

n-3 Fatty acids and gene expression¹⁻⁴

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ABSTRACT

Accumulating evidence in both humans and animal models clearly indicates that a group of very-long-chain polyunsaturated fatty acids, the n-3 fatty acids (or omega-3), have distinct and important bioactive properties compared with other groups of fatty acids. n-3 Fatty acids are known to reduce many risk factors associated with several diseases, such as cardiovascular diseases, diabetes, and cancer. The mechanisms whereby n-3 fatty acids affect gene expression are complex and involve multiple processes. As examples, n-3 fatty acids regulate 2 groups of transcription factors, such as sterol-regulatory-element binding proteins and peroxisome proliferator-activated receptors, that are critical for modulating the expression of genes controlling both systemic and tissue-specific lipid homeostasis. Modulation of specific genes by n-3 fatty acids and cross-talk between these genes are responsible for many effects of n-3 fatty acids. *Am J Clin Nutr* 2006;83(suppl):1520S-5S.

KEY WORDS Fatty acid, gene expression, EPA, eicosapentaenoic acid, DHA, docosahexaenoic acid, SREBP, sterol-responsive-element binding protein, PPAR, peroxisome proliferator-activated receptor

INTRODUCTION

Fat has traditionally been regarded to be important as a calorie-dense nutrient and as a source for essential fatty acids. In recent years, fats and especially one of their key components, fatty acids, have been increasingly recognized as major biological regulators. Varying intake of dietary fatty acids leads to changes in cell membrane structure and function. Fatty acids have differential effects on the production of cytokines, chemotaxis, and other factors relating to the immunologic response (1). Fatty acids modulate the pathways of blood coagulation and, in blood vessels, vascular resistance. Certain fatty acids influence sterol metabolism, signal transduction, enzyme activities, cell proliferation and differentiation, and receptor expression (2). Many of the effects of fatty acids in both cell biology and human health and disease relate to their abilities to regulate gene expression and subsequent downstream events. In many of the above pathways, a particular group of fatty acids are especially potent, the n-3 (or omega-3) fatty acids.

n-3 FATTY ACIDS

A growing body of epidemiologic and experimental studies indicates that increased n-3 fatty acid consumption ameliorates or decreases the risk of a variety of diseases (3). n-3 Fatty acids have definite roles in cognitive development and learning, visual

function, the immune-inflammatory response, pregnancy outcomes, neurologic degeneration, cardiovascular disease, and cancer (3). n-3 Fatty acids appear to have distinct capacities to modulate both cellular metabolic functions and gene expression. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are 2 key biological regulators. Emerging data show that synthesis of EPA and DHA from their 18:3 precursor α -linoleic acid is relatively inefficient (4, 5). Thus, efficient tissue accretion of n-3 fatty acids depends to a significant degree on the delivery of EPA and DHA directly from dietary, ie, marine or industrial, sources.

Why are these longer chain n-3 fatty acids different from other fatty acids in terms of their biological effects? Likely, their longer chain length, high number of double bonds, and the presence of the first double bond (3 carbon atoms from the methyl terminal, thus n-3) gives these fatty acids distinct and unique properties that separate them and their metabolic products from the more common n-6 and n-9 fatty acids. Overall, the positive effects of n-3 fatty acids on health relate to 1) inhibition or modulation of eicosanoid pathways, which leads to alteration of inflammatory responses and related protein expression and activity; 2) modulation of molecules or enzymes associated with various signaling pathways involving normal and pathologic cell function; 3) incorporation of n-3 fatty acids into membrane phospholipids; and 4) direct effects on gene expression. Because the above pathways are highly interactive, the biological potentials of n-3 fatty acids on health and disease must be due to multiple coordinated mechanisms (3).

Although the basis of many of the biological differences is not fully understood, it is clear that the presence of EPA and DHA either as free or unesterified fatty acids, as part of a phospholipid molecule, or as part of a triacylglycerol molecule induces physical effects that change metabolic pathways. For example, in recent published and unpublished studies, our laboratory has shown that model chylomicron-size and VLDL-size triacylglycerol-rich particles containing 5-48% of their fatty

