University of Reading

Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) and hypertension: a review of vasodilatory mechanisms of DHA and EPA

Article

Published Version

Creative Commons: Attribution 4.0 (CC-BY)

Open Access

Bercea, Cristiana-Ioana, Cottrell, Graeme S. ORCID logoORCID: https://orcid.org/0000-0001-9098-7627, Tamagnini, Francesco ORCID logoORCID: https://orcid.org/0000-0002-8741-5094 and McNeish, Alister J. (2021) Omega-3 polyunsaturated fatty acids (ω-3 PUFAs) and hypertension: a review of vasodilatory mechanisms of DHA and EPA. British Journal of Pharmacology, 178 (4). pp. 860-877. ISSN 0007-1188 doi: https://doi.org/10.1111/bph.15336 Available at https://centaur.reading.ac.uk/94951/

It is advisable to refer to the publisher's version if you intend to cite from the work. See Guidance on citing.

To link to this article DOI: http://dx.doi.org/10.1111/bph.15336

Publisher: Wiley

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in the End User Agreement.

University of Reading

www.reading.ac.uk/centaur

CentAUR

Central Archive at the University of Reading

Reading's research outputs online

Received: 19 August 2020 | Revised: 27 November 2020 | Accepted: 29 November 2020

DOI: 10.1111/bph.15336

REVIEW ARTICLE

Omega-3 polyunsaturated fatty acids and hypertension: a review of vasodilatory mechanisms of docosahexaenoic acid and eicosapentaenoic acid

Cristiana-Ioana Bercea | Graeme S. Cottrell | Francesco Tamagnini | Alister J. McNeish

Reading School of Pharmacy, School of Chemistry, Food and Pharmacy, The University of Reading, Reading, UK

Correspondence
Alister J. McNeish, Reading School of Pharmacy, School of Chemistry, Food, and Pharmacy, The University of Reading, Whiteknights, Reading, RG6 6UB, UK.
Email: a.mcneish@reading.ac.uk

Funding information
British Heart Foundation, Grant/Award Number: PG/19/57/34568

Hypertension is often characterised by impaired vasodilation involving dysfunction of multiple vasodilatory mechanisms. ω-3 polyunsaturated fatty acids (PUFAs), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) can reduce blood pressure and vasodilation. In the endothelium, DHA and EPA improve function including increased NO bioavailability. However, animal studies show that DHA- and EPA-mediated vasodilation persists after endothelial removal, indicating a role for vascular smooth muscle cells (VSMCs). The vasodilatory effects of ω-3 PUFAs on VSMCs are mediated via opening of large conductance calcium-activated potassium channels (BK_{Ca}), ATP-sensitive potassium channels (K_{ATP}) and possibly members of the K_v7 family of voltage-activated potassium channels, resulting in hyperpolarisation and relaxation. ω-3 PUFA actions on BK_{Ca} and voltage-gated ion channels involve electrostatic interactions that are dependent on the polyunsaturated acyl tail, cis-geometry of these double bonds and negative charge of the carboxyl headgroup. This suggests structural manipulation of ω-3 PUFA could generate novel, targeted, therapeutic leads.

KEYWORDS
hypertension, DHA docosahexaenoic acid, EPA eicosapentaenoic acid, omega-3 polyunsaturated fatty acids (PUFA), vasodilation, endothelium, vascular smooth muscle cells, Nitric oxide

1 | INTRODUCTION

1.1 | Hypertension is associated with impaired vasodilation

The characteristic manifestation of hypertension is a chronic increase in arterial blood pressure (BP), generally defined as systolic (SBP) and diastolic (DBP) blood pressure values above 139 and 89 mmHg, respectively (McCormack, Boffa, Jones, Carville, & McManus, 2019). 90%–95% of cases represent primary hypertension, which arises independently of other conditions, and is associated with lifestyle and genetic factors (Oparil et al., 2018). Hypertension is divided into categories depending on severity and risk of complications, and small reductions in blood pressure (such as 1 mmHg), even for those

Abbreviations: α-LA, α-linolenic acid; CYP450, cytochrome P450; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; EDHF, endothelium-derived hyperpolarisation factor; eNOS, endothelial NOS; EE, ethyl esters; EPA, eicosapentaenoic acid; LA, linolenic acid; PUFAs, polyunsaturated fatty acids; SBP, systolic blood pressure; SHR, spontaneously hypertensive rat; TAG, triacylglycerides; VSMC, vascular smooth muscle cell.

[Correction added on 8 April 2021, after first online publication: The copyright line was changed.]

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
© 2020 The Authors. British Journal of Pharmacology published by John Wiley & Sons Ltd on behalf of British Pharmacological Society.

considered normotensive, can represent a significant improvement in multiple predicted health outcomes such as all-cause mortality or cardiovascular disease-related mortality (Li et al., 2020; Miller, Van Elswyk, & Alexander, 2014).

Blood pressure is controlled by several factors and a major determinant is peripheral resistance, largely controlled by changes in arterial diameter. The arterial system includes elastic and muscular arteries, which differ in morphology and physiology. Elastic arteries are large diameter arteries also referred to as conduit arteries, for example the aorta. They contain more elastin and regulate the pressure wave coming from the heart in order to stabilise the vascular pulse pressure, reviewed in Wagenseil and Mecham (2012). Muscular arteries are small diameter arteries also referred to as resistance arteries, for example the mesenteric. They contain less elastin, depending on need, vasoconstriction reduces the diameter of the lumen, resisting blood flow and thus generating vascular resistance (Stott, Jepps, & Greenwood, 2014).

Under normal conditions, increased blood flow promotes vasodilation, which is mediated by the two major cell types present in arteries, the inner endothelial cells and the outer smooth muscle cells. Dysfunctions in vasomotor tone mean arteries develop an abnormally high contraction, leading to a chronic narrowing of their lumen, which leads to increased blood pressure. In healthy arteries, vascular smooth muscle cells (VSMCs) are quiescent and non-proliferative, with the blood vessel wall containing large numbers of progenitor cells, see Lacolley, Regnault, Segers, and Laurent (2017) for a review of VSMC function in health and disease. Vascular dysfunction in diseases such as hypertension is marked by vascular remodelling leading to a thickening and narrowing of the lumen caused in part by VSMC migration, hypertrophy and proliferation leading to an increase in volume, reviewed in Hixon and Gualberto (2003). Furthermore, increased production of extracellular matrix proteins such as collagen and endothelial dysfunction lead to arterial stiffness—the loss of elasticity in the arterial wall.

1.2 | General vasodilatory mechanisms

In vivo and in vitro studies have identified a role for endothelial factors and VSMCs in mediating vasodilation, extensively reviewed elsewhere (Brozovich et al., 2016; Chen, Pittman, & Popel, 2008). VSMCs are highly plastic despite being highly differentiated and can therefore change phenotype, for example in the case of certain disorders (see below). VSMCs are stimulated by hypertensive stimuli such as mechanical forces (such as shear stress; Birukov, 2009) and oxidative stress to produce vasoconstriction (Touyz et al., 2018). The contractile machinery in VSMCs is dependent on changes in intracellular Ca^{2+} concentrations (Figure 1) that control the activity of **myosin light chain kinase**, which modulates the phosphorylation of the actin-myosin bridge-cycling. Importantly, VSMC contraction is also dependent on K^+ efflux that regulates membrane potential by hyperpolarization, which reduces voltage gated Ca^{2+} entry this prevents contraction (Brozovich et al., 2016).

The inability of VSMCs to regulate arterial diameter in response to blood flow results in impaired vasodilation and the vessels become stiff. Stiffening of VSMCs leads to endothelial dysfunction (Giles, Sander, Nossaman, & Kadowitz, 2012). Endothelium-produced vasodilators include **nitric oxide** (NO), especially in conduit arteries (Tousoulis et al., 2014), prostaglandins (Durand & Gutterman, 2013) and endothelium-derived hyperpolarization factors, especially in resistance arteries (Feletou & Vanhoutte, 2007; Vanhoutte, Shimokawa, Tang, & Feletou, 2009). The endothelium transmits endothelial hyperpolarization to VSMCs via the myoendothelial gap junctions (Vanhoutte et al., 2009) (Figure 1). Endothelial dysfunction correlating with hypertension includes loss of NO production by **endothelial NO synthase** (eNOS) and increased production of endothelium-derived contracting factors (EDCFs) by **cyclooxygenases** (COX), reviewed in Vanhoutte and Tang (2008).

Finally, vascular tone is also controlled by adipocytes in the perivascular adipose tissue, which produce vasodilators that relax VSMCs through mechanisms involving K^+ channels (Figure 1) and endothelial NOS (Ramirez, O'Malley, & Ho, 2017); see Agabiti-Rosei et al. (2018) for an in-depth review. Inhibition of K^+ channels leads to blockade of the anti-contractile effect of perivascular adipose tissue. Obesity is often accompanied by more perivascular adipose tissue and alterations in the physiology of perivascular adipose tissue, leading to dysfunctions of these mechanisms (Ramirez et al., 2017). In hypertension, there is less perivascular adipose tissue and of smaller size compared to normotensive arteries (Oriowo, 2015), leading to reduced anti-contractile activity. Indeed, it was shown that in artery segments with reduced perivascular adipose tissue, there is reduced hyperpolarisation compared to segments with intact perivascular adipose tissue (Verlohren et al., 2004). Although to our knowledge no research has investigated the effects of ω-3 polyunsaturated fatty acids (PUFAs) on adipocytes in the context of hypertension, some studies report that ω-3 PUFAs regulate adipocyte differentiation, apoptosis and adipose tissue inflammation in subjects with obesity or metabolic syndrome (Martinez-Fernandez, Laiglesia, Huerta, Martinez, & Moreno-Aliaga, 2015).

1.3 | Roles of omega-3 polyunsaturated fatty acids (ω-3 PUFAs) in the cardiovascular system

Attention was first drawn to the potential benefits of seafood and "fish oils" when several epidemiological studies reported a decreased incidence of cardiovascular disease, including hypertension, in regions of the world with a high consumption of these foods (Bang, Dyerberg, & Sinclair, 1980; Kagawa et al., 1982). This led to a large body of clinical, epidemiological, in vivo and in vitro data that identified ω-3 PUFAs derived from marine sources as having cardiovascular effects.

The two main ω-3 PUFAs, **eicosapentaenoic acid** (EPA) and **docosahexaenoic acid** (DHA), form part of the lipid component of cell membranes. In the human body, EPA is produced by desaturases and elongases from **α-linolenic acid** (α-LA), which is present in high amounts in certain seeds and vegetables (Shahidi & Ambigaipalan, 2018) (Figure 2). α-LA is converted into EPA, which can then be metabolised into DHA through the action of elongases (which add carbons to the hydrocarbon chain of the fatty acid) and desaturases (which replace single bonds with double bonds)

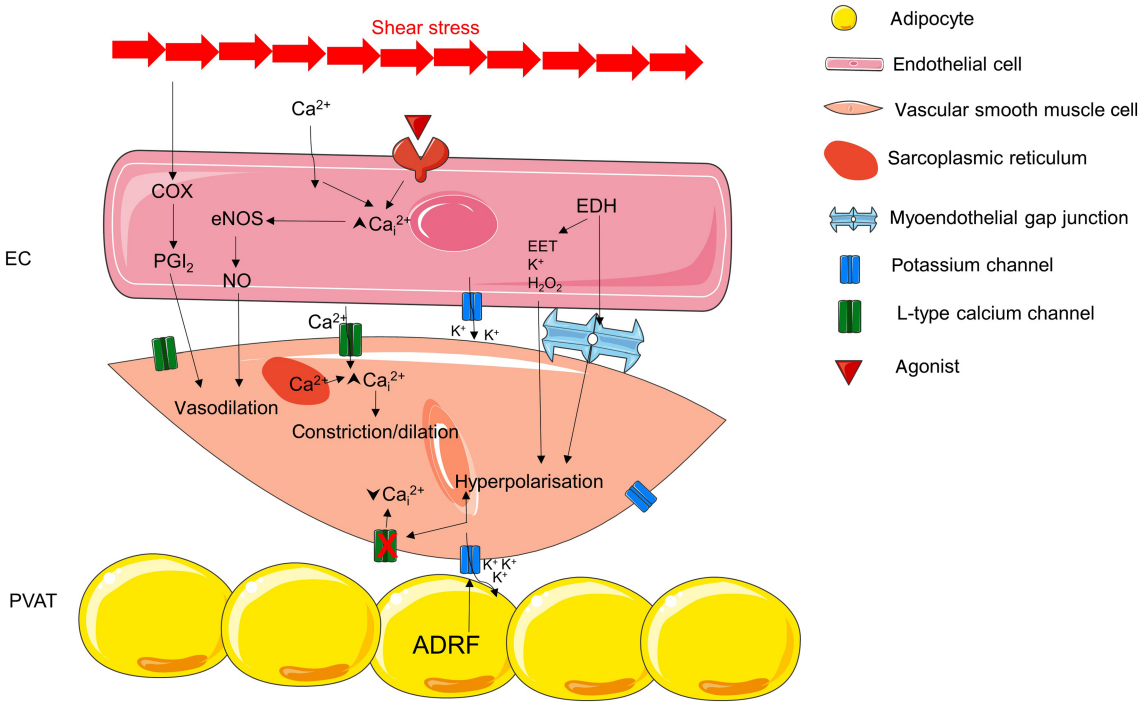

FIGURE 1 General vasodilatory mechanisms. Endothelial factors and vascular smooth muscle cells (VSMCs) mediate vasodilation. The contractile machinery in VSMCs is dependent on changes in intracellular Ca^{2+} concentrations which control myosin light chain kinase and thus the activity of the actin-myosin bridge-cycling. This process is regulated by membrane potential depolarisation increases calcium entry via L-type VGCC a process opposed by hyperpolarization due to K^+ efflux. Endothelium-dependent vasodilation is initiated by mechanical forces such as shear stress or by agonists binding to receptors, both of which increase endothelial cell calcium concentration. This leads to production of vasodilators such as NO, PGs (PGI_2) and endothelium-derived hyperpolarization (EDH). The endothelium can also transmit endothelial hyperpolarization to VSMCs via the myoendothelial gap junctions as well as any diffusible factors such as EETs and H_2O. Finally, vascular tone is also controlled by adipocytes in the perivascular adipose tissue (PVAT), which produce vasodilators that instruct VSMCs to relax through mechanisms involving K^+ channels. Abbreviations: ADRF, adipose derived relaxing factor(s); COX, cyclooxygenase; EC, endothelial cell, EET, epoxyeicosatrienoic acids. eNOS, endothelial nitric oxide synthase Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License

(Sokola-Wysoczanska et al., 2018). DHA and EPA can be further metabolised, as we will discuss in future sections.

