Mechanisms of Action of (n-3) Fatty Acids$^{1,2}$

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Abstract

(n-3) PUFA are a family of biologically active fatty acids. The simplest member of this family, α-linolenic acid, can be converted to the more biologically active very long-chain (n-3) PUFA EPA and DHA; this process occurs by a series of desaturation and elongation reactions, with stearidonic acid being an intermediate in the pathway. Biological activity of α-linolenic and stearidonic acids most likely relates to their conversion to EPA. The very long-chain (n-3) PUFA have a range of physiological roles that relate to optimal cell membrane structure and optimal cell function and responses. Thus, (n-3) PUFA play a key role in preventing, and perhaps treating, many conditions of poor health and well-being. The multiple actions of (n-3) PUFA appear to involve multiple mechanisms that connect the cell membrane, the cytosol, and the nucleus. For some actions, (n-3) PUFA appear to act via receptors or sensors, so regulating signaling processes that influence patterns of gene expression. Some effects of (n-3) PUFA seem to involve changes in cell membrane fatty acid composition. Changing membrane composition can in turn affect membrane order, formation of lipid rafts, intracellular signaling processes, gene expression, and the production of both lipid and peptide mediators. Under typical Western dietary conditions, human cells tend to have a fairly high content of the (n-6) fatty acid arachidonic acid. Increased oral intake of EPA and DHA modifies the content of arachidonic acid as well as of EPA and DHA. Arachidonic acid is the substrate for eicosanoids involved in physiology and pathophysiology. The eicosanoids produced from EPA frequently have properties that are different from those that are produced from arachidonic acid. EPA and DHA are also substrates for production of resolvin and protectins, which seem to be biologically extremely potent. Increasing the contents of EPA and DHA in membranes modifies the pattern of production of these different lipid mediators. J. Nutr. doi: 10.3945/jn.111.155259.

Structure, Naming, and Metabolic Relationships of (n-3) Fatty Acids

The term (n-3) is a structural descriptor for a family of PUFA (1); (n-3) describes the position of the double bond that is closest to the methyl terminus of the acyl chain. In all (n-3) fatty acids, this double bond is on carbon 3, counting the methyl carbon as carbon number 1. As with all fatty acids, (n-3) fatty acids have systematic and common names. They are also described by a shorthand nomenclature that is based on the number of carbon atoms in the acyl chain, the number of double bonds, and the position of the first double bond relative to the methyl carbon. The simplest (n-3) fatty acid is α-linolenic acid [18:3(n-3)]. α-Linolenic acid is synthesized from linoleic acid [18:2(n-6)] by desaturation, catalyzed by Δ-15 desaturase. Animals, including humans, do not possess the Δ-15 desaturase enzyme and so cannot synthesize α-linolenic acid. Thus, α-linolenic acid is a classically essential fatty acid, along with linoleic acid. Plants can synthesize α-linolenic acid because they possess Δ-15 desaturase.

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Dietary Sources and Typical Intakes of α-Linolenic Acid and Very Long-Chain (n-3) PUFA

α-Linolenic acid is a common constituent of green leaves, often comprising over 50% of their fatty acids. However, green leaves are not high in fat, so these are not usually a major dietary source of α-linolenic acid. A number of different seeds and their oils and some nuts contain considerable amounts of α-linolenic acid. Flax seeds (flaxseeds) and their oil typically contain 45–55% of fatty acids as α-linolenic acid, whereas soybean oil, rapeseed oil, and walnuts all typically contain ~10% of fatty acids as α-linolenic acid. There is little α-linolenic acid in corn oil, sunflower oil, or safflower oil, which are all rich in linoleic acid. Dietary intakes of α-linolenic acid among Western adults are typically in the range of 0.5–2 g/d (2,7). The (n-6) fatty acid linoleic acid is the main PUFA in most Western diets and is typically consumed in 5- to 20-fold greater amounts than α-linolenic acid (2,7,8).

