261. S. Willoughby, A. Holmes and J. Loscalzo, *European journal of cardiovascular nursing : journal of the Working Group on Cardiovascular Nursing of the European Society of Cardiology*, 2002, **1**, 273-288.
262. N. A. Alarayyed, B. R. Graham, B. N. Prichard and C. C. Smith, *British Journal of Clinical Pharmacology*, 1995, **39**, 369-374.
263. L. G. Gao, J. Cao, Q. X. Mao, X. C. Lu, X. L. Zhou and L. Fan, *Atherosclerosis*, 2013, **226**, 328-334.
264. J. J. Agren, S. Vaisanen, O. Hanninen, A. D. Muller and G. Hornstra, *Prostaglandins Leukot Essent Fatty Acids*, 1997, **57**, 419-421.
265. *The Lancet*, 1999, **354**, 447-455.
266. P. Barter and H. N. Ginsberg, *The American journal of cardiology*, 2008, **102**, 1040-1045.
267. P. G. Swann, D. L. Venton and G. C. Le Breton, *FEBS Lett*, 1989, **243**, 244-246.
268. M. Omura, S. Kobayashi, Y. Mizukami, K. Mogami, N. Todoroki-Ikeda, T. Miyake and M. Matsuzaki, *FEBS Lett*, 2001, **487**, 361-366.
269. C. A. Sargent and R. A. Riemersma, *Biochemical Society transactions*, 1990, **18**, 1077-1078.
270. M. L. Burr, A. M. Fehily, J. F. Gilbert, S. Rogers, R. M. Holliday, P. M. Sweetnam, P. C. Elwood and N. M. Deadman, *Lancet (London, England)*, 1989, **2**, 757-761.
271. D. S. Siscovick, T. E. Raghunathan, I. King, S. Weinmann, K. G. Wicklund, J. Albright, V. Bovbjerg, P. Arbogast, H. Smith, L. H. Kushi and et al., *Jama*, 1995, **274**, 1363-1367.
272. M. de Lorgeril, S. Renaud, N. Mamelle, P. Salen, J. L. Martin, I. Monjaud, J. Guidollet, P. Touboul and J. Delaye, *Lancet (London, England)*, 1994, **343**, 1454-1459.
273. A. Sellmayer, H. Witzgall, R. L. Lorenz and P. C. Weber, *The American journal of cardiology*, 1995, **76**, 974-977.
274. S. S. Nair, J. W. Leitch, J. Falconer and M. L. Garg, *The Journal of nutrition*, 1997, **127**, 383-393.
275. *Lancet (London, England)*, 1999, **354**, 447-455.
276. I. A. Brouwer, P. L. Zock, A. J. Camm, D. Bocker, R. N. Hauer, E. F. Wever, C. Dullemeijer, J. E. Ronden, M. B. Katan, A. Lubinski, H. Buschler and E. G. Schouten, *Jama*, 2006, **295**, 2613-2619.
277. A. Leaf, C. M. Albert, M. Josephson, D. Steinhaus, J. Kluger, J. X. Kang, B. Cox, H. Zhang and D. Schoenfeld, *Circulation*, 2005, **112**, 2762-2768.
278. M. H. Raitt, W. E. Connor, C. Morris, J. Kron, B. Halperin, S. S. Chugh, J. McClelland, J. Cook, K. MacMurdy, R. Swenson, S. L. Connor, G. Gerhard, D. F. Kraemer, D. Oseran, C. Marchant, D. Calhoun, R. Shnider and J. McAnulty, *Jama*, 2005, **293**, 2884-2891.
279. H. Leon, M. C. Shibata, S. Sivakumaran, M. Dorgan, T. Chatterley and R. T. Tsuyuki, *BMJ (Clinical research ed.)*, 2008, **337**, a2931.