The desaturases and elongases involved in the synthesis of EPA and DHA also metabolise linoleic acid (LA) into ω-6 PUFAs (Figure 2). LA has a greater bioavailability than α-LA and competition between α-LA and LA for the active sites of the same enzymatic pathway means the generation of ω-6 PUFAs from LA is more efficient than that of DHA and EPA from α-LA (Arterburn, Hall, & Oken, 2006). In contrast to saturated fatty acids and monounsaturated fatty acids that can be synthesised by the body in sufficient amounts, ω-3 PUFAs are mostly dependent on dietary intake. As between 1% and 10% of α-LA is converted into EPA, this means that both DHA and EPA can be considered essential fatty acids, implicating dietary intake into any expected health benefits (Goyens, Spilker, Zock, Katan, & Mensink, 2005; Hussein et al., 2005). Furthermore, as ω-3 PUFAs compete with ω-6 PUFAs, an imbalance in the diet between ω-6 and ω-3 intake may also lead to an impairment in the cardiovascular effects of DHA and EPA. ω-6 PUFAs have vasoconstrictive, vasodilatory and pro-inflammatory roles. For example, **arachidonic acid** is a highly studied ω-6 PUFA, whose metabolites act as endothelium-derived hyperpolarising factors (EDHFs) to cause vasodilation (Campbell & Falck, 2007).

Numerous reviews and meta-analyses have examined the evidence supporting cardiovascular effects of ω-3 PUFAs (Innes & Calder, 2020; Jain, Aggarwal, & Zhang, 2015; Mozaffarian & Wu, 2011; Saravanan, Davidson, Schmidt, & Calder, 2010). Multiple proposed mechanisms have emerged for these effects (Massaro, Scoditti, Carluccio, & De Caterina, 2008), including the lowering of blood pressure. The blood pressure lowering and vasodilatory effects of ω-3 PUFAs have been investigated, as we will discuss, using randomised controlled studies, epidemiological studies, in vivo animal studies and in vitro studies. The beneficial effects of ω-3 PUFAs on blood pressure in hypertensive or normotensive human subjects have been reviewed elsewhere (AbuMweis, Jew, Tayyem, & Agraib, 2018; Colussi, Catena, Novello, Bertin, & Sechi, 2017; Miller et al., 2014), but to our knowledge, no reviews to date have discussed the vasodilator mechanisms underlying these effects. Here, we will present a summary of the evidence for endothelium-mediated blood pressure lowering effects of ω-3 PUFAs in human studies and discuss possible explanations for

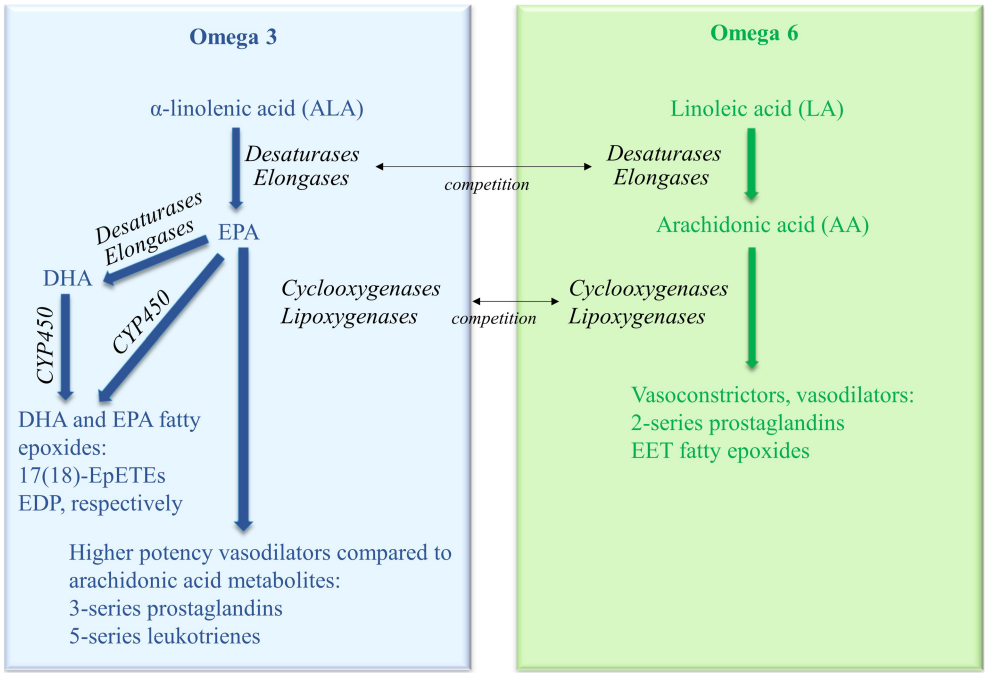

FIGURE 2 ω-3 polyunsaturated fatty acids (PUFAs) and ω-6 PUFA metabolic pathways. α-linolenic acid (α-LA) and linoleic acid (LA) are metabolised by and compete for, the same desaturases and elongases, producing EPA (eicosapentaenoic acid) and arachidonic acid (AA), respectively. EPA can then be metabolised to docosahexaenoic acid (DHA). The same cyclooxygenases and lipoxygenases can then metabolise EPA (to the vasodilatory 3-series PGs and 5-series leukotrienes, respectively) or arachidonic acid (AA; to the mixed vasodilatory and vasoconstrictive 2-series PGs). Although many AA metabolites can have vasodilatory effects, the EPA metabolites are considerably more potent. Finally, DHA and EPA can be metabolised by cytochrome P450 (CYP450) into the fatty epoxides epoxydocosapentaenoic acid (EDP) and 17(18)-EpETEs, respectively; AA can also be converted into fatty epoxides by CYP450 which have both vasodilator and constrictor mechanisms but is not shown here for simplicity

conflicting information. We will then present data from animal studies to discuss endothelium-dependent and endothelium-independent, VSMC-mediated, ω-3 PUFA-dependent mechanisms of vasodilation.

2 | DHA AND EPA ON BLOOD PRESSURE AND VASODILATION IN HUMANS

2.1 | Effects on blood pressure

Long-term cohort studies and short-term randomised controlled trials have identified associations between increased consumption of ω-3 PUFAs and lowered blood pressure in hypertensive individuals (AbuMweis et al., 2018; Colussi et al., 2017; Garcia-Lopez et al., 2016; Miller et al., 2014; Minihane et al., 2016; Ramel, Martinez, Kiely, Bandarra, & Thorsdottir, 2010; Shen et al., 2017; Yang et al., 2019). For example, a meta-analysis of short-term studies lasting several months found that ω-3 PUFA decreased SBP by 1.52 mmHg and DBP by 0.99 mmHg (Miller et al., 2014), whereas in hypertensive subjects they found reductions in SBP of 4.51 mmHg and in DBP of 3.05 mmHg. This finding of a greater effect in hypertensive subjects was supported by a second meta-analysis demonstrating larger decreases in SBP and DBP in hypertensive patients (Tagetti et al., 2015). The data for the benefits of consumption of ω-3 PUFA to lower blood pressure in normotensive individuals are less robust and hence, evidence for primary prevention of hypertension is weaker (Innes & Calder, 2020; Minihane et al., 2016; Root, Collier, Zwetsloot, West, & McGinn, 2013), although one international epidemiological study found significant hypotensive effects of ω-3s in non-hypertensive subjects (Ueshima et al., 2007). Large prospective cohort studies lasting several years that looked at the development of cardiovascular disease in normotensive patients, whose diets contained ω-3 PUFAs, found that diets high in ω-3 PUFAs correlated with a lower risk of developing hypertension. Other meta-analyses, reviewed together with the cohort studies, mostly show reductions in blood pressure only in hypertensive subjects (Colussi et al., 2017). Consistent with this, a recent cohort study (which did not focus on blood pressure) found an overall reduced cardiovascular disease incidence and cardiovascular disease-related mortality in individuals habitually taking supplements where the effect was stronger in subjects with a history of cardiovascular disease events (Li et al., 2020).

While there seems to be a consensus that ω-3 PUFAs can play a role in reducing hypertension the data from individual studies is variable. This likely reflects the high variation in ω-3 PUFA source, dose and duration and the formulation of ω-3PUFAs (fish meals, fish oil, capsules containing ethyl-ester forms of DHA and EPA, and powders). Current guidelines (Minihane, 2013) recommend 0.5 g/day for healthy individuals and between 1 and 1.5 g/day for hypertensive individuals,

coming from two servings of oily fish per week. Most studies, however, use over 1.5 g/day, with less emphasis being placed on doses lower than 1 g/day and there is considerable variation in dosage between studies (Minihane et al., 2016). Furthermore, most guidelines only refer to consumption from fish, not other forms, such as supplements (Siniarski et al., 2018) and the amounts of ω-3 PUFAs in fish can vary. Therefore, intervention studies mostly use higher doses of a combination of DHA and EPA than could normally be reasonably obtained from a meal/diet rich in marine oils and administer DHA and EPA in the form of capsule supplements or drinks. Generally, studies focus on the combined effects of DHA and EPA (of both known and unknown ratios), as occurs naturally in fish oil. The effects have been studied using, for example, capsules containing EPA and DHA to a ratio of about 1:1.2 (Krantz et al., 2015), emulsion drink containing equal amounts (Siniarski et al., 2018), or drink with fish oil concentrate (Newens, Thompson, Jackson, & Williams, 2015). Treatment durations also vary usually between 1 month and 1 year, with some studies using higher doses and some studies investigating acute interventions (see above). Furthermore, as it is possible that DHA has a more potent effect than EPA on BP and as EPA by itself appears to have a strong effect on endothelial function, the ratio of EPA to DHA is likely to have an effect on the results obtained. Interestingly, one major review of over 60 studies (Colussi et al., 2017) and one major meta-analysis of 70 studies (Miller et al., 2014) concluded that there does not appear to be any clear dose-dependence in the effects of ω-3s on blood pressure. These reviews, as well as that by (Innes & Calder, 2018), offer an excellent summary of the various doses used in each study.

Some studies have looked at the individual effect of EPA and DHA. For example a study on normotensive subjects administered supplements of either DHA or EPA found that SBP and DBP were lower after DHA and after the olive oil control, with no difference between the two; EPA *increased* SBP and DBP (Lee et al., 2019). Hence, EPA might exert cardiovascular benefits through other mechanisms than blood pressure lowering (for example heart rate). Other studies comparing the effects of DHA and EPA separately on blood pressure in normotensive subjects have found no effect for either (Asztalos et al., 2016; Grimsgaard, Bonaa, Hansen, & Myhre, 1998; Nestel et al., 2002), as was the case in hypertensive patients also being treated with anti-hypertensive medication (Woodman et al., 2003). Furthermore, DHA alone slightly lowered BP in male but not female normotensive subjects (Singhal et al., 2013) and EPA alone lowered SBP in normotensive subjects (Iketani, Takazawa, & Yamashina, 2013). Therefore, most data point to the fact that individual ω-3 PUFAs have differential effects on blood pressure and DHA has been suggested to be more potent in this respect (Cottin, Sanders, & Hall, 2011; Innes & Calder, 2018; Jacobson, Glickstein, Rowe, & Soni, 2012; Mori & Woodman, 2006; Mozaffarian & Wu, 2012).

Most of the studies informing ω-3 recommendations used EPA and DHA ethyl esters (EEs); fish oils from fish eaten as part of meals are esterified as triacylglycerides (TAGs) and re-esterified, resulting in concentrated fatty acids. Concentrated fish oil capsule preparations mainly contain EPA and DHA as TAGs or EEs. Many studies measuring plasma levels (i.e. short-term bioavailability) found increased bioavailability of DHA and EPA from TAGs compared to EEs, but the results are heterogenous (Neubronner et al., 2011). One study in hyperlipidaemic subjects treated with statins used gelatine-coated soft capsules containing either reesterified triacylglycerols (rTAGs) or EEs with the ω-3 composition being the same in the experimental groups (Neubronner et al., 2011). They found that 6 months of treatment resulted in an increase in relative EPA in red blood cells significantly higher in the rTAG group compared with the EE group. The increase in the ω-3 index (the percentage of ω-3 fatty acids in RBC membranes) was significantly higher in the rTAG group compared with the EE group. Furthermore, in hypertensive young adults on a calorie-restricted diet administered either salmon, cod, or capsules, there is similarly lowered DBP in subjects taking capsules or eating salmon, but it should be noted that they also saw a reduction in controls (Ramel et al., 2010). Hypertensive individuals administered ω-3 PUFAs for 4 weeks in the form of six meals of fish per week fortified with either liquid fish oil or microencapsulated powder found similar, significant, reductions in SBP (Sveinsdottir, Martinsdottir, & Ramel, 2016). Therefore, there is no clear consensus if the mode of administration plays a significant role in lowering of blood pressure.

There are also considerable differences in participants, with many studies using mixed cohorts; comorbidities and treatments affect outcomes, as does the subject's overall diet and most studies feature normotensive or hypertensive patients with other conditions (Minihane et al., 2016; Ramel et al., 2010; Sveinsdottir et al., 2016). Membrane fatty acid composition at the start of a trial or the baseline amount of ω-3 PUFAs in red blood cells can also vary with diet and can depend on the subjects' genetics (Colussi et al., 2017). Finally, as suggested above, it is possible that DHA has a more potent effect than EPA to lower blood pressure and as EPA appears to have a greater effect on endothelial function, the ratio of EPA to DHA is likely to have an effect on the changes in blood pressure observed (Lee et al., 2019).

Despite the confounding factors, the consensus view is that effects of ω-3 PUFAs are probably greater in patients already suffering from hypertension and that DHA has a more potent effect. What remains unclear and is not always investigated in human studies are the physiological and pharmacological mechanisms that potentially underly this reduction in blood pressure, which are discussed below.

2.2 | Effects on the endothelium

Arterial stiffness (i.e. decreased elasticity) is caused by both endothelial dysfunction and dysfunction of the collagen matrix in the artery wall (Diez, 2007). Endothelial dysfunction is marked by endothelial injury and a disruption in repair mechanisms. It is possible that ω-3 PUFAs improve large artery elasticity through their effects of lowering blood pressure via vasodilation, for example, by enhancing NO

production or release. Indeed, there is evidence that acute administration of ω-3 PUFAs improves endothelial function as assessed functionally using flow-mediated dilationa marker of endothelial dependent relaxation attributed to NO generation (Newens, Thompson, Jackson, Wright, & Williams, 2011).

Two meta-analyses suggest that long-term administration of ω-3 PUFAs has a beneficial effect on flow-mediated dilation in subjects with cardiovascular disease (Wang et al., 2012; Xin, Wei, & Li, 2012) including hypertensive subjects, albeit with no significant reduction in BP (Casanova et al., 2017). Furthermore, in patients with coronary artery disease, whose baseline endothelial function is impaired, EPA treatment improves flow-mediated dilation (Sawada et al., 2016). EPA in particular is believed to increase endothelium-dependent vasodilation by producing PGs and increasing NO production in endothelial cells (Iketani et al., 2013). Interestingly, postprandial reductions in flow-mediated dilation seen in normotensive individuals following a high-saturated fat meal is reversed by acute supplementation of ω-3 PUFAs (Fahs et al., 2010; Newens et al., 2011). In contrast, several studies found no differences in flow-mediated dilation between control and treatment groups in hypertensive subjects receiving DHA and EPA capsules (Grenon et al., 2015; Ramirez et al., 2019; Siniarski et al., 2018). In hypertensive individuals receiving ethyl ester capsules, pulse-wave velocity (PWV, another functional measure of endothelial function) appeared to be reduced, but this was not statistically significant (Krantz et al., 2015).