Fish are described as lean or fatty (oily). Lean fish, like cod, store lipid in their liver. Oily fish, like mackerel, herring, salmon, tuna, and sardines, store lipid in their flesh. Fish and other seafood are much better sources of very-long-chain (n-3) PUFA than other foods (7). It is important to note though that different types of fish contain different amounts of these fatty acids and may have different ratios of EPA:DHA (7). This is because the (n-3) PUFA content and type is influenced by a number of factors, including the metabolic characteristics of the fish, their diet, the temperature of the water in which they live, and the season. A single lean fish meal (e.g., one serving of cod) could provide ~0.2–0.3 g very-long-chain (n-3) fatty acids. In contrast, a single oily fish meal (e.g., one serving of salmon or mackerel) could provide 1.5–3.5 g of these fatty acids. Because oily fish consumption among many Western populations is typically low, average (mean) intakes of very-long-chain (n-3) fatty acids among adults in Northern and Eastern Europe, North America, and Australasia are low and estimated to be ~0.15–0.25 g/d (9). However, the distribution of intakes is bimodal, because there are consumers and nonconsumers of oily fish. An Australian study estimated a median intake of very-long-chain (n-3) fatty acids of ~0.03 g/d among adults compared with a mean intake of ~0.19 mg/d (10). Populations such as the Japanese, who consume oily fish in greater amounts and with greater regularity than populations in Europe, North America, and Australasia, have higher intakes of very-long-chain (n-3) PUFA.

Fish oil is obtained from oily fish flesh or lean fish livers (e.g., cod liver). Fish oil is rich in very-long-chain (n-3) fatty acids and in a typical fish oil, EPA and DHA comprise ~30% of the fatty acids present. Thus, a 1-g fish oil capsule can provide ~0.3 g of EPA plus DHA. However, the variations in the amount of (n-3) fatty acids and the relative proportions of the individual very-long-chain (n-3) PUFA (EPA, DPA, and DHA) in fish are reflected in fish oils. As an example, cod liver oil is richer in EPA than DHA, whereas tuna oil is richer in DHA than EPA. Oils that are more concentrated in (n-3) fatty acids than standard fish oils are available.

Overview of the Physiological Roles and Corresponding Health Benefits of (n-3) PUFA

Very-long-chain (n-3) fatty acids have been demonstrated to have a wide range of physiological roles, as summarized in Table 1. These roles include:

- **Cardiovascular Health**: EPA and DHA are known to have anti-inflammatory effects, which can help reduce the risk of heart disease.
- **Blood Pressure Management**: Consumption of EPA and DHA can help lower blood pressure and reduce the risk of stroke.
- **Cerebrovascular Health**: These fatty acids are important for brain health and function.
- **Vision and Eye Health**: Long-chain n-3 fats, especially DHA, are critical for maintaining healthy eyes and vision.
- **Immune Function**: Fish oils containing EPA and DHA are beneficial for the immune system.
- **Developmental Benefits**: During pregnancy and infancy, DHA is essential for healthy brain development.

3 Abbreviations used: COX, cyclooxygenase; DPA, docosapentaenoic acid; GPR, G-protein coupled receptor; IkB, inhibitory subunit of NFκB.
TABLE 1  Summary of the physiological roles and potential health benefits of very long-chain (n-3) fatty acids