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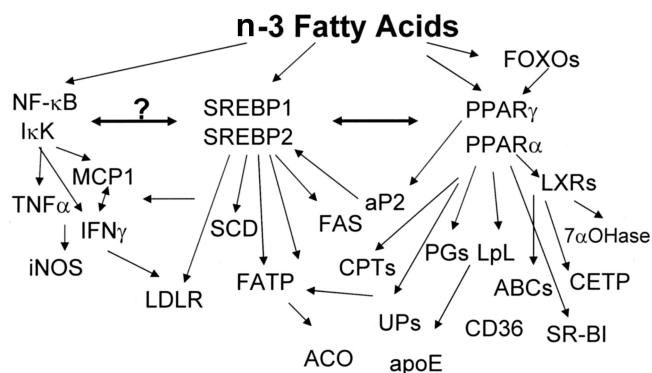


FIGURE 1. Examples of the different gene families regulated by n-3 fatty acids and their interaction. ABCs, ATP binding cassette transporters; ACO, acyl CoA oxidase; aP2, adipocyte fatty acid binding protein; apoE, apolipoprotein E; CETP, cholesteryl ester transfer protein; CPTs, carnitine palmitoyltransferases; FATP, fatty acid transport protein; FAS, fatty acid synthase; FOXOs, forkhead transcription factors; IFN γ , interferon γ ; I κ K, inhibitory κ B kinase; iNOS, inducible nitric oxide synthase; LDLR, LDL receptor; LpL, lipoprotein lipase; LXRs, liver X receptors; MCP1, monocyte chemoattractant protein 1; NF- κ B, nuclear factor κ B; 7 α OHase, 7 α -hydroxylase; PGs, prostaglandins; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl CoA desaturase; SR-BI, scavenger receptor B1; SREBP, sterol-responsive-element binding protein; TNF α , tumor necrosis factor α ; UPs, uncoupling proteins.

acids as EPA and DHA have different blood clearance pathways than do n-6 triacylglycerol-rich particles (6-8). Compared with predominantly n-6 triacylglycerol-rich particles, n-3 triacylglycerol-enriched particles are cleared faster from blood in both humans and animals (6, 7) and are much less dependent on the activities of lipoprotein lipase, the LDL receptor, and apolipoprotein E-dependent pathways for blood removal and tissue targeting (7). Because these changes in blood clearance are obvious within 1 min after injection into animal models, it is most likely that at least some of these differences from n-6 particles result from direct physical effects of n-3 fatty acids or n-3 triacylglycerols.

n-3 FATTY ACIDS AND GENE EXPRESSION

n-3 Fatty acids are major modulators of many genes (**Figure 1**). As illustrated in the figure, n-3 fatty acids affect the expression of several key proteins pertinent to inflammation, lipid metabolism, and energy utilization. Major effects of n-3 fatty acids include their ability to reduce inflammation and promote lipid oxidation for enhanced energy utilization. However, in different tissues, n-3 fatty acids can up-regulate or down-regulate the expression of different proteins (9). For example, a diet enriched in n-3 fatty acids reduces peroxisome proliferator-activated receptor α (PPAR α) expression in adipose tissue without affecting PPAR γ expression. In contrast, the expression of both PPAR α and PPAR γ is induced in the arterial wall in an insulin-resistant mouse model (Chang, Deckelbaum, and Seo, unpublished observations, 2005). Moreover, the n-3 acyl chain position within a triacylglycerol molecule apparently has a significant effect on the potency of n-3 fatty acids in regulating protein expression (10). Other possible mechanisms for these effects include the expression or processing of intermediates, such as transcription factors, nuclear hormone receptors, and lipid second messengers, which then trigger a cascade of other genes or other effects in the cell that then affect gene expression,

eg, producing an n-3 metabolite that affects gene expression. Another hypothetical consideration could be a direct molecular interaction of n-3 fatty acids with certain genes.

n-3 FATTY ACIDS AND STEROL-REGULATORY-ELEMENT BINDING PROTEINS

The ability of polyunsaturated fatty acids to affect sterol-regulatory-element binding protein (SREBP)-dependent gene expression provides an example of the pluripotent effects of n-3 fatty acids on gene expression. Three SREBPs are recognized: SREBP1a and SREBP1c, which largely regulate genes of fatty acid metabolism, and SREBP2, which regulates genes involved in cholesterol metabolism; however, there is a crossover in their activities. SREBPs are regulated posttranscriptionally, and the inactive precursor form is located in the endoplasmic reticulum, where it is linked to SREBP cleavage-activating protein (SCAP). The complex is anchored by the Insig proteins (11). The landmark studies of the Brown and Goldstein group have shown that under conditions of sterol deprivation, Insigs dissociate from the SREBP/SCAP complex, which then translocates to the Golgi via vesicular transport (11). In the Golgi, SREBP dissociates from SCAP and undergoes a two-step proteolytic cleavage, and the transcriptionally active amino-terminal fragment of SREBP, n-SREBP, is released. SREBP binds to sterol regulatory elements in the promoter region of many genes of lipid metabolism. Cholesterol and oxysterols regulate this pathway by end-product feedback inhibition (12).