Studies in healthy individuals have generally found no improvement in flow-mediated dilation after ω-3 PUFA treatment (Sanders et al., 2011; Singhal et al., 2013). Interestingly, an acute dose of ω-3 PUFAs in the form of a drink containing fish oil concentrate found ω-3 PUFAs enhance endothelial function independently of NO production (Newens et al., 2015). Moreover, Wu, Mayneris-Perxachs, Lovegrove, Todd, and Yaqoob (2014) studied normotensive subjects at moderate risk for cardiovascular disease and found that a combination of DHA and EPA had no effect on BP, but increased the number of endothelial progenitor cells and decreased the number of endothelial microparticles, without affecting the concentration of circulating NO; this might indicate improved maintenance and repair and decreased damage. As 2017 review (Colussi et al., 2017) concluded that in animal and human studies, ω-3 PUFAs improve the function of both normal and damaged endothelium, mainly through an increase in NO availability, via the activation of eNOS. Despite this, it seems that ω-3 PUFAs do not have large effects on the healthy endothelium in humans except in conditions of stress and that their effects of on the endothelium are predominately in those suffering from CVD.

In addition to the method of delivery, another major limitation to assessing the effect of these fatty acids on the human endothelium is that endothelium-dependent flow-mediated dilation measurements are an indirect measurement, conducted in large arteries where vasodilation is largely mediated via NO. Few investigate other vasodilator mechanisms; there is a lack of investigation of resistance vascular responses and mechanisms including those independent of the endothelium. Indeed, the exact mechanisms of vasodilation are difficult to study in human subjects, and currently, both **in vivo** and in vitro animal studies provide most mechanistic evidence for ω-3 PUFAs action on blood pressure and vasodilatation.

3 | DHA AND EPA VASODILATORY MECHANISMS IN ANIMAL OR IN VITRO STUDIES

3.1 | Overview

Animal studies typically use doses of DHA or EPA (or a combination of both) that result in free fatty acid concentrations of approximately 10–60 μM, obtaining higher concentrations is restricted by lack of solubility or by vehicle effects. However, these doses are clinically relevant as they reflect the concentration of ω-3 PUFAs in their free fatty form present in human plasma after a meal rich in fish oil, which is around 70 μM (Newens et al., 2011).

Vasorelaxation is normally studied on isolated arteries (most often the aorta) following precontraction with a vasoconstrictor. The most commonly used is **U46619**, a mimetic of the powerful vasoconstrictor **TXA_2** (Otsuka, Tanaka, Tanaka, Koike, & Shigenobu, 2005). A large number of studies using different model animals have confirmed that DHA and EPA induce relaxation in isolated arteries following U46619 contraction in a concentration-dependent manner (Hoshi, Tian, Xu, Heinemann, & Hou, 2013; Hoshi et al., 2013; Limbu, Cottrell, & McNeish, 2018; Omura et al., 2001; Sato et al., 2014; Wang, Chai, Lu, & Lee, 2011). Other constricting agents have been used to induce vasoconstriction, such as noradrenaline or high [K^+] solutions (Engler & Engler, 2000; Engler, Engler, Browne, Sun, & Sievers, 2000); with these constrictor agents, the response to ω-3 PUFAs is less consistent. For example, DHA (10 μM) caused complete relaxation of normotensive rat aorta pre-contracted with U46619 but not those pre-constricted with **noradrenaline** (α-**adrenoceptor** stimulation) or high [K^+] (depolarising stimulus) (Sato et al., 2013). In fact, Otsuka et al. (2005) and Sato et al. (2013) both identified that DHA causes greater vasodilation after **TP** receptor-mediated contractions than after α_1-adrenoceptor contractions. The smaller ω-3 PUFA response in the presence of high [K^+] solutions (Sato et al., 2013) may also potentially indicate role for K^+ channels—as previously described for **acetylcholine**-mediated dilatation in rats (Adeagbo & Triggle, 1993; McNeish, Dora, & Garland, 2005; McNeish, Wilson, & Martin, 2001). The further mechanistic implications of high [K^+] will be discussed below.

Multiple studies have used in vivo approaches to study the effects of ω-3 PUFAs on blood pressure with mixed results. Acute administration of intravenous DHA (62.5 μmol/kg) transiently lowered BP in normotensive mice (Hoshi, Wissuwa, et al., 2013). However, when normotensive orchidectomised rats were fed chow containing 2 g/100 g DHA for 2 months (i.e. chronic administration), there was no change in systolic blood pressure (Villalpando et al., 2015). Interestingly chronic feeding of ω-3 PUFAs can prevent **angiotensin II**-induced hypertension in rats (Niazi et al., 2017). Furthermore,

chronic feeding of DHA has no effect on blood pressure in control WKY rats but reduced the development of hypertension in the stroke-prone spontaneously hypertensive rat model (Kimura et al., 2002). These results suggest that ω-3 PUFAs may not have a major impact on blood pressure in healthy animals but have greater effects in models of hypertension, which seems to agree with the human clinical data where effects seem to be larger in hypertension.

3.2 | Endothelial mechanisms—NO production, epoxides and eicosanoids

As discussed above, human studies implicate endothelium-dependent mechanisms, particularly NO, in ω-3 PUFA-mediated relaxation. This also appears to be the case in animal studies, many of which demonstrate ω-3 PUFAs improve the function of both normal and damaged endothelium, mainly through an increase in NO availability, especially via the up-regulation of eNOS (Colussi et al., 2017). However, as in human studies, there is considerable variation in both study methodology, results and reported mechanisms.

When ω-3 PUFAs are included in the diet or supplemented long term, endothelial NO production is often enhanced, as is endothelial function and vasodilation. For example, dietary EPA enhances endothelium-dependent relaxation in porcine coronary arteries (Shimokawa, Aarhus, & Vanhoutte, 1988) and chronic exposure of cultured porcine aortic endothelial cells to EPA increases agonist-induced NO release (Boulanger, Schini, Hendrickson, & Vanhoutte, 1990). Long-term supplementation of ω-3 PUFAs restores reduced NO production in orchidectomised rats (Villalpando et al., 2015) and in the **AngII**-induced hypertension model in rats (Niazi et al., 2017). Chronic fish oil supplementation increases the expression of eNOS at both gene and protein level in the rat aorta with resultant increase in NO bioavailability (Lopez et al., 2004). Short-term administration of ω-3 PUFAs also increases endothelial function; acute administration of EPA evokes NO production in bovine aorta and endothelium removal inhibited this EPA-induced relaxation (Omura et al., 2001). In human umbilical vein endothelial cells (HUVEC), EPA led to translocation of eNOS from caveole fractions to soluble fractions and increased eNOS activity in a concentration-dependent manner (Li et al., 2007). Finally, in porcine coronary arteries and in human mammary arteries from bypass surgery patients, EPA and DHA combined in the ratio 6:1 elicit endothelium-dependent relaxation through formation of NO (Zgheel et al., 2014; Zgheel et al., 2019) and in cultured endothelial cells they induce an increase in ROS (discussed below) (Zgheel et al., 2014). In this latter study, ω-3 PUFAs indirectly induced eNOS phosphorylation at the activator site. In addition, the authors found that a ratio of EPA to DHA of 6:1 and 9:1 has a more potent effect than lower ratios of EPA to DHA, similar ratios of DHA to EPA or either PUFA on its own. Together, these results indicate that these endothelial effects are not limited to long-term supplementation.

In contrast, multiple studies with short-term or acute addition of ω-3 PUFAs to evoke relaxation suggest that the endothelium only plays a small role in DHA or EPA-mediated vasodilation. For example in rat aorta and mesenteric arteries, DHA and EPA induce relaxation, which is only slightly reduced after endothelium removal, is unaffected by inhibition of NOS (Limbu et al., 2018). Effects similar to those previously observed for EPA-induced relaxation in the rat aorta that were not affected by either L-NAME or endothelium removal (Engler et al., 2000). Furthermore, DHA-induced relaxation responses are independent of NO in aorta of spontaneously hypertensive rats (SHR) (Engler & Engler, 2000) and of both NO and the endothelium in Wistar rats (Sato et al., 2013).

Calcium homeostasis is partly regulated by reactive oxygen species (ROS), the major source of which is **NADPH oxides**. In hypertensive blood vessels, increased ROS and oxidation/reduction signalling lead to enhanced calcium signalling, contraction and tone, and increased ROS can reduce NO production; a redox state where pro-oxidants are in excess of anti-oxidants leads to oxidative stress, which leads to arterial remodelling (Touyz et al., 2018). In porcine coronary arteries as well as cultured endothelial cells treated with EPA and DHA rations of 6:1 relaxation was accompanied by an increase in endothelial ROS and was reduced by the anti-oxidant N-acetylcysteine and by inhibitors of intracellular stress, indicating ω-3 PUFAs might have a pro-oxidant effect leading to increased eNOS (Zgheel et al., 2014). In contrast, rats fed a high fructose diet, the addition of fish oils prevented increased oxidative stress (Nyby et al., 2005). Additionally, in human aortic endothelial cells, EPA and DHA reduced ROS and increased the mRNA levels of anti-oxidant molecules (Sakai et al., 2017).

As described in Section 1.3, EPA is metabolised from the ω-3 α-LA and the same enzymes also metabolise ω-6 **linoleic acid** (LA) into ω-6 PUFAs such as arachidonic acid, with LA having a greater bioavailability than α-LA. After synthesis, ω-3s and ω-6s are also necessary for the downstream synthesis of eicosanoids (Figure 2), which play contrasting roles in vascular physiology (Bagga, Wang, Farias-Eisner, Glaspy, & Reddy, 2003). There are three main pathways of eicosanoid synthesis (Figure 2), with ω-3s competing with arachidonic acid for the active sites, **cytochrome P450** enzymes (CYP450), COX and **lipoxygenase** enzymes (LOX) with CYP lipid mediators being the most sensitive to changes in dietary intake of fatty acids (Fer et al., 2008). EPA is metabolized by the COX pathway into 3-series PGs and thromboxanes and by 5-lipoxygenase into 5-series leukotrienes. EPA and DHA are metabolized by CYP450 epoxygenases into fatty epoxides (Wang et al., 2011) and the epoxide molecules cause vasodilation by activating Ca^{2+}-activated K^+ channels, as will be discussed below (Hoshi, Wissuwa, et al., 2013). The anti-hypertensive actions of ω-3 supplements or ω-3-rich foods might occur by reducing the ratio of ω-6 to ω-3, thus balancing the competition with arachidonic acid as a substrate for CYP450 and production of more vasodilator mediators (Tagetti et al., 2015).

An example of a CYP450 DHA-metabolite is epoxydocosapentaenoic acid (EDP), which in porcine coronary arteries activates large conductance Ca^{2+}-activated K^+ channels in VSMCs, leading to hyperpolarisation and vasodilation (Engler et al., 2000). Metabolites of EPA obtained via CYP450, 17(18)-EpETEs, act in

human pulmonary arteries (Morin, Sirois, Echave, Rizcallah, & Rousseau, 2009) and rat cerebral and mesenteric arteries (Hercule et al., 2007) promoting vasodilation. Furthermore, DHA-mediated dilation of rat coronary arteries is reduced upon CYP450 inhibition (Wang et al., 2011). In contrast, some studies have observed that inhibition of CYP450 does not affect DHA-mediated dilation of rat aorta or mesenteric artery (Limbu et al., 2018; Sato et al., 2014). Interestingly, in our study (Limbu et al., 2018), we found that while inhibition of CYP450 did not block DHA-mediated dilation in either aorta or mesenteric artery, it did partially block EPA-mediated dilation, supporting the idea that DHA and EPA likely cause vasodilation by different mechanisms.

Studies from several groups conclude that COX-derived metabolites do not contribute to DHA-induced vasodilation of rat aorta and mesenteric artery (Limbu et al., 2018; Lopez et al., 2004; Sato et al., 2013). Interestingly, the study by Lopez et al. (2004) found that ω-3 PUFAs incorporate into the phospholipids of cellular membranes, leading to changes in eicosanoid metabolites that affect the production of NO. On the other hand, others have reported that ω-3 PUFA vasodilatory effects in resistance vessels are modulated by inhibition of COX (Engler et al., 2000; Engler & Engler, 2000). In aorta from SHR and normotensive Wistar rats (Engler & Engler, 2000) also found involvement of vasodilatory prostanoids at high concentrations of DHA. Furthermore, in orchidectomised rats, DHA reversed the increase in release of the prostanoids TXA_2, PGI_2, $PGF_{2\alpha}$ and PGE_2 induced by orchidectomy (Villalpando et al., 2015); in contrast, in control male rats DHA only decreased PGE_2 release.

As described in Section 1.2, endothelium-derived hyperpolarisation (EDH) is a major vasodilator pathway, especially in resistance arteries. EDH is characterised by the involvement of small- and intermediate-conductance Ca^{2+}-activated K^+ channels in the endothelium (SK_{Ca} and IK_{Ca}, respectively) and large-conductance Ca^{2+}-activated K^+ channels in VSMCs (BK_{Ca}) (Feletou & Vanhoutte, 2007) (Figure 3). In pig coronary arteries, vasodilation to a combination of EPA and DHA seems to involve EDH as combined inhibition of NOS and both SK_{Ca} and IK_{Ca} are required to fully inhibit relaxation (Zgheel et al., 2019). Our group has identified that in aorta and the mesenteric resistance artery, SK_{Ca} inhibition had no effect on vasodilation induced by DHA or by EPA, and additional inhibition of IK_{Ca} led to partial reduction in relaxation only in mesenteric arteries and that inhibition of NOS had no additional effect (Limbu et al., 2018).

