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ORIGINAL PAPER

Regulation of Neutrophil Function by Marine n-3 Fatty Acids—A Mini Review

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Abstract

While normal functioning neutrophils contribute in various, critical ways to the maintenance of a stable immune system, their hypo- or hyper-activation has been implicated in the onset or exacerbation of multiple inflammatory conditions often affecting the vulnerable, aging population. As such, many would benefit from interventions capable of targeting neutrophils in disease-specific ways without disrupting their primary role in maintaining immune function. After consumption, marine omega-3 fatty acids are rapidly incorporated into the phospholipid bilayer of neutrophils, changing the fatty acid composition and consequently modifying neutrophil function. In addition to eicosanoid synthesis, the mechanisms by which marine n-3 fatty acids and their metabolites alter neutrophil function involve blockage of transcription factors that subsequently reduce pro-inflammatory gene expression by neutrophils and through the disruption of lipid rafts. In the current mini-review, a brief explanation of marine n-3 fatty acid metabolism is provided and the subsequent impact on neutrophil function is discussed. In addition, current evidence of the effects of marine n-3 fatty acid supplementation on neutrophil function from clinical trials conducted in the past 15 years is summarized.

Keywords Neutrophils · n-3 fatty acids · Polyunsaturated fatty acids · Eicosapentaenoic acid · Docosahexaenoic acid

Introduction

Neutrophils (or polymorphonuclear cells) are the most abundant leukocytes in human blood (50–70% of white blood cells) and carry out a variety of functions as part of the innate immune system [1, 2]. Neutrophils are phagocytic cells with one of their main functions being to protect the host by killing invading microorganisms, including bacteria and fungi [3, 4]. On a daily basis, about 10^{11} neutrophils are produced and mature in the bone marrow via a series of progressive and interdependent, highly regulated steps [5]. Multiple elements, such as cytokines, growth factors, and granulocyte-macrophage colony-stimulating factor, coordinate neutrophil production under homeostatic and infectious circumstances [6]. Mature neutrophils are located in the vascular system in a circulating blood pool and in a marginated blood pool present in various tissues, the major sites being the liver, spleen, and bone marrow [2, 6, 7]. Marginated neutrophils may patrol for microbial invasion or tissue damage within these organs or they may be swiftly transported back to the peripheral circulation, responding to inflammatory signals coming from other infectious or damaged areas [2, 8].

Under homeostatic circumstances, neutrophils are the first cells to be recruited to sites of inflammation, where they clear pathogens by intra- and extra-cellular means [4]. Within neutrophils, pathogens may be encapsulated in phagosomes and then killed by cytotoxic proteins, peptides, and enzymes that are released when neutrophil granules fuse with phagosomes [9] or via NADPH oxygenase-dependent actions (reactive oxygen species [ROS]) [10]. Similar mechanisms may also be used in the extracellular space, but the fusion of the neutrophil granules and activation of NADPH oxidase occurs at the plasma membrane [11, 12]. Neutrophils may also eliminate extra-cellular pathogens by releasing neutrophil extracellular traps (NETs), structures containing histones, proteins, and enzymes attached to chromatin DNA [13]. While it has long been known that neutrophils exhibit the critical innate defense function of killing pathogens, there is more recent evidence they are involved in other

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specialized functions including coordinating the immune response by releasing pro-inflammatory mediators and contributing to the crosstalk between signaling pathways of the innate and adaptive immune systems [14–16]. They have also been shown to play an active role in inflammation resolution [3, 4].

While normal functioning neutrophils contribute in various, critical ways to the maintenance of a stable immune system, malfunctioning neutrophils have been implicated in the onset or exacerbation of multiple inflammatory conditions. For example, hypo-activation of neutrophils occurs during compensatory anti-inflammatory response syndrome after significant trauma and sepsis, and hyper-activation of neutrophils is evident in many chronic inflammatory diseases such as inflammatory arthritis and chronic obstructive pulmonary disease, acute inflammatory conditions such as acute respiratory distress syndrome, and reperfusion injury [17–20].