Our laboratory and other groups later showed that polyunsaturated fatty acids alone significantly decrease transcriptionally active concentrations of n-SREBP (13-15). However, the mechanisms by which polyunsaturated fatty acids regulate SREBP are less clear. Interestingly, the longer chain polyunsaturated fatty acids, eg, EPA, DHA, and the n-6 fatty acid arachidonic acid, have far more inhibitory capacity on SREBP processing than do the shorter chain polyunsaturated fatty acids (eg, 18:1, 18:2); saturated fatty acids have no or almost no effect on SREBP processing (14). We postulated that the ability of polyunsaturated fatty acids to inhibit SREBP conversion from its inactive to its active form relates to both physical and biochemical effects and showed that both are likely to occur. Using large and small unilamellar vesicles as a model for plasma and intracellular membranes, respectively, we showed that addition of fatty acids to these model membranes decreases the affinity of cholesterol for phospholipid and this in turn results in enhanced transfer from cholesterol-rich regions (such as the plasma membrane) to cholesterol-poor regions (such as the endoplasmic reticulum), a condition that would lead to decreased SREBP transport out of the endoplasmic reticulum to the Golgi (16).

Another possible mechanism by which polyunsaturated fatty acids decrease SREBP is by affecting the cellular composition of membranes. We showed that polyunsaturated fatty acids increase the hydrolysis of plasma membrane sphingomyelin to ceramide (17). Sphingomyelin has a higher affinity for free cholesterol than do other phospholipids in the cell membrane. Hydrolysis of sphingomyelin to ceramide and phosphocholine affects cellular cholesterol homeostasis and gene transcription by 2 different mechanisms. One, lower amounts of sphingomyelin result in a decreased ability to solubilize free cholesterol, which results in intracellular displacement of cholesterol and a consequent decrease in SREBP-mediated gene transcription. Two, we

showed in separate experiments that ceramide itself is a potent inhibitor of SREBP processing through effects on sphingolipid synthesis (17), a process that can regulate endoplasmic reticulum–Golgi vesicular transport (18). We then went on to show that ceramide synthesis is obligatory in the regulation of SREBP processing (19). Thus, polyunsaturated fatty acids can interact with different steps of sphingolipid metabolism that affect SREBP processing.

The ability of longer chain polyunsaturated but not shorter chain monounsaturated or saturated fatty acids to suppress the LXRE enhancer complex in the SREBP1c promoter region further exemplifies the multiple mechanisms by which fatty acids can affect gene transcription. Although all the above changes can be observed over a relatively short time period (eg, 4–18 h), longer term effects of n–3 fatty acids have also been shown. For example, chronic feeding of n–3-rich diets to rats increases the degradation of SREBP messenger RNA, which results in overall decreases in cellular concentrations of both precursor and mature forms of SREBP (20). In summary, multiple interactive mechanisms exist whereby long-chain polyunsaturated fatty acids, especially n–3 fatty acids, can affect SREBP-dependent gene expression.

n–3 FATTY ACIDS, PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS, AND OTHER TRANSCRIPTION FACTORS

A substantial amount of data, primarily from in vitro studies, indicate that n–3 fatty acids are important regulators of PPAR. There are at least 4 PPAR isoforms: alpha, beta, delta, and gamma, which have distinct, but interrelated functions. Activation of the PPAR superfamily is critical for controlling key proteins that are crucial for lipid homeostasis pathways. n–3 Fatty acids have been reported to bind to at least PPAR α and PPAR γ to induce physiologic responses such as promoting β -oxidation and adipogenesis. Nevertheless, the effects of n–3 fatty acids on PPARs vary depending on cell types. For example, n–3 fatty acids stimulate *trans*-activation of a PPAR response element in HepG2 cells, whereas the same n–3 fatty acids suppress PPAR responses in MCF-7 human breast cancer cells (9).