It is also possible that ω-3 PUFAs as being able to reduce endothelium-dependent constrictor responses produced by endothelium-derived contracting factors (EDCFs) by competing with arachidonic acid for COXs (Vanhoutte & Tang, 2008). Indeed, this may be the case as age-dependent endothelial dysfunction in rats is reversed in part by ω-3 PUFAs reducing EDCF production (Farooq et al., 2020). While further investigation of the effect on EDCFs is

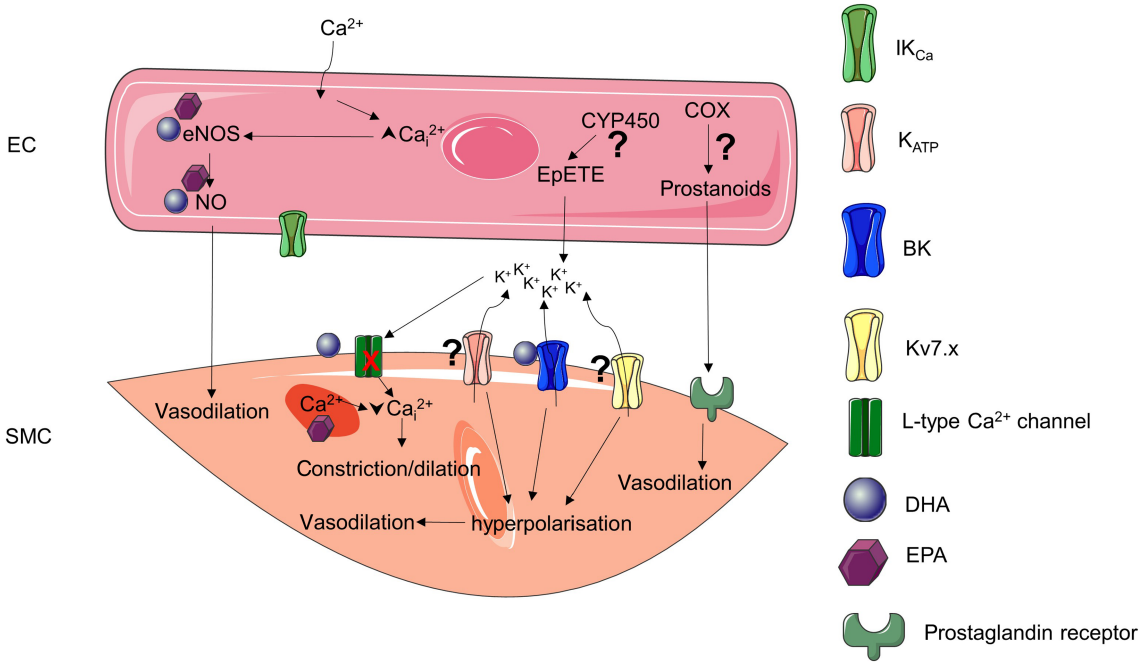

FIGURE 3 Vasodilatory pathways where ω-3 polyunsaturated fatty acids (PUFAs) are believed to be involved. Docosahexaenoic acid (DHA; grey circles) and eicosapentaenoic acid (EPA; purple hexagons) have been suggested to evoke vasodilation by a variety of mechanisms that are shown on the diagram—where the evidence is limited/suggested or contradictory we have indicated this by a "?" symbol. Suggested endothelium-dependent mechanisms include stimulation of endothelial nitric oxide synthase (eNOS) or increasing NO bioavailability (DHA and EPA) and generation metabolites of ω-3 PUFAs by CYP450 or cyclooxygenase (COX) (contradictory results in the literature for both DHA and EPA). Removal of the endothelium often has a limited effect on ω-3 PUFA-induced vasodilation and several potential smooth muscle targets have been identified; these include blockade of L-type Ca^{2+} channels (DHA), activation of K_{ATP} (DHA and EPA), activation of BK_{Ca} (DHA) and putatively K_v7 channels (DHA and EPA) Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License

warranted, these mechanisms are unlikely to contribute to the acute vasodilator effects of ω-3 PUFAs seen in heathy and diseased tissues.

Despite the differences in findings between studies, it is clear that the endothelium, NO and COX contribute to ω-3 PUFA-mediated vasodilation upon chronic exposure but they do not seem to be involved in the main vasodilatory mechanisms upon acute exposure to ω-3 PUFAs in healthy tissues (see Figure 3 for a summary of the mechanisms). As discussed above, an alternative interpretation is that endothelium-dependent mechanisms only have a major contribution to ω-3 PUFA-induced relaxations in arteries where endothelial function is already impaired.

3.3 | The role of smooth muscle cells: hyperpolarising effects and activation of K^+ channels

Many studies performed in blood vessels isolated from animals show that DHA- or EPA-mediated vasodilation occurs even after NOS inhibition or endothelial removal (Limbu et al., 2018; Sato et al., 2013; Singh, Kathirvel, Choudhury, Garg, & Mishra, 2010), indicating a direct role on VSMCs. Moreover, as discussed above, several human studies show that in subjects where DHA and EPA reduced BP, the effect on endothelial function was unclear, as measured using flow-mediated dilation or Doppler flow analyses (Newens et al., 2011; Ramirez et al., 2019; Singhal et al., 2013). Very few human studies have investigated the specific role of SMCs. Typically senescent, the growth and proliferation of VSMCs are manifestations of hypertension or vascular dysfunction (Lacolley et al., 2017. Studies on isolated human VSMCs found that ω-3 PUFAs might affect SMC proliferation (Mizutani et al., 1997), migration (Goua et al., 2008; Mizutani et al., 1997) and contraction (Zhang, Zhang, Lyu, Kishi, & Kobayashi, 2017). Indeed, there is strong evidence of a role for VSMCs in ω-3 PUFA-mediated dilation in animal studies (see below).

VSMCs control vessel diameter and in hypertensive vessels VSMCs are dysfunctional; the constantly rigid vascular wall and increased contraction lead to increased blood pressure. A major controller of contraction in VSMCs, especially in smaller resistance arteries, is Ca^{2+} homeostasis, which is largely controlled by depolarisation and resultant Ca^{2+} influx and modulated by hyperpolarising K^+ efflux (Nelson & Quayle, 1995).

As Ca^{2+} influx via **L-type Ca^{2+} channels** is one of the major mechanisms in vascular contraction, it is possible that their inhibition could be the reason why ω-3 PUFAs can evoke vasodilation. Extensive research has shown that L-type calcium channels can be inhibited by PUFAs, as reviewed by Elinder and Liin (2017). DHA at high concentrations has been shown to negatively regulate L-type Ca^{2+} channels in aortas from SHRs (Engler & Engler, 2000), similarly EPA-induced relaxation might rely on reduction of extracellular Ca^{2+} influx in the sheep pulmonary circulation (Singh et al., 2010). However, few other studies implicate this channel type in ω-3 PUFA-induced relaxation, for example in rat aorta EPA-induced relaxation does not involve influx of Ca^{2+} through L-type Ca^{2+} channel (Engler et al., 2000). Furthermore, as mentioned above, ω-3 PUFAs fail to evoke large relaxations in conditions where constriction is elicited by depolarizing concentrations of $[K^+]$ where L-Type Ca^{2+} channels would be expected to be open (Sato et al., 2013; Sato et al., 2014). Therefore, there is only weak evidence that inhibition of Ca^{2+} channels is a primary mechanism in ω-3 PUFA-induced vascular relaxation. The results of Sato et al. (2013) also indicate there could be a significant role for the hyperpolarising K^+ efflux, which would normally reduce the open probability of L-type Ca^{2+} channels, causing relaxation as these conditions of high $[K^+]$ effectively abolish any dilation dependent upon potassium channels by altering their reversal potential (Adeagbo & Triggle, 1993) (Figure 3).

In contrast to Ca^{2+} channels, K^+ channels are often activated by PUFAs (Elinder & Liin, 2017), an effect that would elicit vasodilation. Some of the major classes of potassium channels in VSMCs linked to the control of vascular tone are Ca^{2+}-activated (e.g., BK_{Ca}), **voltage-gated** (including the Kv7 family), **ATP-sensitive and inward rectifier Potassium channels**. Opening of potassium channels leads to efflux of K^+ and repolarisation/hyperpolarisation of the membrane resulting closure of voltage-activated Ca^{2+} channels and subsequent vasodilation (Nelson & Quale, 1995). Voltage-gated channels are activated by depolarisation and in the case of BK_{Ca} channels regulated by other factors independently, such as calcium.

Reviews have offered insights of how ω-3 PUFAs are believed to interact with several types of K^+ channels (Elinder & Liin, 2017; Moreno, de la Cruz, & Valenzuela, 2016). Briefly, the structure of ω-3 PUFAs is important for its effect on ion channels, including the length of the carbon chain and the degree of unsaturation, and we will discuss several structural characteristics of DHA and EPA relevant for their vasodilatory effect. Furthermore, reviews by Nieves-Cintron, Syed, Nystoriak, and Navedo (2018), Sobey (2001) and Baker (2000) have reviewed the functions of different ion channels in the regulation of blood pressure. Here, we will discuss how vasodilatory effects of ω-3 PUFAs are potentially mediated via opening of BK_{Ca} channels, the Kv7 family of voltage-activated potassium channels and ATP-sensitive potassium channels (K_{ATP}), all of which have been implicated in ω-3 PUFA-mediated responses.

3.3.1 | BK_{Ca} channels

BK_{Ca} channels are densely expressed in VSMCs, have a high conductance and are key to controlling resting membrane potential (Lai et al., 2009). Multiple studies offer evidence that ω-3 PUFAs act on BK_{Ca} to cause hyperpolarisation. For example, in patients with pulmonary arterial hypertension, the pulmonary arterial SMC membrane potential is depolarised compared to healthy cells; DHA activates BK_{Ca} and returns the resting membrane potential to levels observed in healthy subjects (Nagaraj et al., 2016). Blocking BK_{Ca} reduces DHA-induced relaxation in these arteries and in mice, lacking the α-subunit of BK_{Ca}, DHA-induced relaxation was reduced (Nagaraj et al., 2016). Inhibition of BK_{Ca} leads to a significant reduction in DHA-mediated relaxation in rat aorta and mesenteric artery (Limbu et al., 2018). In rat coronary artery, DHA produced BK_{Ca} mediated-vasodilation and

reversibly increased outward BK_{Ca} currents (Wang et al., 2011); these DHA-dependent effects on BK_{Ca} were prevented when cells were treated with inhibitors of CYP450, indicating that CYP450-derived metabolites of DHA (and EPA) also act on BK_{Ca}. This is consistent with the effects observed in pig coronary arteries treated with DHA-derived EDPs (Ye et al., 2002), as well as EPA-derived 17(18)-EpETEs in human pulmonary artery (Morin et al., 2009) and rat mesenteric arteries (Hercule et al., 2007).

The structure of ω-3 PUFAs is important for their effect on BK_{Ca} channels and it is thought to involve electrostatic or "lipoelectric" interactions. The pore-forming Slo1 subunit of BK_{Ca} plays a role in the hypotensive effects of DHA in aortic SMCs. DHA reversibly activates Slo1 when in association with the auxiliary subunit β1 and accelerates its activation kinetics in Ca^{2+}-free cell-free patches, suggesting that DHA binds directly to the channel as opposed to modulating the channel via a signalling cascade (Hoshi, Wissuwa, et al., 2013). The mechanism through which DHA opens this conduction gate involves destabilising its closed conformation without the need for voltage sensors or Ca^{2+} binding (Hoshi, Wissuwa, et al., 2013). Interestingly, the non-polar DHA ethyl-ester does not replicate the effects of DHA on current, activation kinetics or animal blood pressure, indicating that both the aliphatic tail and the polar carboxylic headgroups are important; the same is true for EPA versus EPA ethyl-esters (Hoshi, Wissuwa, et al., 2013). A comprehensive screening of mutations (Hoshi, Tian, et al., 2013) identified residues fundamental for the response to DHA. Positively charged Arg11 and positively charged Cys18 (or any pair of oppositely charged Arg and Glu, Lys and Asp, and Lys and Glu) in the N terminus and transmembrane domain of β1, respectively. Interestingly, other groups have proposed electrostatic interactions in the S4 pore forming regions of Slo1 also contribute to BK_{Ca} activation by DHA (Tian et al., 2016).

There is very little evidence of ω-3 PUFAs having effects on other members of the K_{Ca} family. As described above, in rat mesenteric artery lacking NOS production, we found inhibition of SK_{Ca} had no effect on DHA or EPA-induced relaxation, with only slight decrease in relaxation occurring after inhibition of IK_{Ca} channels (Limbu et al., 2018). Here, additional inhibition of BK_{Ca} led to significant inhibition of relaxation, supporting a direct action of DHA on VSMCs by acting on BK_{Ca} channels (Limbu et al., 2018). However, it may also be possible that ω-3 PUFAs can act indirectly to activate K_{Ca}. For example, in human endothelial cells EPA and its metabolite 17,18-EEQ enhance **TRPV4** (transient receptor potential cation channel subfamily V member 4) currents (Caires et al., 2017). TRPV4 channels are involved in reducing BP, with activation leading to an increase in endothelial intracellular Ca^{2+}, the release of NO and hyperpolarisation via activation of K_{Ca} (Earley et al., 2009).

3.3.2 | K_v7 channels

K_v7 channels are widely expressed in the cardiovascular system; **$K_v7.1$**, **7.4** and **7.5** are the predominate subtypes expressed in VSMCs, with $K_v7.4$ and 7.5 thought to be key in the regulation of vascular tone and relaxation (Fosmo & Skraastad, 2017; Stott et al., 2014). In terms of interaction with ω-3 PUFAs, $K_v7.1$ is the subtype most studied; as well being expressed in vascular tissue, it forms the pore subunit of the cardiac I_{KS} channel that is a major component of repolarisation in cardiac cells (Liin et al., 2015). ω-3 PUFAs activate $K_v7.1$ channels in rat cardiac myocytes and in xenopus oocytes (Elinder & Liin, 2017; Liin et al., 2015), leading to increased conductance and more negative voltage activation values.

To activate $K_v7.1$, the negatively charged headgroup and the polyunsaturated acyl tale of DHA and EPA are critical for increasing current amplitudes and shifting conductance versus voltage curves (IV curves) in the negative direction (Larsson, Larsson, & Liin, 2018). Similar to that observed in BK_{Ca} channels, the action of ω-3 PUFAs on $K_v7.1$ is dependent on an electrostatic "lipoelectric" interaction, with the S4 voltage sensing domain (Figure 4), leading to S4 movement and channel opening (Larsson et al., 2018). The PUFAs bind to the outer leaflet of the cell membrane close to the transmembrane segments S3 and S4 (Figure 4). The negatively charged DHA carboxylic acid head group enhances $K_v7.1$ opening by electrostatic interaction with the positively charged S4 helix, shifting the voltage dependence towards more negative voltages. The outermost positive charge of S4 and a non-polar amino acid in the S3-S4 loop (R228 and G219, respectively, see Figure 4) contribute to this DHA affinity (Liin et al., 2015). Structural characteristics of fatty acids required to do this are reviewed in depth by Elinder and Liin (2017) and Moreno et al. (2016), briefly at least two double bonds with all cis-geometry are required to embed into a membrane hydrophobic pocket located near the voltage sensing S4 domain of $K_v7.1$, where they can influence the segment movements and affect channel gating (Borjesson, Hammarstrom, & Elinder, 2008; Elinder & Liin, 2017). Therefore, similar to what is observed at BK_{Ca} channels, both the negatively charged headgroup and the polyunsaturated acyl tale of DHA and EPA are fundamental for increasing current amplitudes and negatively shifting IV curves, properties not shared with uncharged, methyl esters and ethyl esters (Liin et al., 2015).