While it is well-established that neutrophils are critical to maintaining healthy innate immune responses and that hypo- or hyper-activation of neutrophils can have harmful effects in inflammatory conditions, the conventional opinion that neutrophils are a homogeneous population of short-lived cells has recently been questioned. Emerging evidence suggests multiple neutrophil phenotypes with diverse functions may exist and that the neutrophil lifespan may vary depending on the resident tissue type and acute versus chronic inflammatory states [2, 20, 21]. Although the neutrophil function is complex and not entirely clear, it is apparent that multiple inflammatory conditions often affecting the vulnerable, aging population would benefit from interventions capable of targeting neutrophils in disease-specific ways without disrupting their primary role in maintaining immune function.

Testing interventions containing the marine-derived omega-3 (n-3) polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to target and modulate cells such as neutrophils that are involved in inflammatory responses have been of interest to researchers since the first report by Bang et al. in Greenlandic Eskimos suggesting that diets high in EPA and DHA are cardioprotective [22]. Subsequently, studies have shown that after consumption, EPA and DHA are rapidly incorporated into the phospholipid bilayer of neutrophils, changing the fatty acid composition and consequently modifying neutrophil function.

In the current mini-review, we provide a brief explanation of EPA and DHA metabolism and discuss the subsequent impact of EPA and DHA on neutrophil function. In addition, current evidence of the effects of EPA and/or DHA supplementation on neutrophil function from human studies conducted in the past 15 years is summarized. For more comprehensive information on n-3 PUFA metabolism and their impact on the immune system and immune-related diseases, we suggest additional reviews [23–27].

Marine-derived n-3 PUFAs EPA and DHA

The two main families of PUFAs are n-6 and n-3. While the impact of n-6 and n-3 PUFAs on inflammatory cell functions has been studied for many years, n-3 EPA and DHA have been of special interest because of their anti-inflammatory effects [26, 28]. EPA and DHA are obtained in the diet from seafood, mainly fatty fish like tuna, herring, sardines, salmon, and mackerel [29]. They are also generated by their parent n-3 PUFA α-linolenic acid (ALA), present in varieties of nuts and seeds. However, the synthesis of EPA and DHA from ALA via a series of desaturation and elongation reactions happens at a low rate in mammals [30]. Thus levels of EPA and DHA in the body are largely dependent on the dietary intake of fatty fish or fish oil supplements. Generally, people consuming a conventional Western diet have low intake levels of EPA and DHA, <0.2 g/d, unless eating fatty fish or consuming fish oil supplements regularly [23].

The parent n-6 PUFA, linoleic (LA) is metabolized to other n-6 PUFAs, such as arachidonic acid (ARA). Additional sources of ARA include red meat, eggs, and organ meats [23]. Of note, the n-6 and n-3 PUFA families use the same enzymes in the metabolic process; thus there is competition for metabolism into the other n-6 and n-3 PUFAs, respectively [23].

EPA, DHA, and ARA are common components of the membranes of neutrophils and nearly all other cell types, and the comparative amounts present are contingent on several factors including dietary intake and metabolic processes. In Western populations, ARA is the predominant PUFA found in the membranes of the majority of cells [23, 31]. However, when dietary intake of EPA and DHA increases, the amount of EPA and DHA in cell membranes also rises in a time- and the dose-dependent way [31–34], at the expense of ARA to some extent [31, 32, 34, 35]. The proportion of EPA, DHA, and ARA in neutrophil membranes impacts inflammation, given that when ARA, EPA, and DHA are released from cell membrane phospholipids in response to inflammatory stimuli, they act as substrates for cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 enzymes to generate eicosanoids, critical mediators and controllers of inflammatory processes [23, 36–38]. The eicosanoids synthesized in the ARA pathway (e.g., 2-series prostaglandins, 4-series leukotrienes) are generally pro-inflammatory while those produced in the EPA and DHA pathway are weaker biologically (e.g., 3-series prostaglandins, 5-series leukotrienes) or inflammation-resolving [39]. The more recently identified specialized pro-resolving mediators (SPM)

generated via the enzymatic conversion of EPA, DHA, and n-3 docosapentaenoic acid (DPA) that demonstrate inflammation resolving actions include resolvins, protectins, and maresins [25, 40].