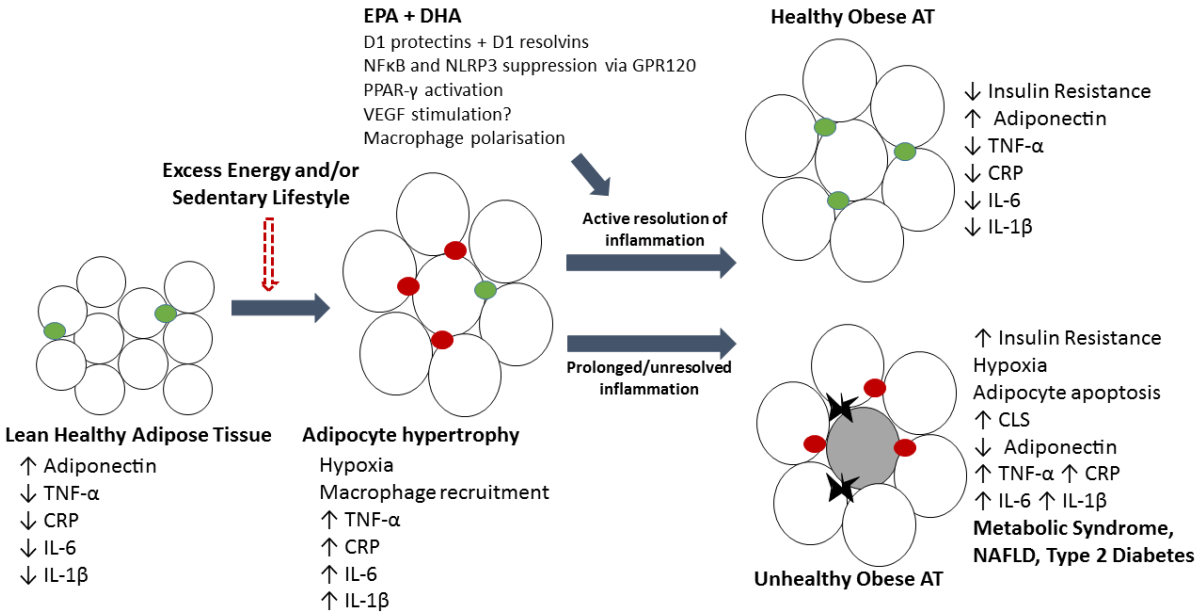

Figure 1. Anti-inflammatory effects of long-chain omega-3 polyunsaturated fatty acids in adipose tissue functioning. :Anti-inflammatory M2 macrophages. :Pro-inflammatory M1 macrophages. : Crown-like structures. EPA: Eicosapentaenoic Acid. DHA: Docosahexaenoic Acid. AT: Adipose tissue. NFκB: Nuclear Factor Kappa B. NLRP3: NLRP3 inflammasome. TNF-α: Tumour Necrosis Factor-Alpha. CRP: c-reactive protein. IL: Interleukin. CLS: Crown-like structures. VEGF: Vascular endothelial growth factor.

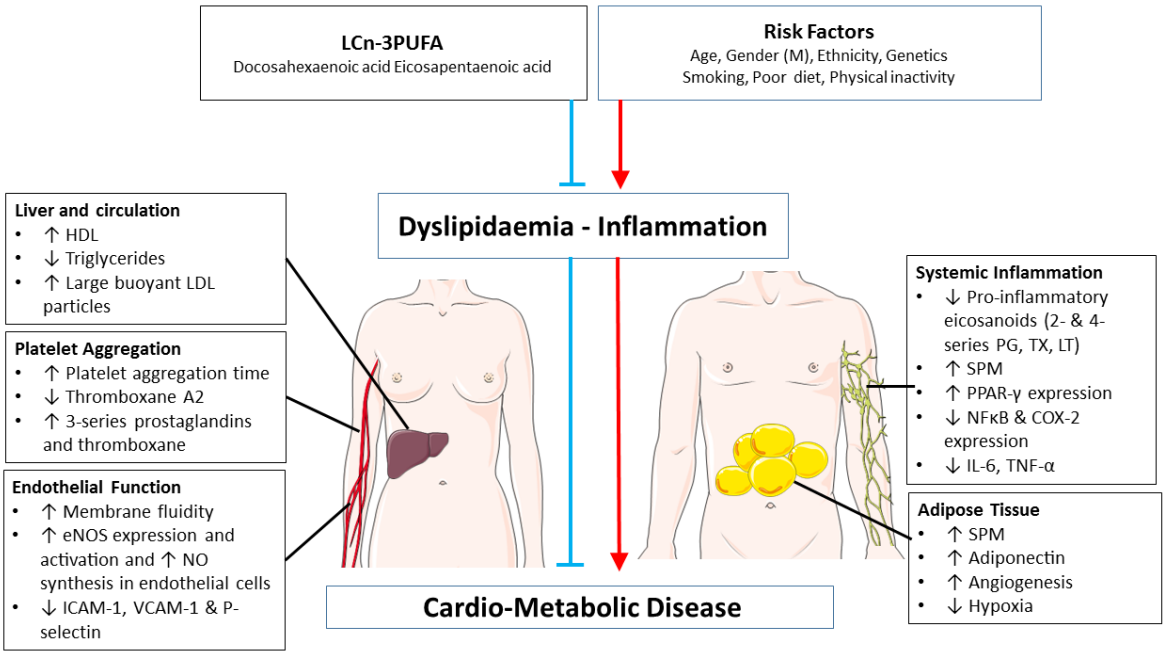

Figure 2. Overview of the localised and systemic effects of long-chain omega-3 polyunsaturated fatty acids for the prevention of cardio-metabolic disease. LCn-3PUFA: Long-chain omega-3 polyunsaturated fatty acids. CRP: c-reactive protein. HDL: High density lipoprotein. LDL: Low density lipoprotein. ICAM: Intracellular adhesion molecule. VCAM: Vascular cell adhesion molecule. PG: Prostaglandin. TX: Thromboxane. LT: Leukotriene. SPM: Specialised pro-resolving mediators. PPAR-γ: Peroxisome proliferator-activated receptor-gamma. TNF-α: Tumour Necrosis Factor-Alpha. IL-6: Interleukin-6. NFκB: Nuclear Factor Kappa B. COX: Cyclooxygenase.