Despite extensive evidence that ω-3 PUFAs can activate $K_v7.1$, to date, no group has investigated the possibility that these channels or other K_v7 subtypes are involved in vascular responses to ω-3 PUFAs. This is an exciting potential avenue of research as the Kv7 family is very highly conserved across members and species, particularly in the S4 voltage sensing domain required for the activation of $K_v7.1$ (Figure 4). Indeed, the amino acid residues found to be involved, as well as the putative ω-3 PUFA binding domain of $K_v7.1$ are conserved between $K_v7.1$, 7.4 and 7.5 (Figure 4). Therefore, it is possible, if not likely, that ω-3 PUFA also activate the subtypes implicated in regulation of vascular tone, relaxation and regulation of blood pressure, that is $K_v7.4$ and $K_v7.5$ (Barrese et al., 2018; Stott et al., 2014).

3.3.3 | K_{ATP} channels

K_{ATP} channels are typically activated by an increase in the ratio of ADP to ATP. Vascular K_{ATP} channels are a predominantly smooth

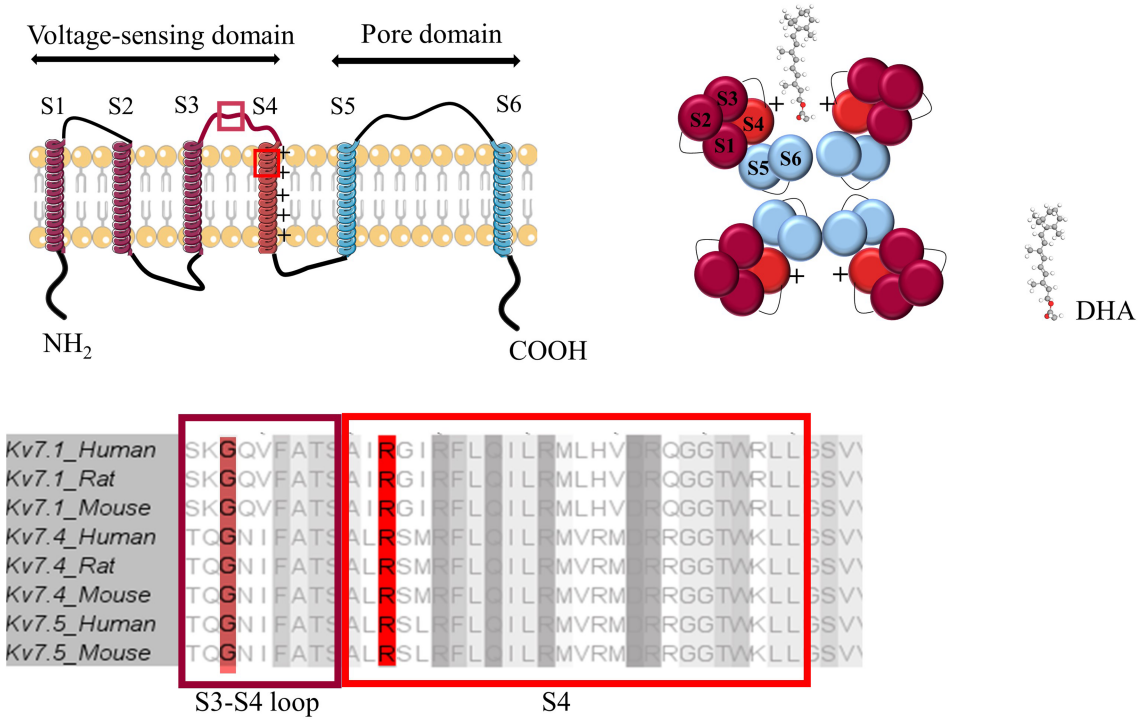

FIGURE 4 Putative ω-3 polyunsaturated fatty acid (PUFA) binding domains in $K_v7.1$, $K_v7.4$ and $K_v7.5$ are highly conserved across humans, rats and mice. The tertiary and quaternary structure of $K_v7.1$ inferred from the crystal structure of $K_v1.2$ (Smith, Vanoye, George, Meiler, & Sanders, 2007). This channel is a tetramer consisting of four identical monomers. Each monomer consists of 6 subunits: subunits S1-S4 form the voltage sensing domain and subunits S5–S6 form the pore domain. The outermost positive charge of S4 is an arginine (R228), marked in a bright red square and contributes to docosahexaenoic acid (DHA) affinity. This essential residue is conserved across species and channel subtype. The non-polar glycine in the S3-S4 loop (G219), also marked in a dark red square, which is also essential for DHA affinity and is also highly conserved Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License

muscle cell potassium channel shown to be involved in responses to many vasodilators (Kubo, Quayle, & Standen, 1997; Nelson, Huang, Brayden, Hescheler, & Standen, 1990; Quayle, Nelson, & Standen, 1997; Quinn, Giblin, & Tinker, 2004). The major vascular K_{ATP} consists of the **Kir6.1** pore subunit and SUR2B accessory subunit, but as opposed to channels consisting of SUR2B and **Kir6.2**, do not open in the absence of intracellular ATP (Yamada et al., 1997). Global deletion of either channel subunit leads to hypertension and coronary artery vasospasm leading to death due to lack of hyperpolarising currents (Miki et al., 2002) (Chutkow et al., 2002). Furthermore, mutations in K_{ATP} are associated with cardiac dysfunction in patients (Haissaguerre et al., 2009; Medeiros-Domingo et al., 2010; Tester et al., 2011). Vascular K_{ATP} channels are also a currently exploited target for pharmacological regulation of blood pressure; agents that act on these channels such as **minoxidil** (Knutsen et al., 2018) and **pinacidil** (Friedel & Brogden, 1990) are used clinically to treat hypertension and certain formulations show promise for the treatment of ischaemic stroke (Sheth et al., 2018).

K_{ATP} channels are expressed both in the endothelium and in SMCs, and studies suggest that knocking out in either tissue has detrimental effects on coronary artery circulation (Kakkar et al., 2006; Malester et al., 2007). As stated above, vasodilators seem to stimulate these channels; for example in rabbit mesenteric arteries, NO leads to hyperpolarisation in SMCs by activating K_{ATP} channels (Murphy & Brayden, 1995). Prostanoids also activate K_{ATP} channels, as seen with PGs PGE_0 (Hide, Ney, Piper, Thiemermann, & Vane, 1995) and **PGE_1** (Eguchi et al., 2007; Ney & Feelisch, 1995). As dietary intake of fish oils alters prostanoid production (Chin, Gust, & Dart, 1993) and ω-3 PUFAs themselves are substrates for production of prostanoids that are likely to activate K_{ATP}, they are an attractive potential mechanism for ω-3 PUFA-induced relaxation.

Despite this, very few studies have investigated the role of K_{ATP} channels in ω-3 PUFA-mediated vasodilation. In rat aorta, DHA and EPA-derived prostanoids induced **glibenclamide** (a K_{ATP} selective blocker)-sensitive vasodilation in an endothelium-independent manner (Engler & Engler, 2000). Likewise, K_{ATP} inhibition using PNU37883A suppresses DHA-induced relaxation in rat aorta with the endothelium removed (Sato et al., 2014). In rats, orchiectomy reduces vasodilatory responses stimulated by K_{ATP} channel openers, an effect that is recovered in DHA-fed animals (Villalpando et al., 2015). Conversely, Wang et al. (2011) found that DHA reversibly increased outward K^+ current in rat VSMCs and this effect was reduced when BK_{Ca}, IK_{Ca}, SK_{Ca} and Kv, channels were inhibited, but not when K_{ATP} channels were inhibited using glibenclamide.

The mechanism by which ω-3 PUFA might activate K_{ATP} is not clear, neither is whether the mechanism is like the apparent electrostatic "lipoelectric" effects observed in BK_{Ca} and K_V7 channels discussed above. Interestingly, in mouse pancreatic β-cells,

polyunsaturated acyl-CoAs (the intracellular esters of free fatty acids) of PUFAs, but not saturated fatty acids activate these pancreatic K_{ATP} channels in a mechanism dependent on electrostatic interactions with acyl CoA (Riedel & Light, 2005). An alternative mechanism that may explain apparent ω-3 PUFA activation of K_{ATP} is that ω-3 PUFAs have been found to activate PKA in human adipocytes and rat cardiac cells (Mies, Shlyonsky, Goolaerts, & Sariban-Sohraby, 2004; Tai et al., 2009); PKA is known to induce vasodilation by activating vascular K_{ATP} (Yang et al., 2008). Regardless, it appears that K_{ATP} channels in vascular SMCs may play a role in ω-3 PUFA-mediated vasodilation (see Figure 3 for a summary of the mechanisms) and further research needs to be conducted to investigate the role of these channels in regulation of vascular tone and blood pressure.

4 | CONCLUSIONS

Human studies indicate that ω-3 PUFAs lower blood pressure particularly in hypertensive individuals and there is sufficient data to suggest that they do this, in part, by having a beneficial effect on dysfunctional endothelium and NO-dependent responses. Mechanistic insights from both human and animal studies indicate that despite a role for the endothelium and NO in ω-3 PUFA mediated vasodilation, there are clearly other mechanisms involved. VSMC ion channels seem to contribute to the ω-3 PUFA vasodilator responses, consistent with the large body of knowledge regarding PUFA interactions with such channels. Unfortunately, the precise mechanisms remain elusive. Therefore, a systematic pharmacological strategy to characterise ω-3 PUFA vasodilatory properties is required to progress our understanding of the key ω-3 PUFA-induced vasodilatory mechanisms. We propose such pharmacological investigations require in vitro data from multiple vascular beds coupled with in vivo data. Furthermore, characterisation of effects on different specific ion channels is required, with K_v7 and K_{ATP} being particularly interesting novel targets. These studies should include biophysical characterisation coupled with genetic manipulation of the channels to isolate which amino acids are required to stimulate channel activation by ω-3 PUFAs. It is also vital to assess both natural and synthetic structural analogues of ω-3 PUFAs to elucidate structure/activity relationships for evoking vasodilator responses. Indeed, exploiting known structural determinants for ω-3 PUFA action such as chain length, degree of saturation and charge of the polar head could lead to development of novel compounds with the potential for treating cardiovascular diseases such as hypertension.

4.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

ACKNOWLEDGEMENTS

There is no new novel data in this review article. Cristiana Bercea is a Postdoctoral Research Associate, funded by the British Heart Foundation PG/19/57/34568. C.B. researched, wrote, edited and designed the figures for the manuscript, G.C. wrote and edited the manuscript, F.T. wrote and edited the manuscript. A.Mc. researched, wrote and edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

No material has been reproduced from other sources.

ETHICS APPROVAL

There was no requirement of ethical approval for this review article.

REFERENCES

AbuMweis, S., Jew, S., Tayyem, R., & Agraib, L. (2018). Eicosapentaenoic acid and docosahexaenoic acid containing supplements modulate risk factors for cardiovascular disease: A meta-analysis of randomised placebo-control human clinical trials. *Journal of Human Nutrition and Dietetics*, *31*(1), 67–84. https://doi.org/10.1111/jhn.12493

Adeagbo, A. S., & Triggle, C. R. (1993). Varying extracellular [K^+]: A functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *Journal of Cardiovascular Pharmacology*, *21*(3), 423–429. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7681503. https://doi.org/10.1097/00005344-199303000-00011

Agabiti-Rosei, C., Paini, A., De Ciuceis, C., Withers, S., Greenstein, A., Heagerty, A. M., & Rizzoni, D. (2018). Modulation of vascular reactivity by perivascular adipose tissue (PVAT). *Current Hypertension Reports*, *20*(5), 44. https://doi.org/10.1007/s11906-018-0835-5

Alexander, S. P. H., Mathie, A., Peters, J. A., Veale, E. L., Striessnig, J., Kelly, E., … Collaborators, C. (2019). The concise guide to pharmacology 2019/20: Ion channels. *British Journal of Pharmacology*, *176*(Suppl 1), S142–S228. https://doi.org/10.1111/bph.14749

Arterburn, L. M., Hall, E. B., & Oken, H. (2006). Distribution, interconversion, and dose response of n-3 fatty acids in humans. *The American Journal of Clinical Nutrition*, *83*(6 Suppl), 1467S–1476S. https://doi.org/10.1093/ajcn/83.6.1467S

Asztalos, I. B., Gleason, J. A., Sever, S., Gedik, R., Asztalos, B. F., Horvath, K. V., … Schaefer, E. J. (2016). Effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular disease risk factors: A randomized clinical trial. *Metabolism*, *65*(11), 1636–1645. https://doi.org/10.1016/j.metabol.2016.07.010

Bagga, D., Wang, L., Farias-Eisner, R., Glaspy, J. A., & Reddy, S. T. (2003). Differential effects of prostaglandin derived from omega-6 and omega-3 polyunsaturated fatty acids on COX-2 expression and IL-6 secretion. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(4), 1751–1756. https://doi.org/10.1073/pnas.0334211100

Baker, E. H. (2000). Ion channels and the control of blood pressure. *British Journal of Clinical Pharmacology*, *49*(3), 185–198. https://doi.org/10.1046/j.1365-2125.2000.00159.x

Bang, H. O., Dyerberg, J., & Sinclair, H. M. (1980). The composition of the Eskimo food in north western Greenland. *The American Journal of Clinical Nutrition*, *33*(12), 2657–2661. https://doi.org/10.1093/ajcn/33.12.2657

Barrese, V., Stott, J. B., Figueiredo, H. B., Aubdool, A. A., Hobbs, A. J., Jepps, T. A., … Greenwood, I. A. (2018). Angiotensin II promotes KV7.4

channels degradation through reduced interaction with HSP90 (heat shock protein 90). *Hypertension*, *71*(6), 1091–1100. https://doi.org/10.1161/HYPERTENSIONAHA.118.11116

Birukov, K. G. (2009). Cyclic stretch, reactive oxygen species, and vascular remodeling. *Antioxidants & Redox Signaling*, *11*(7), 1651–1667. https://doi.org/10.1089/ARS.2008.2390

Borjesson, S. I., Hammarstrom, S., & Elinder, F. (2008). Lipoelectric modification of ion channel voltage gating by polyunsaturated fatty acids. *Biophysical Journal*, *95*(5), 2242–2253. https://doi.org/10.1529/biophysj.108.130757

Boulanger, C., Schini, V. B., Hendrickson, H., & Vanhoutte, P. M. (1990). Chronic exposure of cultured endothelial cells to eicosapentaenoic acid potentiates the release of endothelium-derived relaxing factor(s). *British Journal of Pharmacology*, *99*(1), 176–180. https://doi.org/10.1111/j.1476-5381.1990.tb14673.x

Brozovich, F. V., Nicholson, C. J., Degen, C. V., Gao, Y. Z., Aggarwal, M., & Morgan, K. G. (2016). Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. *Pharmacological Reviews*, *68*(2), 476–532. https://doi.org/10.1124/pr.115.010652

Caires, R., Sierra-Valdez, F. J., Millet, J. R. M., Herwig, J. D., Roan, E., Vasquez, V., & Cordero-Morales, J. F. (2017). Omega-3 fatty acids modulate TRPV4 function through plasma membrane remodeling. *Cell Reports*, *21*(1), 246–258. https://doi.org/10.1016/j.celrep.2017.09.029