In addition to eicosanoid synthesis, the mechanisms by which EPA and DHA and their metabolites alter neutrophil function is through the blockage of transcription factors that subsequently reduces pro-inflammatory gene expression by neutrophils and through the disruption of lipid rafts. Lipid rafts are areas of plasma membrane enhanced with sphingolipids and cholesterol, and contain signaling molecules that impact cellular signal transduction, and are known to affect neutrophil function [41, 42]. Singularly and collectively, these mechanisms can modulate neutrophil recruitment and migration, phagocytic potency, and the formation of ROS.

Impact of EPA and/or DHA on Neutrophil Recruitment and Migration

In vitro experiments by Tull et al. demonstrated that neutrophil recruitment and migration across vascular endothelial cells into inflamed tissue is a tightly regulated process requiring a signal generated by the metabolism of n-6 ARA into the eicosanoid prostaglandin-D2 (PGD2) by COX enzymes [43]. EPA blocks this interaction between PGD2 and its receptor on neutrophils by generating PGD3, subsequently inhibiting neutrophil migration across the endothelium [43]. DHA metabolites have also been shown to reduce neutrophil migration. Using synthetic materials and in vivo experiments, Dalli et al. demonstrated that exposure of human neutrophils to DHA-derived resolvin D3 (RvD3) or aspirin-triggered-RvD3 at concentrations as low at 10^{-11} M reduced transmigration ~25%, suggesting that both RvD3 epimers wield strong anti-inflammatory and pro-resolving actions with leukocytes [44]. Similarly, in a microfluidic platform duplicating key features of focal inflammation sites, another DHA-derived resolvin, RvD1, was also observed to reduce neutrophil trafficking [45]. Maresins, a family of DHA-derived lipid mediators, also display both anti-inflammatory and pro-resolving activities by inhibiting neutrophil infiltration [46, 47].

While numerous studies using in vitro methods and/or animal models of inflammation have demonstrated the inhibitory effects of EPA and/or DHA on neutrophil trafficking, findings from clinical trials have been mixed. Luu et al. isolated monocytes from the blood of donors >45 years of age with peripheral arterial disease (PAD) ($n = 19$) or control donors ($n = 29$), before and after 12 weeks of supplementation with 1.02 g/d EPA and 0.69 g/d DHA [48]. While monocytes from both groups stimulated the endothelial cells to support the adhesion and migration of neutrophils, EPA+DHA supplementation reduced the potency of monocytes from the control donors, but not those from patients with PAD, to induce recruitment, suggesting effects may depend on the medical status and medication usage [48]. Conversely, using ex vivo experiments, Gorjao et al. found that neutrophil chemotaxis was increased by 128% in ten male volunteers after consuming 3 g/day of fish oil containing 26% EPA and 54% DHA for 2 months [49]. In addition, in a randomized, placebo-controlled study hypothesizing that exercise would enhance the anti-inflammatory effects of n-3 PUFAs, neutrophil chemotaxis and adherence were not significantly affected by exercise, n-3 PUFAs, or the combination of the two in 50 volunteers (25–65 years of age) with at least one CVD risk factor allocated to consume either sunflower oil or DHA-rich fish oil (1.6 g/d of DHA) for 12 weeks [50]. The variation in findings from the paucity of clinical trials that have investigated the effects of EPA and/or DHA supplementation on neutrophil recruitment and migration may be explained by the differences in doses and duration of supplementation, and variations in age, sex, medication usage, and health status of the participants across studies.