<table>
<thead>
<tr>
<th>Physiological role of very long-chain (n-3) fatty acids</th>
<th>Potential health benefit</th>
<th>Disease target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulation of blood pressure</td>
<td>Decreased blood pressure</td>
<td>Hypertension, CVD</td>
</tr>
<tr>
<td>Regulation of platelet function</td>
<td>Decreased likelihood of thrombosis</td>
<td>Thrombosis, CVD</td>
</tr>
<tr>
<td>Regulation of blood coagulation</td>
<td>Decreased likelihood of thrombosis</td>
<td>Thrombosis, CVD</td>
</tr>
<tr>
<td>Regulation of plasma TG concentrations</td>
<td>Decreased plasma TG concentrations</td>
<td>Hypertriglyceridemia, CVD</td>
</tr>
<tr>
<td>Regulation of vascular function</td>
<td>Improved vascular reactivity</td>
<td>CVD</td>
</tr>
<tr>
<td>Regulation of cardiac rhythm</td>
<td>Decreased cardiac arrhythmias</td>
<td>CVD</td>
</tr>
<tr>
<td>Regulation of heart rate</td>
<td>Increased heart rate variability</td>
<td>CVD</td>
</tr>
<tr>
<td>Regulation of inflammation</td>
<td>Decreased inflammation</td>
<td>Inflammatory diseases, psoriasis, lupus, arthritis, cystic fibrosis, dermatitis, neurodegeneration, etc., CVD</td>
</tr>
<tr>
<td>Regulation of immune function</td>
<td>Improved immune function</td>
<td>Compromised immunity</td>
</tr>
<tr>
<td>Regulation of fatty acid and TG metabolism</td>
<td>Decreased TG synthesis and storage</td>
<td>Weight gain, weight loss, obesity</td>
</tr>
<tr>
<td>Regulation of bone turnover</td>
<td>Maintained bone mass</td>
<td>Osteoporosis</td>
</tr>
<tr>
<td>Regulation of insulin sensitivity</td>
<td>Improved insulin sensitivity</td>
<td>Type-2 diabetes</td>
</tr>
<tr>
<td>Regulation of tumor cell growth</td>
<td>Decreased tumor cell growth and survival</td>
<td>Some cancers</td>
</tr>
<tr>
<td>Regulation of visual signaling</td>
<td>Optimized visual signaling</td>
<td>Poor infant visual development</td>
</tr>
<tr>
<td>Structural component of brain and central nervous system</td>
<td>Optimized brain development leading</td>
<td>Poor infant and childhood cognitive</td>
</tr>
</tbody>
</table>

1 CVD, cardiovascular disease. Reproduced with permission of (1).

1, where these roles are linked to certain health or clinical benefits. They have been demonstrated to beneficially modify a number of risk factors for cardiovascular disease. Risk factors improved include blood pressure (11), platelet reactivity and thrombosis (12), plasma TG concentrations (13), vascular function (14), cardiac arrhythmias (15), heart rate variability (15), and inflammation (16). Due to these effects, increased intake of very long-chain (n-3) fatty acids is associated with a reduced risk of cardiovascular morbidity and mortality (17,18). Supplementation of at-risk patients with very long-chain (n-3) fatty acids reduced mortality (19–23). In addition, several noncardiovascular actions of these fatty acids have been described (Table 1), suggesting that increasing their intake could reduce the risk of (i.e., protect against) a number of conditions and may even be used as a treatment. As an example, very long-chain (n-3) PUFA have been used successfully in rheumatoid arthritis (24) and, to a lesser extent, in inflammatory bowel diseases (25) and asthma (26). DHA has an important structural role in the eye and brain. Consequently, ensuring a good DHA supply early in life when the brain and eye are developing is vitally important to optimize visual and neurological development (26,27). Very long-chain (n-3) fatty acids may contribute to enhanced mental development (28) and improved childhood learning and behavior (29) and may lower the burden of psychiatric illnesses in adults (30). There also appears to be a role for very long-chain (n-3) PUFA, especially DHA, in preventing neurodegenerative disease of ageing (31). The effects of very long-chain (n-3) PUFA on health outcomes are likely to be dose dependent, but clear dose response data have not been identified in most cases.

Plant (n-3) PUFA that are precursors of EPA and DHA (e.g., α-linolenic acid and stearidonic acid) appear to share some of the physiological and functional attributes of very long-chain (n-3) PUFA (2,4,32,33). These actions of the plant (n-3) PUFA appear to derive from their conversion to the biologically active EPA (2,4,32,33). Therefore, because this conversion is limited, although greater for stearidonic acid than α-linolenic acid (4), the biological potency of the plant (n-3) PUFA is less than that of the very long-chain (n-3) PUFA (2).

Overview of Mechanisms of Action of (n-3) PUFA

As outlined in the previous section, (n-3) PUFA have multiple actions. Therefore, they are likely to act via multiple mechanisms. There are 4 general mechanisms by which (n-3) PUFA could affect cell and tissue behavior to elicit their physiological actions: 1) (n-3) PUFA could influence metabolite and/or hormone concentrations that in turn influence cell and tissue behavior; 2) (n-3) PUFA could influence other factors (e.g., oxidation of LDL; oxidative stress) that in turn influence cell and tissue behavior; 3) direct effects of (n-3) PUFA on cell behavior via surface or intracellular fatty acid “receptors” or “sensors”; 4) effects of (n-3) PUFA on cell behavior mediated via changes in the composition of cell membrane phospholipids.