How do n–3 fatty acids activate PPARs? Although the mechanisms by which n–3 fatty acids activate PPAR α and PPAR γ remain elusive, the oxidized linoleate and arachidonate metabolites 13-HODE, 15-HETE, prostaglandin J₂, and 15-deoxy- Δ 12,14-prostaglandin J₂ are generally believed to be natural PPAR ligands (21, 22). Also, at least in vitro, EPA binds to all PPAR isoforms at a K_d of 1–4 μ mol/L (23). Thus, it is likely that oxidative derivatives of n–3 free fatty acids, and their eicosanoid products, bind PPARs (24). Recent elegant studies by Hostetler et al (25) showed that polyunsaturated fatty acids as well as their coenzyme A derivatives, but not saturated fatty acids, can directly bind to PPAR α . The studies also showed that free fatty acid binding subsequently induces conformational changes that correlate functionally with coactivator binding. Although these studies were not performed with n–3 fatty acids, it is expected that similar mechanisms likely operate for PPAR activation.

Activation of PPARs by n–3 fatty acids unequivocally influences many critical cellular functions at multiple levels. Thus, together with their ability to regulate SREBPs, n–3 fatty acids

can serve as master switches. Although in vivo data on the interaction of PPARs and SREBPs are scarce, accumulating evidence for a variety of chemical agonists suggests that substantial “cross-talk” likely exists between PPAR signaling, SREBP expression, and liver X receptor (26–29). These interactions could, in turn, affect overall cell lipid homeostasis in a highly complex but coordinated manner. The downstream effects of these interactions are also complicated, because the characteristics and potency of each n–3 fatty acid differ and can exhibit transcriptional regulatory properties. For example, it has been shown that DHA can bind to RXRs (a transcription factor that heterodimerizes with PPARs) and influence RXR-mediated transcription in brain (30). In contrast, another n–3 fatty acid, EPA, failed to activate RXR-mediated transcription even though both DHA and EPA activate PPARs. In summary, n–3 fatty acids and likely their metabolites unquestionably serve as agonists for nuclear hormone and other receptors and SREBPs, but detailed mechanistic information regarding how and when fatty acids regulate responsive genes in vivo is still lacking.

n–3 FATTY ACIDS IN DISEASE PREVENTION AND THERAPEUTICS

Because of their ability to inhibit inflammatory pathways and suppress the expression of a large number of genes related to lipid metabolism, n–3 fatty acids are being considered as therapeutic agents in dyslipidemia, the metabolic syndrome, type 2 diabetes, and steatohepatitis. Although their role in lowering elevated blood triacylglycerol concentrations has been unequivocally proven, data in humans relating to the other areas of abnormalities in lipid metabolism remain to be proven.

Nevertheless, it is of interest that n–3 fatty acids, in particular EPA and DHA, have been shown to have similar regulatory

Inflammatory Proteins	Energy/Lipid Metabolism
<i>NF-κB</i> (34-37)	PPAR α
<i>IKKs</i> (35, 38)	PPAR δ
iNOS (34, 39)	<i>PPARγ</i>
<i>IFNγ</i> (34, 40, 41)	SREBPs (14)
<i>IL-1b</i> (42-44)	<i>aP2</i>
<i>IL-2</i> (37, 41, 45-47)	<i>ACO</i>
<i>IL-6</i> (42, 48-52)	<i>CPT1</i> (73)
<i>IL-8</i> (42, 53, 54)	<i>UCP1</i> (74)
<i>IL-12</i> (40, 55, 56)	<i>UCP2</i> (75)
<i>E-selectin</i> (52, 57-59)	Leptin
<i>VCAM1</i> (52, 58, 60)	PDK4
<i>ICAM1</i> (52, 58, 60)	GLUT4 (73)
<i>MCPI</i> (61-63)	Caveolin-1 (76, 77)
<i>CRP</i> (52, 59)	Caveolin-2 (77)
<i>vWF</i> (59, 64, 65)	CD36 (73)
<i>MMP9</i> (66-68)	SCD1 (73)
<i>TNF-α</i> (35, 41-43, 60)	<i>ABCA1</i>
<i>COX2</i> (69-72)	LpL
	LXR α
	apoE

FIGURE 2. Genes influenced by n–3 fatty acids and by glitazones. Key representative genes critical for inflammation and lipid metabolism are listed. In general, proinflammatory genes (left column) are suppressed by n–3 fatty acids, whereas genes critical for lipid peroxidation, energy utilization, and lipid homeostasis are increased by n–3 fatty acids (right column). Note that many genes indicated by italic letters are activated or suppressed by n–3 fatty acids and glitazones in the same direction. This figure is based on data in references 31–75. COX2, cyclooxygenase 2; CRP, C-reactive protein; GLUT4, glucose transporter 4; ICAM, intercellular adhesion molecule; IL, interleukin; MMP9, matrix metalloproteinase 9; PDK4, pyruvate dehydrogenase kinase 4; UCP, uncoupling protein; VCAM, vascular cell adhesion molecule; vWF, von Willebrand factor. Other abbreviations are as defined in the legend to Figure 1.