Impact of EPA and/or DHA on Phagocytic Activity

Some human studies have shown amplification of phagocytic activity in neutrophils after EPA and/or DHA supplementation. Gorjao et al. reported an increase in the n-3/n-6 ratio from 0.15 to 0.70 in neutrophils and an increase in phagocytic activity of neutrophils to zymosan particles by 62% in a sample of adult males ($n = 10$) after consuming 3 g/day of fish oil containing 26% EPA and 54% DHA for 2 months [49]. Bonatto et al. also found that fish oil supplementation providing approximately 0.3 g/d of EPA plus 0.4 g/d of DHA for 8 weeks in chemotherapy patients significantly increased the EPA and DHA content in PMNC, mainly neutrophils [51]. Moreover, while phagocytosis of zymosan declined by 45% over 8 weeks in the group receiving chemotherapy only ($n = 19$), it increased by 15% in the fish oil group ($n = 19$). Moreover, phagocytic activity was higher at 8 weeks in the fish oil group than in the control group at this time, suggesting that there may be a role for EPA+DHA therapy in maintaining innate immune function in patients receiving chemotherapy [51].

Short-term supplementation with n-3 enriched oils has also been shown to enhance the phagocytic activity of neutrophils. A recent prospective, open-label, nonblinded pilot study of ten healthy adults and ten patients with PAD evaluated the effects of three escalating doses of enriched marine oil (corresponding to ~1.5 g, 3.0 g, and 6.0 g

per day + SPM precursors) on lipid mediators and leukocyte function [52]. The supplement was given daily for 5 days, followed by a 9-day washout interval, for a total of 33 days of study interval. Over the course of treatment, supplementation led to a significant increase in phagocytic activity of peripheral neutrophils in the healthy participants and a trend toward increased neutrophil phagocytosis in the PAD cohort, a process shown to impact the shift from inflammation to resolution [52, 53]. Likewise, Souza et al. found that in healthy volunteers 19–37 years of age ($n = 22$) enrolled in a double-blinded, placebo-controlled, crossover study, there was a dose-dependent increase in neutrophil phagocytosis of *S. aureus and E. coli* following supplementation at the early intervals (2–4 h post supplementation), but that the greatest increases were observed at the 24-h interval [54]. Taken together, these recent findings indicate that supplementation with marine n-3 PUFAs leads to rapid neutrophil responses.

However, some earlier studies have reported null effects of n-3 PUFA supplementation on neutrophil function. A double-blind and placebo-controlled study of healthy older ($n = 62$) and younger men ($n = 93$), showed no significant impact of age or treatment group (placebo, low-, moderate-, and high-EPA) or interaction between these factors on phagocytic activity of neutrophils tested with *E. coli* in vitro. The low-, moderate-, and high-EPA supplements also contained 0.3, 0.6, and 0.9 g/d of DHA, respectively, and varying amounts of n-6 linoleic acid [34]. Mukaro et al. also did not detect any difference in the bactericidal activity of neutrophils in healthy participants ($n = 42$) when comparing neutrophil responses between the n-3 PUFA-supplemented participants (1 g/d of EPA and DHA in enriched foods) and placebo participants after six months in a randomized double-blind study [55]. The collective findings suggest that different compositions of EPA and DHA may exert diverse effects on the phagocytic activity of neutrophils in humans.

Impact of EPA and DHA on Formation of ROS

Findings from previous human studies evaluating ROS production by neutrophils in response to EPA and/or DHA have also been mixed and suggest that results may be dose- and age-dependent. Gorjao et al. found that supplementing diets of healthy males 25–45 years of age with DHA-rich fish oil (1.62 g/d of DHA and 0.78 g/day of EPA) for 2 months led to a significant increase in ROS production by phorbol-myristate-acetate-stimulated neutrophils [49]. Similarly, in an RCT of cancer patients receiving chemotherapy (men and women aged 51–58 years; $n = 28$), superoxide production by PMNC (mostly neutrophils) increased on average by 28% after 8 weeks of low-dose fish oil supplementation containing 0.3 g/d of EPA and 0.4 g/d of DHA; no change was noted in the control group [51]. Conversely, in a placebo-controlled, double-blind study, consumption of EPA (ranging from 1.35 to 4.05 g/day for 12 weeks) in healthy younger and older men caused a dose-dependent decrease in neutrophil respiratory burst only in the older men in comparison to the placebo group [34]. Hill et al. [50] also observed a significant reduction of ROS production by neutrophils after study participants ingested DHA-rich fish oil (1.56 g/d of DHA and 0.36 g/d of EPA) for 12 weeks. Similarly, plasma myeloperoxidase (MPO; a peroxidase enzyme released primarily by neutrophils) was significantly reduced with n-3 fatty acid supplementation alone in patients with chronic kidney disease randomly assigned to n-3 fatty acids (1.8 g/d of EPA + 1.42 g/d of DHA + 0.15 g/d of DPA), coenzyme Q10 supplementation, both, or control (olive oil) for 8 weeks [56].