Actions of (n-3) Fatty Acids Mediated via Surface or Intracellular Fatty Acid Receptors or Sensors

Involvement of PPAR

PPAR are transcription factors. They regulate gene expression and so have a role in cell and tissue responses to the environment. PPAR act by forming a heterodimer with the retinoic-X-receptor. The ligand for the retinoic-X-receptor is cis-9-retinoic acid. PPARα and PPARγ are the most well-understood PPAR
endothelial cells (46,47), monocytes (48,49), macrophages (50), and dendritic cells (41,51,52). These inhibitory effects of (n-3) PUFA seem to involve decreased IκB phosphorylation and decreased activation of NFκB (50,53), effects that appear to involve a reduction in the activation of key early signaling proteins such as mitogen-activated protein kinases (54). In agreement with the in vitro studies, fish oil feeding to laboratory animals also decreases NFκB activation (55) and production of inflammatory cytokines (56–58). Some studies in healthy human volunteers involving fish oil supplementation showed decreased production of TNFα, IL-1β, IL-6, and various growth factors by monocytes or mononuclear cells that were stimulated with bacterial endotoxin (59–63). These findings indicate that very long-chain (n-3) PUFA act on inflammatory processes through inhibiting the activation of NFκB that occurs in response to exogenous inflammatory stimuli. Although the antiinflammatory effects of (n-3) PUFA on NFκB may involve actions on the signaling process leading to its activation (54), there is also an interaction between PPARγ and NFκB (Fig. 2). Ligand-bound (i.e., activated) PPAR interact physically with NFκB, preventing its translocation to the nucleus (64). Thus, through binding to PPAR, (n-3) PUFA could act to inhibit upregulation of NFκB target genes.

Involvement of G-protein coupled surface receptors

Some GPR can bind certain fatty acids. GPR40 and GPR120 are both able to bind long-chain fatty acids and both these GPR activate intracellular signaling pathways. Whereas GPR120 is highly expressed on adipocytes and inflammatory macrophages, GPR40 is not expressed on the latter (65). GW9508, which is an agonist of GPR120, was found to inhibit the response of cultured macrophages to endotoxin, and this involved maintenance of cytosolic IκB and a decrease in production of TNFα and IL-6 production, effects that are similar to those of EPA and DHA. Thus, it appears that GPR120 is involved in antiinflammatory signaling. GPR120-mediated gene activation was enhanced by EPA and DHA. Furthermore, the ability of DHA to inhibit responsiveness to endotoxin was abolished in GPR120 knockdown cells. These findings indicate that the inhibitory effect of DHA (and probably also EPA) on NFκB occurs via GPR120. Thus, there appear to be at least 2 mechanisms by which (n-3) PUFA act to inhibit NFκB activation and production of inflammatory mediators.

Interaction with NFκB

NFκB is a key transcription factor involved in inducing the expression of genes encoding a range of proteins involved in inflammation, including several cytokines, adhesion molecules, COX-2, and inducible NO synthase (43,44). In its inactive state, NFκB is in the form of a trimer present in the cytosol. Activation of NFκB occurs in response to certain extracellular signals, including bacterial endotoxin, inflammatory cytokines, UV irradiation, and oxidative stress. The activation of NFκB requires that its inhibitory subunit, IκB, is phosphorylated. This leads to dissociation of IκB from the NFκB trimer. IκB is then degraded, while the remaining NFκB dimer translocates to the nucleus (45). Pure EPA and DHA can inhibit the production of a range of inflammatory proteins, including COX-2, inducible NO synthase, TNFα, IL-1, IL-6, IL-8, and IL-12 in cultured adipose tissue, where it regulates adipocyte differentiation and regulates the metabolic responses of adipocytes, including promoting insulin sensitivity. PPARγ is also expressed in inflammatory cells, where it is involved in regulating the production of inflammatory mediators, having an antiinflammatory action (35) (Fig. 2B). PPAR are activated by noncovalent binding of ligands that include (n-3) PUFA and various eicosanoids mediators (36–40). In dendritic cells, DHA induced PPARγ (41) and a number of known PPARγ target genes (42). These effects were linked to decreased production of the inflammatory cytokines TNFα and IL-6 upon endotoxin stimulation (41). Thus, through activation of PPAR, (n-3) PUFA are able to regulate metabolism and other cell and tissue responses, including adipocyte differentiation and inflammation (Fig. 2). This mechanism might explain some of physiological actions of (n-3) PUFA, e.g., their ability to lower fasting plasma TG concentrations, increase insulin sensitivity, and reduce inflammation (Fig. 3).