Campbell, W. B., & Falck, J. R. (2007). Arachidonic acid metabolites as endothelium-derived hyperpolarizing factors. *Hypertension*, *49*(3), 590–596. https://doi.org/10.1161/01.HYP.0000255173.50317.fc

Casanova, M. A., Medeiros, F., Trindade, M., Cohen, C., Oigman, W., & Neves, M. F. (2017). Omega-3 fatty acids supplementation improves endothelial function and arterial stiffness in hypertensive patients with hypertriglyceridemia and high cardiovascular risk. *Journal of the American Society of Hypertension*, *11*(1), 10–19. https://doi.org/10.1016/j.jash.2016.10.004

Chen, K., Pittman, R. N., & Popel, A. S. (2008). Nitric oxide in the vasculature: Where does it come from and where does it go? A quantitative perspective. *Antioxidants & Redox Signaling*, *10*(7), 1185–1198. https://doi.org/10.1089/ars.2007.1959

Chin, J. P., Gust, A. P., & Dart, A. M. (1993). Indomethacin inhibits the effects of dietary supplementation with marine oils on vasoconstriction of human forearm resistance vessels in vivo. *Journal of Hypertension*, *11*(11), 1229–1234. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8301104

Chutkow, W. A., Pu, J., Wheeler, M. T., Wada, T., Makielski, J. C., Burant, C. F., & McNally, E. M. (2002). Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K (ATP) channels. *The Journal of Clinical Investigation*, *110*(2), 203–208. https://doi.org/10.1172/JCI15672

Colussi, G., Catena, C., Novello, M., Bertin, N., & Sechi, L. A. (2017). Impact of omega-3 polyunsaturated fatty acids on vascular function and blood pressure: Relevance for cardiovascular outcomes. *Nutrition, Metabolism, and Cardiovascular Diseases*, *27*(3), 191–200. https://doi.org/10.1016/j.numecd.2016.07.011

Cottin, S. C., Sanders, T. A., & Hall, W. L. (2011). The differential effects of EPA and DHA on cardiovascular risk factors. *The Proceedings of the Nutrition Society*, *70*(2), 215–231. https://doi.org/10.1017/S0029665111000061

Diez, J. (2007). Arterial stiffness and extracellular matrix. *Advances in Cardiology*, *44*, 76–95. https://doi.org/10.1159/000096722

Durand, M. J., & Gutterman, D. D. (2013). Diversity in mechanisms of endothelium-dependent vasodilation in health and disease. *Microcirculation*, *20*(3), 239–247. https://doi.org/10.1111/micc.12040

Earley, S., Pauyo, T., Drapp, R., Tavares, M. J., Liedtke, W., & Brayden, J. E. (2009). TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. *American Journal of Physiology. Heart and Circulatory Physiology*, *297*(3), H1096–H1102. https://doi.org/10.1152/ajpheart.00241.2009

Eguchi, S., Kawano, T., Tanaka, K., Yasui, S., Mawatari, K., Takahashi, A., … Nakajo, N. (2007). Effects of prostaglandin E1 on vascular ATP-sensitive potassium channels. *Journal of Cardiovascular Pharmacology*, *50*(6), 686–691. https://doi.org/10.1097/FJC.0b013e3181583d9b

Elinder, F., & Liin, S. I. (2017). Actions and mechanisms of polyunsaturated fatty acids on voltage-gated ion channels. *Frontiers in Physiology*, *8*(43). https://doi.org/10.3389/fphys.2017.00043

Engler, M. B., & Engler, M. M. (2000). Docosahexaenoic acid–induced vasorelaxation in hypertensive rats: Mechanisms of action. *Biological Research for Nursing*, *2*(2), 85–95. https://doi.org/10.1177/109980040000200202

Engler, M. B., Engler, M. M., Browne, A., Sun, Y. P., & Sievers, R. (2000). Mechanisms of vasorelaxation induced by eicosapentaenoic acid (20:5n-3) in WKY rat aorta. *British Journal of Pharmacology*, *131*(8), 1793–1799. https://doi.org/10.1038/sj.bjp.0703754

Fahs, C. A., Yan, H., Ranadive, S., Rossow, L. M., Agiovlasitis, S., Wilund, K. R., & Fernhall, B. (2010). The effect of acute fish-oil supplementation on endothelial function and arterial stiffness following a high-fat meal. *Applied Physiology, Nutrition, and Metabolism*, *35*(3), 294–302. https://doi.org/10.1139/H10-020

Farooq, M. A., Gaertner, S., Amoura, L., Niazi, Z. R., Park, S. H., Qureshi, A. W., … Auger, C. (2020). Intake of omega-3 formulation EPA:DHA 6:1 by old rats for 2 weeks improved endothelium-dependent relaxations and normalized the expression level of ACE/-AT1R/NADPH oxidase and the formation of ROS in the mesenteric artery. *Biochemical Pharmacology*, *173*, 113749. https://doi.org/10.1016/j.bcp.2019.113749

Feletou, M., & Vanhoutte, P. M. (2007). Endothelium-dependent hyperpolarizations: Past beliefs and present facts. *Annals of Medicine*, *39*(7), 495–516. https://doi.org/10.1080/07853890701491000

Fer, M., Dreano, Y., Lucas, D., Corcos, L., Salaun, J. P., Berthou, F., & Amet, Y. (2008). Metabolism of eicosapentaenoic and docosahexaenoic acids by recombinant human cytochromes P450. *Archives of Biochemistry and Biophysics*, *471*(2), 116–125. https://doi.org/10.1016/j.abb.2008.01.002

Fosmo, A. L., & Skraastad, O. B. (2017). The Kv7 channel and cardiovascular risk factors. *Frontiers in Cardiovascular Medicine*, *4*(75). https://doi.org/10.3389/fcvm.2017.00075

Friedel, H. A., & Brogden, R. N. (1990). Pinacidil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in the treatment of hypertension. *Drugs*, *39*(6), 929–967. https://doi.org/10.2165/00003495-199039060-00008

Garcia-Lopez, S., Villanueva Arriaga, R. E., Najera Medina, O., Rodriguez Lopez, C. P., Figueroa-Valverde, L., Cervera, E. G., … Rosas-Nexticapa, M. (2016). One month of omega-3 fatty acid supplementation improves lipid profiles, glucose levels and blood pressure in overweight schoolchildren with metabolic syndrome. *Journal of Pediatric Endocrinology & Metabolism*, *29*(10), 1143–1150. https://doi.org/10.1515/jpem-2015-0324

Giles, T. D., Sander, G. E., Nossaman, B. D., & Kadowitz, P. J. (2012). Impaired vasodilation in the pathogenesis of hypertension: Focus on nitric oxide, endothelial-derived hyperpolarizing factors, and prostaglandins. *Journal of Clinical Hypertension (Greenwich, Conn.)*, *14*(4), 198–205. https://doi.org/10.1111/j.1751-7176.2012.00606.x

Goua, M., Mulgrew, S., Frank, J., Rees, D., Sneddon, A. A., & Wahle, K. W. (2008). Regulation of adhesion molecule expression in human endothelial and smooth muscle cells by omega-3 fatty acids and conjugated linoleic acids: Involvement of the transcription factor NF-κB? *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *78*(1), 33–43. https://doi.org/10.1016/j.plefa.2007.10.004

Goyens, P. L., Spilker, M. E., Zock, P. L., Katan, M. B., & Mensink, R. P. (2005). Compartmental modeling to quantify α-linolenic acid conversion after longer term intake of multiple tracer boluses. *Journal of Lipid*

Research, *46*(7), 1474–1483. https://doi.org/10.1194/jlr.M400514-JLR200

Grenon, S. M., Owens, C. D., Nosova, E. V., Hughes-Fulford, M., Alley, H. F., Chong, K., … Conte, M. S. (2015). Short-term, high-dose fish oil supplementation increases the production of omega-3 fatty acid-derived mediators in patients with peripheral artery disease (the OMEGA-PAD I trial). *Journal of the American Heart Association*, *4*(8), e002034. https://doi.org/10.1161/JAHA.115.002034

Grimsgaard, S., Bonaa, K. H., Hansen, J. B., & Myhre, E. S. (1998). Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans. *The American Journal of Clinical Nutrition*, *68*(1), 52–59. https://doi.org/10.1093/ajcn/68.1.52

Haissaguerre, M., Chatel, S., Sacher, F., Weerasooriya, R., Probst, V., Loussouarn, G., … Schott, J. J. (2009). Ventricular fibrillation with prominent early repolarization associated with a rare variant of KCNJ8/KATP channel. *Journal of Cardiovascular Electrophysiology*, *20*(1), 93–98. https://doi.org/10.1111/j.1540-8167.2008.01326.x

Hercule, H. C., Salanova, B., Essin, K., Honeck, H., Falck, J. R., Sausbier, M., … Gollasch, M. (2007). The vasodilator 17,18-epoxyeicosatetraenoic acid targets the pore-forming BK α channel subunit in rodents. *Experimental Physiology*, *92*(6), 1067–1076. https://doi.org/10.1113/expphysiol.2007.038166

Hide, E. J., Ney, P., Piper, J., Thiemermann, C., & Vane, J. R. (1995). Reduction by prostaglandin E1 or prostaglandin E0 of myocardial infarct size in the rabbit by activation of ATP-sensitive potassium channels. *British Journal of Pharmacology*, *116*(5), 2435–2440. https://doi.org/10.1111/j.1476-5381.1995.tb15092.x

Hixon, M. L., & Gualberto, A. (2003). Vascular smooth muscle polyploidization—From mitotic checkpoints to hypertension. *Cell Cycle*, *2*(2), 105–110. https://doi.org/10.4161/cc.2.2.341

Hoshi, T., Tian, Y., Xu, R., Heinemann, S. H., & Hou, S. (2013). Mechanism of the modulation of BK potassium channel complexes with different auxiliary subunit compositions by the omega-3 fatty acid DHA. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(12), 4822–4827. https://doi.org/10.1073/pnas.1222003110

Hoshi, T., Wissuwa, B., Tian, Y., Tajima, N., Xu, R., Bauer, M., … Hou, S. (2013). Omega-3 fatty acids lower blood pressure by directly activating large-conductance Ca^{2+}-dependent K^+ channels. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(12), 4816–4821. https://doi.org/10.1073/pnas.1221997110

Hussein, N., Ah-Sing, E., Wilkinson, P., Leach, C., Griffin, B. A., & Millward, D. J. (2005). Long-chain conversion of [^{13}C]linoleic acid and α-linolenic acid in response to marked changes in their dietary intake in men. *Journal of Lipid Research*, *46*(2), 269–280. https://doi.org/10.1194/jlr.M400225-JLR200

Iketani, T., Takazawa, K., & Yamashina, A. (2013). Effect of eicosapentaenoic acid on central systolic blood pressure. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *88*(2), 191–195. https://doi.org/10.1016/j.plefa.2012.11.008

Innes, J. K., & Calder, P. C. (2018). The differential effects of eicosapentaenoic acid and docosahexaenoic acid on cardiometabolic risk factors: A systematic review. *International Journal of Molecular Sciences*, *19*(2), 532. https://doi.org/10.3390/ijms19020532

Innes, J. K., & Calder, P. C. (2020). Marine omega-3 (N-3) fatty acids for cardiovascular health: An update for 2020. *International Journal of Molecular Sciences*, *21*(4), 1362. https://doi.org/10.3390/ijms21041362

Jacobson, T. A., Glickstein, S. B., Rowe, J. D., & Soni, P. N. (2012). Effects of eicosapentaenoic acid and docosahexaenoic acid on low-density lipoprotein cholesterol and other lipids: A review. *Journal of Clinical Lipidology*, *6*(1), 5–18. https://doi.org/10.1016/j.jacl.2011.10.018

Jain, A. P., Aggarwal, K. K., & Zhang, P. Y. (2015). Omega-3 fatty acids and cardiovascular disease. *European Review for Medical and Pharmacological Sciences*, *19*(3), 441–445. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25720716

Kagawa, Y., Nishizawa, M., Suzuki, M., Miyatake, T., Hamamoto, T., Goto, K., … Ebihara, A. (1982). Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular diseases. *Journal of Nutritional Science and Vitaminology (Tokyo)*, *28*(4), 441–453. https://doi.org/10.3177/jnsv.28.441

Kakkar, R., Ye, B., Stoller, D. A., Smelley, M., Shi, N. Q., Galles, K., … McNally, E. M. (2006). Spontaneous coronary vasospasm in KATP mutant mice arises from a smooth muscle-extrinsic process. *Circulation Research*, *98*(5), 682–689. https://doi.org/10.1161/01.RES.0000207498.40005.e7

Kimura, S., Saito, H., Minami, M., Togashi, H., Nakamura, N., Ueno, K., … Parvez, H. (2002). Docosahexaenoic acid attenuated hypertension and vascular dementia in stroke-prone spontaneously hypertensive rats. *Neurotoxicology and Teratology*, *24*(5), 683–693. https://doi.org/10.1016/s0892-0362(02)00219-2

Knutsen, R. H., Beeman, S. C., Broekelmann, T. J., Liu, D., Tsang, K. M., Kovacs, A., … Kozel, B. A. (2018). Minoxidil improves vascular compliance, restores cerebral blood flow, and alters extracellular matrix gene expression in a model of chronic vascular stiffness. *American Journal of Physiology. Heart and Circulatory Physiology*, *315*(1), H18–H32. https://doi.org/10.1152/ajpheart.00683.2017

Krantz, M. J., Havranek, E. P., Pereira, R. I., Beaty, B., Mehler, P. S., & Long, C. S. (2015). Effects of omega-3 fatty acids on arterial stiffness in patients with hypertension: A randomized pilot study. *Journal of Negative Results in Biomedicine*, *14*, 21. https://doi.org/10.1186/s12952-015-0040-x

Kubo, M., Quayle, J. M., & Standen, N. B. (1997). Angiotensin II inhibition of ATP-sensitive K^+ currents in rat arterial smooth muscle cells through protein kinase C. *The Journal of Physiology*, *503*(Pt 3), 489–496. https://doi.org/10.1111/j.1469-7793.1997.489bg.x

Lacolley, P., Regnault, V., Segers, P., & Laurent, S. (2017). Vascular smooth muscle cells and arterial stiffening: Relevance in development, aging, and disease. *Physiological Reviews*, *97*(4), 1555–1617. https://doi.org/10.1152/physrev.00003.2017

Lai, L. H., Wang, R. X., Jiang, W. P., Yang, X. J., Song, J. P., Li, X. R., & Tao, G. (2009). Effects of docosahexaenoic acid on large-conductance Ca^{2+}-activated K^+ channels and voltage-dependent K^+ channels in rat coronary artery smooth muscle cells. *Acta Pharmacologica Sinica*, *30*(3), 314–320. https://doi.org/10.1038/aps.2009.7