In addition, an RCT that assigned 15 healthy male soccer players to either a placebo or almond-based beverage enriched with DHA (1.4 g/d) for 8 weeks found no significant effects in the production of ROS by neutrophils after their activation with zymosan or PMA [57]. However, DHA diet supplementation significantly reduced the time at which the greatest ROS production by neutrophils was reached after physical activity [57]. Andersen et al. also found no effect of marine n-3 fatty acids on plasma levels of MPO in healthy adults after 12 weeks of supplementation [58].

Additional Effects of EPA and/or DHA on Neutrophil Function and Numbers

In addition to neutrophils' immune and inflammatory functions involving migration, phagocytosis of bacteria, and ROS production, they also secrete cytokines, small proteins that help coordinate inflammatory and immune responses. However, we could find only two clinical trial reports in the past 15 years that included measures of neutrophil-specific cytokine production before and after n-3 PUFA supplementation. In a randomized double-blind study by Capo et al., 15 male athletes ingested a beverage enriched with DHA (mean intake of 1.14 g/d) or placebo for 8 weeks. The DHA diet supplementation prevented PMA effects on neutrophil expression of inflammatory genes interleukin 8 (IL-8) and tumor necrosis factor α (TNF-α) and resulted in a reduced rate of IL-6 production [59]. However, n-3 PUFA supplementation had no effect on IL-8, TNF-α, IL-1ra, IL-1∝, or IL-4 production by unstimulated and stimulated neutrophils in a study of 8 male basketball wheelchair athletes supplemented with 1500 mg DHA, 300 mg EPA

(5:1 DHA:EPA), and 6 mg natural vitamin E per day for 30 days [60].

In addition to affecting neutrophil function, some clinical trials have reported that dietary supplementation with EPA and/or DHA modifies the number of circulating neutrophils. In patients receiving chemotherapy ($n = 38$) randomized into a control group or a fish oil (FO) group receiving 0.3 g/d EPA plus 0.4 g/d DHA supplementation for 8 weeks, the PMNC number (primarily neutrophils) declined in the control group ($p = 0.06$) but not in the FO group [51]. Moreover, after 8 weeks the number of PMNC was higher by ~30% in the FO group, suggesting low-dose EPA+DHA may be helpful in averting chemotherapy-induced decline in neutrophil numbers [51]. However, in a double-blind and placebo-controlled study, there were no differences in the number of circulating neutrophils between groups after 44 healthy subjects aged 23–63 years consumed either standard or n-3 PUFA-enriched foods equaling 1 g/d of combined EPA and DHA for 6 months [55]. Similarly, Bartelt et al. found no effect of FO containing 0.85 g/d of EPA and 0.60 g/d of DHA for 8 weeks on the neutrophil count in 58 healthy young men 18–40 years of age randomly assigned to supplementation with FO or olive oil [61]. Likewise, in another RCT, treatment with n-3 fatty acids for 8 weeks in patients with chronic kidney disease did not affect neutrophil counts [56]; neither did treatment with a DHA-enriched drink for 8 weeks in young adults athletes [57].