FIGURE 2 The PPARα and -γ pathways. ACO, acyl CoA oxidase; Adipo, adiponectin; ADRP, adipose differentiation related protein; aP2, adipocyte protein 2; ApoA, apolipoprotein A; C/EBP, CCAAT/enhancer-binding proteins; COX, cyclooxygenase; CYP4A, cytochrome P450 4A; FABP, fatty acid binding protein; L, ligand; LPL, lipoprotein lipase; RXR, retinoic acid receptor.

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which (n-3) PUFA inhibit NFκB activation, one involving GPR120 and the other involving PPARγ, although these may be linked. Oh et al. (65) also demonstrated that DHA promoted the translocation of the glucose transporter GLUT4 to the surface of cultured adipocytes and that this was associated with enhanced glucose uptake. These effects were abolished by GPR120 knockout, suggesting that GPR120 mediates some of the metabolic actions of DHA.

**Actions of (n-3) Fatty Acids Mediated via Changes in the Composition of Cell Membrane Phospholipids**

The roles of fatty acids in cell membrane phospholipids

Fatty acids in the phospholipids of cell membranes play important roles in assuring the correct environment for membrane protein function (66), maintaining membrane order (fluidity) (67), and influencing lipid raft formation (68). Second messengers like diacylglycerol are produced by enzyme-catalyzed hydrolysis of membrane phospholipids; the fatty acid composition of such second messengers, which is determined by that of the precursor phospholipid, can influence their activity (69). In addition, membrane phospholipids are substrates for the release of (non-esterified) PUFA; the released PUFA can act as signaling molecules, ligands (or precursors of ligands) for transcription factors like PPAR, or precursors for biosynthesis of lipid mediators, which are involved in regulation of many cell and tissue responses. Thus, as summarized in Figure 4, changes in membrane phospholipid fatty acid composition can influence the function of cells via alterations in the physical properties of the membrane such as membrane order and raft structure; effects on cell signaling pathways that lead to altered transcription factor activity and changes in gene expression; and alterations in the pattern of lipid mediators produced, with the different mediators having different biological activities and potencies.

**Cell membrane fatty acid composition is modified by exposure to (n-3) PUFA**

Different plasma lipid pools, cells, and tissues have different, characteristic, fatty acid compositions. These compositions are influenced by the availability of different fatty acids but also by the metabolic characteristics of the particular pool, cell, or tissue. Dietary supplementation with fish oil results in modification of fatty acid profiles, with an increase in the content of EPA and DHA in plasma lipids, platelets, erythrocytes, leukocytes, colonic tissue, cardiac tissue, and liver tissue. The incorporation of EPA and DHA occurs in a dose-response fashion (70–76) and these fatty acids often replace (n-6) PUFA such as arachidonic acid.

**Eicosanoids produced from the (n-6) PUFA arachidonic acid include various prostaglandins, thromboxanes, and leukotrienes. These have well-established roles in regulation of inflammation, immunity, platelet aggregation, smooth muscle contraction, and renal function (77). Excess or inappropriate production of these eicosanoids is associated with disease processes. For example, cysteinyl-leukotrienes derived from arachidonic acid have a recognized role in the pathology of asthma. Very long-chain (n-3) PUFA decrease the availability of arachidonic acid as a substrate for eicosanoid synthesis and they also inhibit arachidonic acid metabolism. Thus, very long-chain (n-3) PUFA decrease production of eicosanoids from arachidonic acid and so can affect the actions regulated by those mediators (16).**

**EPA is a substrate for the synthesis of alternative eicosanoids, which have a different structure from those produced from arachidonic acid and which are typically less potent (16). This lower potency may be related to the generally lower ability of the
EPA-derived mediators to interact with relevant eicosanoid receptors (78). A recent study reported that the EPA-derived PG_D3 could inhibit the action of the arachidonic acid-derived PG_D2 through the DP1 receptor (79). Interestingly, Wada et al. (78) reported that PG_D3 interacted more strongly with the DP1 receptor than did PG_D2.