Larsson, J. E., Larsson, H. P., & Liin, S. I. (2018). KCNE1 tunes the sensitivity of KV7.1 to polyunsaturated fatty acids by moving turret residues close to the binding site. *eLife*, *7*, e37257. https://doi.org/10.7554/eLife.37257

Lee, J. B., Notay, K., Klingel, S. L., Chabowski, A., Mutch, D. M., & Millar, P. J. (2019). Docosahexaenoic acid reduces resting blood pressure but increases muscle sympathetic outflow compared with eicosapentaenoic acid in healthy men and women. *American Journal of Physiology. Heart and Circulatory Physiology*, *316*(4), H873–H881. https://doi.org/10.1152/ajpheart.00677.2018

Li, Q., Zhang, Q., Wang, M., Zhao, S., Ma, J., Luo, N., … Li, J. (2007). Eicosapentaenoic acid modifies lipid composition in caveolae and induces translocation of endothelial nitric oxide synthase. *Biochimie*, *89*(1), 169–177. https://doi.org/10.1016/j.biochi.2006.10.009

Li, Z. H., Zhong, W. F., Liu, S., Kraus, V. B., Zhang, Y. J., Gao, X., … Mao, C. (2020). Associations of habitual fish oil supplementation with cardiovascular outcomes and all cause mortality: Evidence from a large population based cohort study. *BMJ*, *368*, m456. https://doi.org/10.1136/bmj.m456

Liin, S. I., Silvera Ejneby, M., Barro-Soria, R., Skarsfeldt, M. A., Larsson, J. E., Starck Harlin, F., … Elinder, F. (2015). Polyunsaturated fatty acid analogs act antiarrhythmically on the cardiac IKs channel. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(18), 5714–5719. https://doi.org/10.1073/pnas.1503488112

Limbu, R., Cottrell, G. S., & McNeish, A. J. (2018). Characterisation of the vasodilation effects of DHA and EPA, n-3 PUFAs (fish oils), in rat aorta and mesenteric resistance arteries. *PLoS ONE*, *13*(2), e0192484. https://doi.org/10.1371/journal.pone.0192484

Lopez, D., Orta, X., Casos, K., Saiz, M. P., Puig-Parellada, P., Farriol, M., & Mitjavila, M. T. (2004). Upregulation of endothelial nitric oxide synthase in rat aorta after ingestion of fish oil-rich diet. *American Journal of Physiology. Heart and Circulatory Physiology*, *287*(2), H567–H572. https://doi.org/10.1152/ajpheart.01145.2003

Malester, B., Tong, X., Ghiu, I., Kontogeorgis, A., Gutstein, D. E., Xu, J., … Coetzee, W. A. (2007). Transgenic expression of a dominant negative K (ATP) channel subunit in the mouse endothelium: Effects on coronary flow and endothelin-1 secretion. *The FASEB Journal*, *21*(9), 2162–2172. https://doi.org/10.1096/fj.06-7821com

Martinez-Fernandez, L., Laiglesia, L. M., Huerta, A. E., Martinez, J. A., & Moreno-Aliaga, M. J. (2015). Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. *Prostaglandins & Other Lipid Mediators*, *121*(Pt A), 24–41. https://doi.org/10.1016/j.prostaglandins.2015.07.003

Massaro, M., Scoditti, E., Carluccio, M. A., & De Caterina, R. (2008). Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *79*(3–5), 109–115. https://doi.org/10.1016/j.plefa.2008.09.009

McCormack, T., Boffa, R. J., Jones, N. R., Carville, S., & McManus, R. J. (2019). The 2018 ESC/ESH hypertension guideline and the 2019 NICE hypertension guideline, how and why they differ. *European Heart Journal*, *40*(42), 3456–3458. https://doi.org/10.1093/eurheartj/ehz681

McNeish, A. J., Dora, K. A., & Garland, C. J. (2005). Possible role for K^+ in endothelium-derived hyperpolarizing factor-linked dilatation in rat middle cerebral artery. *Stroke*, *36*(7), 1526–1532doi:01.STR.0000169929.66497.73 [pii]. https://doi.org/10.1161/01.STR.0000169929.66497.73

McNeish, A. J., Wilson, W. S., & Martin, W. (2001). Dominant role of an endothelium-derived hyperpolarizing factor (EDHF)-like vasodilator in the ciliary vascular bed of the bovine isolated perfused eye. *British Journal of Pharmacology*, *134*(4), 912–920. https://doi.org/10.1038/sj.bjp.0704332

Medeiros-Domingo, A., Tan, B. H., Crotti, L., Tester, D. J., Eckhardt, L., Cuoretti, A., … Ackerman, M. J. (2010). Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K (ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. *Heart Rhythm*, *7*(10), 1466–1471. https://doi.org/10.1016/j.hrthm.2010.06.016

Mies, F., Shlyonsky, V., Goolaerts, A., & Sariban-Sohraby, S. (2004). Modulation of epithelial Na^+ channel activity by long-chain n-3 fatty acids. *American Journal of Physiology. Renal Physiology*, *287*(4), F850–F855. https://doi.org/10.1152/ajprenal.00078.2004

Miki, T., Suzuki, M., Shibasaki, T., Uemura, H., Sato, T., Yamaguchi, K., … Seino, S. (2002). Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nature Medicine*, *8*(5), 466–472. https://doi.org/10.1038/nm0502-466

Miller, P. E., Van Elswyk, M., & Alexander, D. D. (2014). Long-chain omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid and blood pressure: A meta-analysis of randomized controlled trials. *American Journal of Hypertension*, *27*(7), 885–896. https://doi.org/10.1093/ajh/hpu024

Minihane, A. M. (2013). Fish oil omega-3 fatty acids and cardio-metabolic health, alone or with statins. *European Journal of Clinical Nutrition*, *67*(5), 536–540. https://doi.org/10.1038/ejcn.2013.19

Minihane, A. M., Armah, C. K., Miles, E. A., Madden, J. M., Clark, A. B., Caslake, M. J., … Calder, P. C. (2016). Consumption of fish oil providing amounts of eicosapentaenoic acid and docosahexaenoic acid that can be obtained from the diet reduces blood pressure in adults with systolic hypertension: A retrospective analysis. *The Journal of Nutrition*, *146*(3), 516–523. https://doi.org/10.3945/jn.115.220475

Mizutani, M., Asano, M., Roy, S., Nakajima, T., Soma, M., Yamashita, K., & Okuda, Y. (1997). Omega-3 polyunsaturated fatty acids inhibit migration of human vascular smooth muscle cells in vitro. *Life Sciences*, *61*(19), PL269–PL274. https://doi.org/10.1016/s0024-3205(97)00838-2

Moreno, C., de la Cruz, A., & Valenzuela, C. (2016). In-depth study of the interaction, sensitivity, and gating modulation by PUFAs on K^+ channels; interaction and new targets. *Frontiers in Physiology*, *7*, 578. https://doi.org/10.3389/fphys.2016.00578

Mori, T. A., & Woodman, R. J. (2006). The independent effects of eicosapentaenoic acid and docosahexaenoic acid on cardiovascular risk factors in humans. *Current Opinion in Clinical Nutrition and Metabolic Care*, *9*(2), 95–104. https://doi.org/10.1097/01.mco.0000214566.67439.58

Morin, C., Sirois, M., Echave, V., Rizcallah, E., & Rousseau, E. (2009). Relaxing effects of 17(18)-EpETE on arterial and airway smooth muscles in human lung. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, *296*(1), L130–L139. https://doi.org/10.1152/ajplung.90436.2008

Mozaffarian, D., & Wu, J. H. (2011). Omega-3 fatty acids and cardiovascular disease: Effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*, *58*(20), 2047–2067. https://doi.org/10.1016/j.jacc.2011.06.063

Mozaffarian, D., & Wu, J. H. (2012). (n-3) fatty acids and cardiovascular health: Are effects of EPA and DHA shared or complementary? *The Journal of Nutrition*, *142*(3), 614S–625S. https://doi.org/10.3945/jn.111.149633

Murphy, M. E., & Brayden, J. E. (1995). Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. *The Journal of Physiology*, *486*(Pt 1), 47–58. https://doi.org/10.1113/jphysiol.1995.sp020789

Nagaraj, C., Tang, B., Nagy, B. M., Papp, R., Jain, P. P., Marsh, L. M., … Olschewski, A. (2016). Docosahexaenoic acid causes rapid pulmonary arterial relaxation via KCa channel-mediated hyperpolarisation in pulmonary hypertension. *The European Respiratory Journal*, *48*(4), 1127–1136. https://doi.org/10.1183/13993003.01814-2015

Nelson, M. T., Huang, Y., Brayden, J. E., Hescheler, J., & Standen, N. B. (1990). Arterial dilations in response to calcitonin gene-related peptide involve activation of K^+ channels. *Nature*, *344*(6268), 770–773. https://doi.org/10.1038/344770a0

Nelson, M. T., & Quayle, J. M. (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *The American Journal of Physiology*, *268*(4), C799–C822. https://doi.org/10.1152/ajpcell.1995.268.4.C799

Nestel, P., Shige, H., Pomeroy, S., Cehun, M., Abbey, M., & Raederstorff, D. (2002). The n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. *The American Journal of Clinical Nutrition*, *76*(2), 326–330. https://doi.org/10.1093/ajcn/76.2.326

Neubronner, J., Schuchardt, J. P., Kressel, G., Merkel, M., von Schacky, C., & Hahn, A. (2011). Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters. *European Journal of Clinical Nutrition*, *65*(2), 247–254. https://doi.org/10.1038/ejcn.2010.239

Newens, K. J., Thompson, A. K., Jackson, K. G., & Williams, C. M. (2015). Endothelial function and insulin sensitivity during acute non-esterified fatty acid elevation: Effects of fat composition and gender. *Nutrition, Metabolism, and Cardiovascular Diseases*, *25*(6), 575–581. https://doi.org/10.1016/j.numecd.2015.03.004

Newens, K. J., Thompson, A. K., Jackson, K. G., Wright, J., & Williams, C. M. (2011). DHA-rich fish oil reverses the detrimental effects of saturated fatty acids on postprandial vascular reactivity. *The American Journal of Clinical Nutrition*, *94*(3), 742–748. https://doi.org/10.3945/ajcn.110.009233

Ney, P., & Feelisch, M. (1995). Vasodilator effects of PGE1 in the coronary and systemic circulation of the rat are mediated by ATP-sensitive potassium (K^+) channels. *Agents and Actions. Supplements*, *45*, 71–76. https://doi.org/10.1007/978-3-0348-7346-8_11

Niazi, Z. R., Silva, G. C., Ribeiro, T. P., Leon-Gonzalez, A. J., Kassem, M., Mirajkar, A., … Auger, C. (2017). EPA:DHA 6:1 prevents angiotensin II-induced hypertension and endothelial dysfunction in rats: Role of NADPH oxidase- and COX-derived oxidative stress. *Hypertension Research*, *40*(12), 966–975. https://doi.org/10.1038/hr.2017.72

Nieves-Cintron, M., Syed, A. U., Nystoriak, M. A., & Navedo, M. F. (2018). Regulation of voltage-gated potassium channels in vascular smooth muscle during hypertension and metabolic disorders. *Microcirculation*, *25*(1), e12423. https://doi.org/10.1111/micc.12423

Nyby, M. D., Matsumoto, K., Yamamoto, K., Abedi, K., Eslami, P., Hernandez, G., … Tuck, M. L. (2005). Dietary fish oil prevents vascular dysfunction and oxidative stress in hyperinsulinemic rats. *American Journal of Hypertension*, *18*(2), 213–219. https://doi.org/10.1016/j.amjhyper.2004.08.030

Omura, M., Kobayashi, S., Mizukami, Y., Mogami, K., Todoroki-Ikeda, N., Miyake, T., & Matsuzaki, M. (2001). Eicosapentaenoic acid (EPA) induces Ca^{2+}-independent activation and translocation of endothelial nitric oxide synthase and endothelium-dependent vasorelaxation. *FEBS Letters*, *487*(3), 361–366. https://doi.org/10.1016/s0014-5793(00)02351-6

Oparil, S., Acelajado, M. C., Bakris, G. L., Berlowitz, D. R., Cifkova, R., Dominiczak, A. F., … Whelton, P. K. (2018). Hypertension. *Nature Reviews. Disease Primers*, *4*, 18014. https://doi.org/10.1038/nrdp.2018.14

Oriowo, M. A. (2015). Perivascular adipose tissue, vascular reactivity and hypertension. *Medical Principles and Practice*, *24*(Suppl 1), 29–37. https://doi.org/10.1159/000356380

Otsuka, K., Tanaka, Y., Tanaka, H., Koike, K., & Shigenobu, K. (2005). Comparison of the inhibitory effects of docosahexaenoic acid (DHA) on U46619- and phenylephrine-induced contractions in guinea-pig aorta. *Biological & Pharmaceutical Bulletin*, *28*(7), 1298–1300. https://doi.org/10.1248/bpb.28.1298

Quayle, J. M., Nelson, M. T., & Standen, N. B. (1997). ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiological Reviews*, *77*(4), 1165–1232. https://doi.org/10.1152/physrev.1997.77.4.1165

Quinn, K. V., Giblin, J. P., & Tinker, A. (2004). Multisite phosphorylation mechanism for protein kinase A activation of the smooth muscle ATP-sensitive K^+ channel. *Circulation Research*, *94*(10), 1359–1366. https://doi.org/10.1161/01.RES.0000128513.34817.c4

Ramel, A., Martinez, J. A., Kiely, M., Bandarra, N. M., & Thorsdottir, I. (2010). Moderate consumption of fatty fish reduces diastolic blood pressure in overweight and obese European young adults during energy restriction. *Nutrition*, *26*(2), 168–174. https://doi.org/10.1016/j.nut.2009.04.002

Ramirez, J. G., O'Malley, E. J., & Ho, W. S. V. (2017). Pro-contractile effects of perivascular fat in health and disease. *British Journal of Pharmacology*, *174*(20), 3482–3495. https://doi.org/10.1111/bph.13767

Ramirez, J. L., Gasper, W. J., Khetani, S. A., Zahner, G. J., Hills, N. K., Mitchell, P. T., … Grenon, S. M. (2019). Fish oil increases specialized pro-resolving lipid mediators in PAD (the OMEGA-PAD II trial). *The Journal of Surgical Research*, *238*, 164–174. https://doi.org/10.1016/j.jss.2019.01.038

Riedel, M. J., & Light, P. E. (2005). Saturated and cis/trans unsaturated acyl CoA esters differentially regulate wild-type and polymorphic beta-cell ATP-sensitive K^+ channels. *Diabetes*, *54*(7), 2070–2079. https://doi.org/10.2337/diabetes.54.7.2070

Root, M., Collier, S. R., Zwetsloot, K. A., West, K. L., & McGinn, M. C. (2013). A randomized trial of fish oil omega-3 fatty acids on arterial health, inflammation, and metabolic syndrome in a young healthy population. *Nutrition Journal*, *12*, 40. https://doi.org/10.1186/1475-2891-12-40