More recently identified effects of EPA and/or DHA supplementation on neutrophil function involve the potent actions of the SPMs biosynthesized from EPA and DHA. In a pilot study by Schaller et al., short-term supplementation with highly concentrated EPA+DHA+SPM precursors was shown to dramatically remodel downstream lipid mediator pathways and lead to a less inflammatory and more pro-resolution phenotype in circulating leukocytes (including neutrophils) in both healthy people and patients with PAD [52]. In addition, Souza et al. demonstrated dose- and time-dependent increases in SPM concentrations in plasma that were linked with changes in leukocyte responses in healthy participants consuming supplementation with EPA+DHA+SPM precursors over a 24-h interval [54]. Among other changes in leukocyte responses, there was a decline in adhesion molecule expression by leukocytes and a pronounced change in transcript levels of immune and metabolic genes 24 h post supplementation when compared with placebo. Together, these findings suggest that changes in peripheral blood SPM concentrations are associated with reprogramming of neutrophils (and monocytes and platelets) in peripheral blood towards a protective phenotype and show how SPM mediates the immune-focused actions of supplements containing EPA+DHA+SPM precursors [52, 54].

Conclusion

In the current mini-review, a brief explanation of n-3 PUFA metabolism, neutrophil function, and a summary of the primary results from clinical trials in the past 15 years evaluating the effects of supplements containing the n-3 PUFAs EPA and/or DHA on neutrophil function is provided (Table 1). The focus of the review was on clinical trial reports because results from studies using animal models cannot be unequivocally applied to humans for several reasons, including that there are differences in fatty acid metabolism between animals and humans [62].

The reported findings from the clinical trials reviewed were mixed and likely impacted by multiple exogenous factors. Protocols varied significantly across studies in terms of doses of EPA and/or DHA, ratios of EPA to DHA, forms of supplementation, placebo content, and duration of supplementation. In addition, there were wide variations in the characteristics of the target populations that make it difficult to compare findings across studies given that EPA and DHA metabolism is affected by sex, age, alcohol, smoking, and health status [25, 63]. In addition, there were differences in the in vitro methods used to assess some outcome measures across reports and not all studies considered participants' intake of n-6 and n-3 fatty acids from foods in the analyses. Finally, there were differences across studies in the methodology used to determine the effects of dietary supplements containing EPA and/or DHA on fatty acid levels (e.g., some studies measured fatty acid levels in plasma or in erythrocyte membranes or in neutrophil membranes).

While there is a significant number of previous well-designed clinical trials that aimed to clarify the actions of marine n-3 fatty acids on other cell types involved in immune responses such as monocytes, macrophages, and/or T-cells, there is a limited number of studies that have evaluated neutrophils, the principal cells involved in the initial phase of the inflammatory response. As such, additional clinical trials are needed that use standard indices (e.g., n-3 index: a measure of serum EPA+DHA levels) to quantify the extent to which n-3 supplementation affects fatty acid levels and subsequently, neutrophil function [64]. More specifically, the International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends that in future supplement trials, researchers: (1) quantify marine n-3 PUFA levels at baseline and post-intervention, (2) report on the full fatty acid results (expressed both as a percent of total fatty acids and as concentrations), and (3) analyze the results as an intention

Table 1 Summary of the impact of marine *n*-3 PUFAs on neutrophil function from clinical trials conducted from 2006–2020 with main references