Relatively recently, a new family of lipid mediators, termed resolvins, synthesized from both EPA (E-series resolvins) and DHA (D-series resolvins) has been described; related DHA-derived mediators called protectins have also been described. The synthesis of resolvins and protectins involves the COX and lipoxigenase pathways. Cell culture and animal models have shown potent antiinflammatory, inflammation-resolving, and immunomodulatory actions of resolvins (80–82). For example, resolin E1, resolin D1, and protectin D1 all inhibit trans-endothelial migration of neutrophils, so preventing the infiltration of neutrophils into sites of inflammation; resolin D1 inhibits IL-1β production and protectin D1 inhibits TNFα and IL-1β production (80–82). Protectin D1 appears to have an important role in protecting tissue, including neuronal tissue, from excessive damage in a variety of experimental situations (81) and may be important in preventing neurodegeneration (83).

**(n-3) PUFA influence formation of membrane signaling platforms called rafts**

Lipid rafts are subdomains of cell membranes. They have a distinct, characteristic composition, being rich in sphingolipids and cholesterol, and the side chains of the phospholipids present are usually highly enriched in SFA compared with the surrounding nonraft regions of the membrane (84). Many proteins involved in signal transduction are predominantly found in lipid rafts, which appear to act as signaling platforms by bringing together (i.e., colocalizing) various signaling components in response to a stimulus, so facilitating their interaction (84). Lipid rafts are potentially modifiable by dietary (n-3) fatty acids, but it seems that dietary PUFA are not incorporated into raft lipids, because their low affinity to cholesterol does not allow this to occur. However, they are incorporated into nonraft regions and seem to influence raft formation and function from outside of rafts. In vitro and animal studies suggest that (n-3) PUFA modulate the structure and composition of lipid rafts in immune cells, displacing key signaling proteins and altering intracellular protein trafficking (85–94). There is some evidence that very long-chain (n-3) PUFA affect endothelial function through mechanisms involving rafts (95,96), whereas exposure of specific cancer cell lines to (n-3) PUFA results in disruption of lipid rafts, generation of ceramide from sphingomyelin hydrolysis, expulsion of key receptors, and induction of apoptosis (68,97). This highlights that (n-3) PUFA might inhibit tumor growth through effects on membrane rafts.

**Summary and Conclusions**

(n-3) PUFA are a family of biologically active fatty acids. The simplest member of this family, α-linolenic acid, can be converted to the more biologically active very long-chain (n-3) PUFA EPA and DHA; this process occurs by a series of desaturation and elongation reactions, with stearidonic acid being an intermediate in the pathway. Biological activity of α-linolenic and stearidonic acids most likely relates to their conversion to EPA. The very long-chain (n-3) PUFA have a range of physiological roles that relate to optimal cell membrane structure and cell function and responses. Thus, (n-3) PUFA play a key role in preventing, and perhaps in treating, many conditions of poor health and well-being. The multiple actions of (n-3) PUFA appear to involve multiple mechanisms that connect the cell membrane, cytosol, and nucleus (Fig. 4). Thus, (n-3) PUFA act via cell surface and intracellular receptors/sensors that control cell signaling and gene expression patterns. Some effects of (n-3) PUFA appear to be mediated by, or at least are associated with, changes in fatty acid composition of cell membranes. Changes in these compositions can modify membrane order, lipid raft formation, cell signaling leading to altered gene expression, and the pattern of lipid and peptide mediator production. Under typical Western dietary conditions, human cells are rich in the (n-6) fatty acid arachidonic acid, but the contents of arachidonic acid and of the (n-3) fatty acids EPA and DHA can be altered through oral administration of EPA and DHA. Eicosanoids produced from arachidonic acid have physiological and pathophysiological roles. EPA also gives rise to eicosanoids and these may have differing properties from those of arachidonic acid-derived eicosanoids. EPA and DHA give rise to newly discovered resolvins and protectins that appear to be extremely potent. Increased membrane content of EPA and DHA (and decreased arachidonic acid content) results in a changed pattern of production of eicosanoids and probably also of resolvins and protectins. Thus, the (n-3) fatty acid content of human cells influences their function.

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