Sakai, C., Ishida, M., Ohba, H., Yamashita, H., Uchida, H., Yoshizumi, M., & Ishida, T. (2017). Fish oil omega-3 polyunsaturated fatty acids attenuate oxidative stress-induced DNA damage in vascular endothelial cells. *PLoS ONE*, *12*(11), e0187934. https://doi.org/10.1371/journal.pone.0187934

Sanders, T. A., Hall, W. L., Maniou, Z., Lewis, F., Seed, P. T., & Chowienczyk, P. J. (2011). Effect of low doses of long-chain n-3 PUFAs on endothelial function and arterial stiffness: A randomized controlled trial. *The American Journal of Clinical Nutrition*, *94*(4), 973–980. https://doi.org/10.3945/ajcn.111.018036

Saravanan, P., Davidson, N. C., Schmidt, E. B., & Calder, P. C. (2010). Cardiovascular effects of marine omega-3 fatty acids. *Lancet*, *376*(9740), 540–550. https://doi.org/10.1016/S0140-6736(10)60445-X

Sato, K., Chino, D., Kobayashi, T., Obara, K., Miyauchi, S., & Tanaka, Y. (2013). Selective and potent inhibitory effect of docosahexaenoic acid (DHA) on U46619-induced contraction in rat aorta. *Journal of Smooth Muscle Research*, *49*, 63–77. https://doi.org/10.1540/jsmr.49.63

Sato, K., Chino, D., Nishioka, N., Kanai, K., Aoki, M., Obara, K., … Tanaka, Y. (2014). Pharmacological evidence showing significant roles for potassium channels and CYP epoxygenase metabolites in the relaxant effects of docosahexaenoic acid on the rat aorta contracted with U46619. *Biological & Pharmaceutical Bulletin*, *37*(3), 394–403. https://doi.org/10.1248/bpb.b13-00746

Sawada, T., Tsubata, H., Hashimoto, N., Takabe, M., Miyata, T., Aoki, K., … Yokoyama, M. (2016). Effects of 6-month eicosapentaenoic acid treatment on postprandial hyperglycemia, hyperlipidemia, insulin secretion ability, and concomitant endothelial dysfunction among newly-diagnosed impaired glucose metabolism patients with coronary artery disease. An open label, single blinded, prospective randomized controlled trial. *Cardiovascular Diabetology*, *15*(1), 121. https://doi.org/10.1186/s12933-016-0437-y

Shahidi, F., & Ambigaipalan, P. (2018). Omega-3 polyunsaturated fatty acids and their health benefits. *Annual Review of Food Science and Technology*, *9*, 345–381. https://doi.org/10.1146/annurev-food-111317-095850

Shen, T., Xing, G., Zhu, J., Zhang, S., Cai, Y., Li, D., … Shi, R. (2017). Effects of 12-week supplementation of marine omega-3 PUFA-based formulation Omega3Q10 in older adults with prehypertension and/or elevated blood cholesterol. *Lipids in Health and Disease*, *16*(1), 253. https://doi.org/10.1186/s12944-017-0617-0

Sheth, K. N., Petersen, N. H., Cheung, K., Elm, J. J., Hinson, H. E., Molyneaux, B. J., … Kimberly, W. T. (2018). Long-term outcomes in patients aged </=70 years with intravenous glyburide from the phase II GAMES-RP study of large hemispheric infarction: An exploratory analysis. *Stroke*, *49*(6), 1457–1463. https://doi.org/10.1161/STROKEAHA.117.020365

Shimokawa, H., Aarhus, L. L., & Vanhoutte, P. M. (1988). Dietary omega 3 polyunsaturated fatty acids augment endothelium-dependent relaxation to bradykinin in coronary microvessels of the pig. *British Journal of Pharmacology*, *95*(4), 1191–1196. https://doi.org/10.1111/j.1476-5381.1988.tb11755.x

Singh, T. U., Kathirvel, K., Choudhury, S., Garg, S. K., & Mishra, S. K. (2010). Eicosapentaenoic acid-induced endothelium-dependent and -independent relaxation of sheep pulmonary artery. *European Journal of Pharmacology*, *636*(1–3), 108–113. https://doi.org/10.1016/j.ejphar.2010.02.041

Singhal, A., Lanigan, J., Storry, C., Low, S., Birbara, T., Lucas, A., & Deanfield, J. (2013). Docosahexaenoic acid supplementation, vascular function and risk factors for cardiovascular disease: A randomized controlled trial in young adults. *Journal of the American Heart Association*, *2*(4), e000283. https://doi.org/10.1161/JAHA.113.000283

Siniarski, A., Haberka, M., Mostowik, M., Golebiowska-Wiatrak, R., Poreba, M., Malinowski, K. P., … Gajos, G. (2018). Treatment with omega-3 polyunsaturated fatty acids does not improve endothelial function in patients with type 2 diabetes and very high cardiovascular risk: A randomized, double-blind, placebo-controlled study (omega-FMD). *Atherosclerosis*, *271*, 148–155. https://doi.org/10.1016/j.atherosclerosis.2018.02.030

Smith, J. A., Vanoye, C. G., George, A. L. Jr., Meiler, J., & Sanders, C. R. (2007). Structural models for the KCNQ1 voltage-gated potassium channel. *Biochemistry*, *46*(49), 14141–14152. https://doi.org/10.1021/bi701597s

Sobey, C. G. (2001). Potassium channel function in vascular disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *21*(1), 28–38. Retrieved from. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11145930, https://doi.org/10.1161/01.ATV.21.1.28

Sokola-Wysoczanska, E., Wysoczanski, T., Wagner, J., Czyz, K., Bodkowski, R., Lochynski, S., & Patkowska-Sokola, B. (2018). Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders—A review. *Nutrients*, *10*(10), 1561. https://doi.org/10.3390/nu10101561

Stott, J. B., Jepps, T. A., & Greenwood, I. A. (2014). K_V7 potassium channels: A new therapeutic target in smooth muscle disorders. *Drug Discovery Today*, *19*(4), 413–424. https://doi.org/10.1016/j.drudis.2013.12.003

Sveinsdottir, K., Martinsdottir, E., & Ramel, A. (2016). Blood pressure-lowering effects of long chain n-3 fatty acids from meals enriched with liquid fish oil and from microencapsulated powder. *International Journal of Food Sciences and Nutrition*, *67*(8), 1017–1023. https://doi.org/10.1080/09637486.2016.1208733

Tagetti, A., Ericson, U., Montagnana, M., Danese, E., Almgren, P., Nilsson, P., … Melander, O. (2015). Intakes of omega-3 polyunsaturated fatty acids and blood pressure change over time: Possible interaction with genes involved in 20-HETE and EETs metabolism. *Prostaglandins & Other Lipid Mediators*, *120*, 126–133. https://doi.org/10.1016/j.prostaglandins.2015.05.003

Tai, C. C., Chen, C. Y., Lee, H. S., Wang, Y. C., Li, T. K., Mersamm, H. J., … Wang, P. H. (2009). Docosahexaenoic acid enhances hepatic serum amyloid A expression via protein kinase A-dependent mechanism. *The Journal of Biological Chemistry*, *284*(47), 32239–32247. https://doi.org/10.1074/jbc.M109.024661

Tester, D. J., Tan, B. H., Medeiros-Domingo, A., Song, C., Makielski, J. C., & Ackerman, M. J. (2011). Loss-of-function mutations in the KCNJ8-encoded Kir6.1 K (ATP) channel and sudden infant death syndrome. *Circulation. Cardiovascular Genetics*, *4*(5), 510–515. https://doi.org/10.1161/CIRCGENETICS.111.960195

Tian, Y., Aursnes, M., Hansen, T. V., Tungen, J. E., Galpin, J. D., Leisle, L., … Hoshi, T. (2016). Atomic determinants of BK channel activation by polyunsaturated fatty acids. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(48), 13905–13910. https://doi.org/10.1073/pnas.1615562113

Tousoulis, D., Simopoulou, C., Papageorgiou, N., Oikonomou, E., Hatzis, G., Siasos, G., … Stefanadis, C. (2014). Endothelial dysfunction in conduit arteries and in microcirculation. Novel therapeutic approaches. *Pharmacology & Therapeutics*, *144*(3), 253–267. https://doi.org/10.1016/j.pharmthera.2014.06.003

Touyz, R. M., Alves-Lopes, R., Rios, F. J., Camargo, L. L., Anagnostopoulou, A., Arner, A., & Montezano, A. C. (2018). Vascular smooth muscle contraction in hypertension. *Cardiovascular Research*, *114*(4), 529–539. https://doi.org/10.1093/cvr/cvy023

Ueshima, H., Stamler, J., Elliott, P., Chan, Q., Brown, I. J., Carnethon, M. R., … Group, I. R. (2007). Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: INTERMAP study. *Hypertension*, *50*(2), 313–319. https://doi.org/10.1161/HYPERTENSIONAHA.107.090720

Vanhoutte, P. M., Shimokawa, H., Tang, E. H., & Feletou, M. (2009). Endothelial dysfunction and vascular disease. *Acta Physiologica (Oxford, England)*, *196*(2), 193–222. https://doi.org/10.1111/j.1748-1716.2009.01964.x

Vanhoutte, P. M., & Tang, E. H. (2008). Endothelium-dependent contractions: When a good guy turns bad! *The Journal of Physiology*, *586*(22), 5295–5304. https://doi.org/10.1113/jphysiol.2008.161430

Verlohren, S., Dubrovska, G., Tsang, S. Y., Essin, K., Luft, F. C., Huang, Y., & Gollasch, M. (2004). Visceral periadventitial adipose tissue regulates arterial tone of mesenteric arteries. *Hypertension*, *44*(3), 271–276. https://doi.org/10.1161/01.HYP.0000140058.28994.ec

Villalpando, D. M., Navarro, R., Del Campo, L., Largo, C., Munoz, D., Tabernero, M., … Ferrer, M. (2015). Effect of dietary docosahexaenoic acid supplementation on the participation of vasodilator factors in aorta from orchidectomized rats. *PLoS ONE*, *10*(11), e0142039. https://doi.org/10.1371/journal.pone.0142039

Wagenseil, J. E., & Mecham, R. P. (2012). Elastin in large artery stiffness and hypertension. *Journal of Cardiovascular Translational Research*, *5*(3), 264–273. https://doi.org/10.1007/s12265-012-9349-8

Wang, Q., Liang, X., Wang, L., Lu, X., Huang, J., Cao, J., … Gu, D. (2012). Effect of omega-3 fatty acids supplementation on endothelial function: A meta-analysis of randomized controlled trials. *Atherosclerosis*, *221*(2), 536–543. https://doi.org/10.1016/j.atherosclerosis.2012.01.006

Wang, R. X., Chai, Q., Lu, T., & Lee, H. C. (2011). Activation of vascular BK channels by docosahexaenoic acid is dependent on cytochrome P450 epoxygenase activity. *Cardiovascular Research*, *90*(2), 344–352. https://doi.org/10.1093/cvr/cvq411

Woodman, R. J., Mori, T. A., Burke, V., Puddey, I. B., Barden, A., Watts, G. F., & Beilin, L. J. (2003). Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. *Atherosclerosis*, *166*(1), 85–93. https://doi.org/10.1016/s0021-9150(02)00307-6

Wu, S. Y., Mayneris-Perxachs, J., Lovegrove, J. A., Todd, S., & Yaqoob, P. (2014). Fish-oil supplementation alters numbers of circulating endothelial progenitor cells and microparticles independently of eNOS genotype. *The American Journal of Clinical Nutrition*, *100*(5), 1232–1243. https://doi.org/10.3945/ajcn.114.088880

Xin, W., Wei, W., & Li, X. (2012). Effect of fish oil supplementation on fasting vascular endothelial function in humans: A meta-analysis of randomized controlled trials. *PLoS ONE*, *7*(9), e46028. https://doi.org/10.1371/journal.pone.0046028

Yamada, M., Isomoto, S., Matsumoto, S., Kondo, C., Shindo, T., Horio, Y., & Kurachi, Y. (1997). Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. *The Journal of Physiology*, *499*(Pt 3), 715–720. https://doi.org/10.1113/jphysiol.1997.sp021963

Yang, B., Shi, L., Wang, A. M., Shi, M. Q., Li, Z. H., Zhao, F., … Li, D. (2019). Lowering effects of n-3 fatty acid supplements on blood pressure by reducing plasma angiotensin II in Inner Mongolia hypertensive patients: A double-blind randomized controlled trial. *Journal of Agricultural and Food Chemistry*, *67*(1), 184–192. https://doi.org/10.1021/acs.jafc.8b05463

Yang, Y., Shi, Y., Guo, S., Zhang, S., Cui, N., Shi, W., … Jiang, C. (2008). PKA-dependent activation of the vascular smooth muscle isoform of KATP channels by vasoactive intestinal polypeptide and its effect on relaxation of the mesenteric resistance artery. *Biochimica et Biophysica Acta*, *1778*(1), 88–96. https://doi.org/10.1016/j.bbamem.2007.08.030

Ye, D., Zhang, D., Oltman, C., Dellsperger, K., Lee, H. C., & VanRollins, M. (2002). Cytochrome p-450 epoxygenase metabolites of docosahexaenoate potently dilate coronary arterioles by activating large-conductance calcium-activated potassium channels. *The Journal of Pharmacology and Experimental Therapeutics*, *303*(2), 768–776. https://doi.org/10.1124/jpet.303.2.768

Zgheel, F., Alhosin, M., Rashid, S., Burban, M., Auger, C., & Schini-Kerth, V. B. (2014). Redox-sensitive induction of Src/PI3-kinase/Akt and MAPKs pathways activate eNOS in response to EPA:DHA 6:1. *PLoS ONE*, *9*(8), e105102. https://doi.org/10.1371/journal.pone.0105102

Zgheel, F., Perrier, S., Remila, L., Houngue, U., Mazzucotelli, J. P., Morel, O., … Schini-Kerth, V. B. (2019). EPA:DHA 6:1 is a superior omega-3 PUFAs formulation attenuating platelets-induced contractile responses in porcine coronary and human internal mammary artery by targeting the serotonin pathway via an increased endothelial formation of nitric oxide. *European Journal of Pharmacology*, *853*, 41–48. https://doi.org/10.1016/j.ejphar.2019.03.022

Zhang, Y., Zhang, M., Lyu, B., Kishi, H., & Kobayashi, S. (2017). Omega-3 and omega-6 DPA equally inhibit the sphingosylphosphorylcholine-induced Ca^{2+}-sensitization of vascular smooth muscle contraction via inhibiting Rho-kinase activation and translocation. *Scientific Reports*, *7*, 36368. https://doi.org/10.1038/srep36368

How to cite this article: Bercea C-I, Cottrell GS, Tamagnini F, McNeish AJ. Omega-3 polyunsaturated fatty acids and hypertension: a review of vasodilatory mechanisms of docosahexaenoic acid and eicosapentaenoic acid. *Br J Pharmacol*. 2021;178:860–877. https://doi.org/10.1111/bph.15336