Protocol	Key results
12-weeks supplementation with 1.35 g/d of EPA and 0.3 g/d of DHA or 2.7 g/d of EPA and 0.6 g/d of DHA or 4.05 g/d of EPA and 0.9 g/d of DHA	No significant impact of age or treatment group or interaction between these factors on phagocytic activity of neutrophils tested with *E. coli* in vitro; dose-dependent decrease in neutrophil respiratory burst only in older men versus younger men [34]
12-weeks supplementation with 1.02 g/d EPA and 0.69 g/d DHA	Reduction in the potency of monocytes from normal subjects, but not those from patients with PAD, to induce neutrophil recruitment, suggesting effects may depend on the medical status and medication usage [48]
8-weeks supplementation with 0.78 g/day of EPA and 1.62 g/d of DHA	Increase in neutrophil chemotaxis by 128%; increase in phagocytic activity of neutrophils to zymosan particles by 62%; a significant increase in ROS production by phorbol-myristate-acetate-stimulated neutrophils [49]
12-weeks supplementation with DHA-rich fish oil (1.56 g/d of DHA and 0.36 g/d of EPA)	No significant effects on neutrophil chemotaxis or adherence; significant reduction of ROS production by neutrophils [50]
8-weeks supplementation with 0.3 g/d of EPA and 0.4 g/d of DHA	Increase in phagocytosis of zymosan by neutrophils by 15% in fish oil group; phagocytic activity higher at 8 weeks in fish oil group than in control group; increase in superoxide production by PMNC (mostly neutrophils) on average by 28%; the number of PMNC higher by ~30% on average in fish oil group [51]
5 days supplementation with three escalating doses of enriched marine oil (corresponding to ~1.5 g, 3.0 g, and 6.0 g per day + SPM precursors); a 9-day washout interval, a total of 33 days	Significant increase in phagocytic activity of peripheral neutrophils in healthy participants and a trend toward increase in neutrophil phagocytosis in peripheral arterial disease (PAD) cohort; remodels downstream lipid mediator pathways; less inflammatory and more pro-resolution phenotype in circulating leukocytes (including neutrophils) in both healthy people and patients with PAD [52]
24 h of supplementation; doses: 1.5, 3, and 4.5 g of total fatty acids of which ~30 µg per 1.5 g total fatty acids were composed of AA (~3%), EPA (~46%), n-3 DPA (~18%) and DHA (~33%)	A dose-dependent increase in neutrophil phagocytosis of *S. aureus and E. coli* at the early intervals (2–4 h post supplementation), greatest increases at 24-h interval; reduced adhesion molecule expression by leukocytes and marked change in transcript levels of immune and metabolic genes 24 h post supplementation when compared with placebo [54]
6-month supplementation with marine n-3 fatty acid-enriched foods = 1.0 g/d of combined EPA and DHA	No effect on bactericidal activity of neutrophils or on neutrophil numbers [55]
8-weeks supplementation with 1.8 g/d of EPA+1.42 g/d of DHA + 0.15 g/d of DPA	Significantly reduced plasma levels of myeloperoxidase (a peroxidase enzyme released primarily by neutrophils) in patients with chronic kidney disease; no effect on neutrophil numbers [56]
8-weeks supplementation with almond-based beverage enriched with DHA (1.4 g/d)	No significant effects in the production of ROS by neutrophils after their activation with zymosan or PMA; significantly reduction in time at which maximal ROS production by neutrophils was reached after exercise; no effect on neutrophil counts [57]
12-weeks supplementation with either 6.6 g/d or 2.0 g/d of marine n-3 fatty acids	No effect on plasma levels of myeloperoxidase in healthy adults with low baseline levels of myeloperoxidase [58]
8-weeks supplementation with a beverage enriched with DHA (mean intake of 1.14 g/d)	Prevented PMA effects on neutrophil expression of inflammatory genes interleukin 8 (IL-8) and tumor necrosis factor α (TNF-α), and resulted in a reduced rate of IL-6 production [59]
4-weeks supplementation with 1.5 g/d DHA + 0.3 g/d of EPA (5:1 DHA:EPA)	No effect on IL-8, TNF-α, IL-1ra, IL-1∝, or IL-4 production by unstimulated and stimulated neutrophils [60]
8-week supplementation with 0.85 g/d of EPA + 0.60 g/d of DHA	No effect on neutrophils numbers; zymosan-induced oxidative burse higher in fish oil group [61]

to treat and by the effect of the change of n-3 PUFA levels and the change in the outcome variable(s) where possible to reduce the heterogeneity in trial design and advance the assessment of n-3 supplementation in humans [63].

Compliance with Ethical Standards

Conflict of Interest The author declares no competing Interests.

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