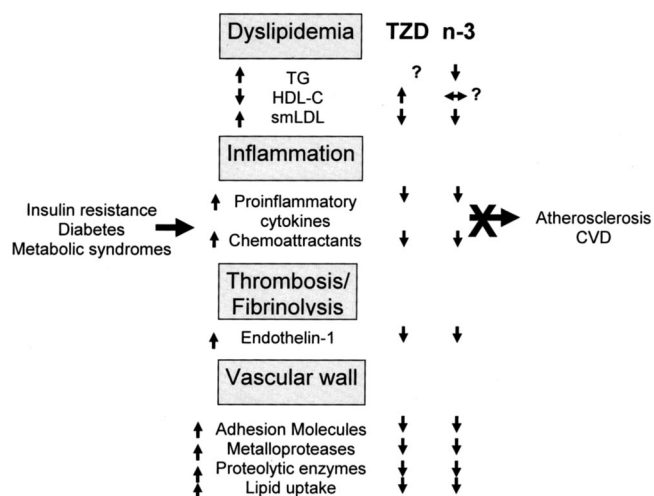


FIGURE 3. Schematic comparison of the effects of thiazolidinediones (TZD, or glitazones) and n-3 fatty acids on different factors contributing to atherosclerosis and cardiovascular disease. Adapted from reference 76. CVD, cardiovascular disease; TG, triacylglycerol.

effects as glitazones, a member of the thiazolidinedione group of drugs, on a large number of genes. The glitazones are potent PPAR γ agonists similar to n-3 fatty acids and have been used or suggested for treatment of a variety of lipid-related disorders. As exemplified in **Figure 2**, n-3 fatty acids are similar to the glitazones in suppressing the expression of a large number of inflammatory proteins and in enhancing the expression and activity of a large number of genes, transcription factors, and proteins that would help to ameliorate adverse pathways related to obesity, the metabolic syndrome, and type 2 diabetes. As a specific disease example, the effects of n-3 fatty acids and thiazolidinedione on several pathways important to atherosclerosis and cardiovascular disease are compared in **Figure 3**. n-3 Fatty acids have been shown to reduce CVD risks (3) and may be especially effective in the setting of other metabolic disorders (77). Although some n-3 fatty acid effects are attributed to reducing plasma triacylglycerol concentrations, the effects are mediated at multiple cellular and genetic levels where n-3 fatty acids diminish key contributors to cardiovascular disease risk. These hypotheses are in agreement with the studies by Li et al (78), which showed that activation of PPAR γ by glitazones can indeed reduce atherosclerosis in LDL-receptor-null mice.

CONCLUSION

In summary, the biologically favorable effects of EPA and DHA are likely mediated through effects on several distinct pathways within cells, tissues, and organs. These include direct interactions in changing the composition of cell membranes and membrane function, activating or suppressing signaling molecules, interacting directly with DNA as well as with proteins that affect the processing of transcription factors, and affecting enzyme activities and vesicular endoplasmic reticulum-Golgi trafficking. It was also recently suggested that because of their high content of double bonds, rather than being prooxidant, EPA and DHA may actually act as scavengers of reactive oxygen species. Indeed, lipid peroxidation appears to be a key mechanism for inducing apoptosis for certain tumor cells (31), whereas similar mechanisms of scavenging reactive oxygen species protect

against apoptosis and neurodegeneration in healthy nerve cells (3). Some of the effects of EPA and DHA may not be directly related to the fatty acid molecule itself but rather to their metabolites, such as eicosanoids. Although it appears that EPA and DHA show similar bioactivity in many instances, frequent differences are described in potency between these n-3 fatty acids in different cells and tissues. Mechanisms underlying the specific responses by cells to EPA and DHA need to be better understood so that we can anticipate better selectivity of DHA compared with EPA, not only in modulating key biological pathways and gene expression, but also in preventing and treating specific diseases.

RJD drafted the original manuscript with substantial input from TSW and TS on the sections related to SREBPs and PPARs, respectively. TS researched the literature to design Figures 1, 2, and 3. All authors contributed to the final report. None of the authors had any conflicts of interest.

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