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Fatty Acid Modulation of Atherosclerosis by Peroxisome Proliferator-Activated Receptors

– Review –

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Abstract

While atherosclerosis is a major killer, there is now concern that mortality from the disease will increase due to the rising incidence of type II diabetes. Because diet can potentially influence both diseases, it is important to elucidate the role of diet in the progression of atherosclerosis. In addition, the mechanisms involved in dietary-related alterations of the disease need to be defined to guide public health recommendations to reduce atherosclerosis incidence and limiting unwanted side effects. Since diet is thought to play a role in atherosclerosis even without added complications due to type II diabetes, reducing the incidence of that metabolic disease will not be enough. While evidence is increasing that high intake of carbohydrate can lead to type II diabetes and atherosclerosis, the preponderance of existing evidence indicates that intake of specific fats as a major dietary causal factor. It has recently been hypothesized that a dietary fat link to atherosclerosis may depend partly on the activity of a transcriptional regulator, the peroxisome proliferator activated receptors (PPAR). Thusfar, PPAR α, β/δ and γ, have been shown to play a major role in metabolism, inflammation, and cancer. Furthermore, PPAR may regulate specific processes associated with atherosclerosis such as triglyceride and low density lipoprotein (LDL) metabolism; the reverse cholesterol transport pathway; lipid accumulation within plaques; the local inflammatory response and plaque stability. Synthetic ligands for PPAR have been developed; however, natural ligands include specific fatty acids and their metabolites. Though the role of PPAR in atherosclerosis has been reported with respect to synthetic ligands, additional studies need to be done with established and possible natural ligands. In this review, we will focus on the relation of dietary fat to PPAR alteration of atherosclerosis.

Key words: dietary fatty acids, endothelial cells, PPAR, cardiovascular disease, transcription factors

INTRODUCTION

The atherosclerotic process

Atherosclerosis is initiated by injury to the vascular endothelium followed by focal intimal thickening due to accumulation of lipids, monocytes, platelets and smooth muscle cells which leads to lesion formation. A schematic representation of some of the steps involved in atheromatous plaque formation is shown in Fig. 1. Short-lived injuries are typically repaired, resulting in restoration of endothelial function, and lesion regression. However, repeated or chronic injuries develop into atheromatous plaques, which can favor thrombosis, yield emboli, or cause ischemic injuries, aneurysms or rupture at the site of affected vasculature. In response to injury and inflammation, vascular endothelial cells become activated and express adhesion molecules. One adhesion molecule, E-selectin interacts with carbohydrate ligands on leukocytes, and is associated with acute inflammation, attracting neutrophils (1). Another adhesion molecule, vascular cell adhesion molecule-1 (VCAM-1), is an immunoglobin protein that interacts with integrin receptors on monocytes (2). VCAM-1 plays a role in the development of atherosclerotic lesions by promoting the adherence of monocytes to endothelial cells. In addition, a gradient of chemotactic factors, including monocyte chemotactic protein (MCP)-1, cause adherent monocytes to enter the intima, where they will differentiate to macrophages, accumulate lipid, produce toxic oxygen species, and further differentiate to foam cells, a characteristic component of atherosclerotic lesions. In this review, we will focus on the initial inflammatory stages of atherosclerosis as we hypothesize that specific dietary fats present repeated chronic injury to the vascular wall. However, some specific fatty acids may protect against the repeated chronic injury.

Dietary fatty acids and cardiovascular disease

While the development of atherosclerosis has a strong genetic component, several epigenetic factors such as smoking and diet have been shown to play a role. Those factors can work together, with the genetic component increasing

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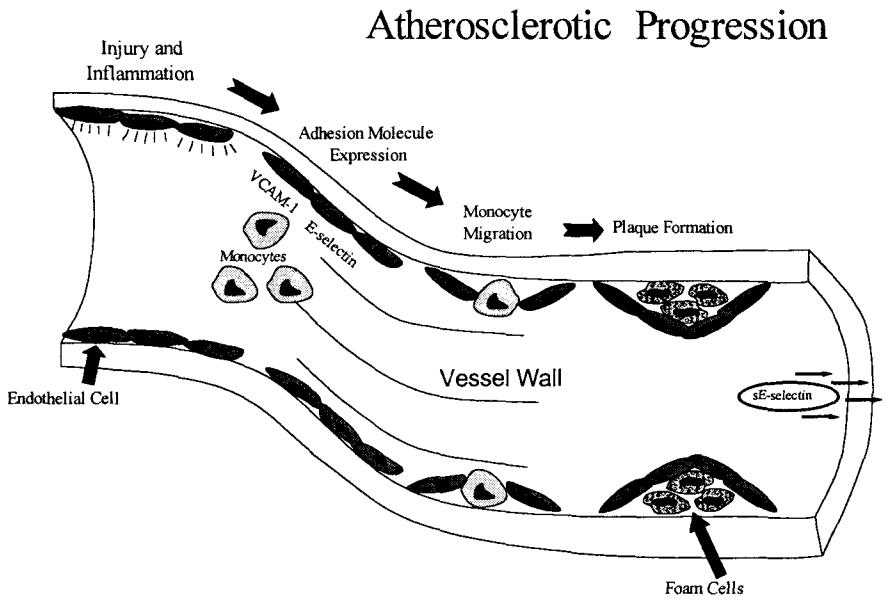

Fig. 1. Schematic representation of progression toward atherosclerotic plaque formation. Chronic injury and the resultant inflammation can start a cascade of events that lead to atherosclerosis.

or decreasing the susceptibility to epigenetic modulation. Specifically, dietary polyunsaturated fatty acids (PUFA) are known to have various antiatherogenic effects, including the lowering of plasma LDL, the increasing of plasma HDL, and the modified production by blood and vascular cells of mediators that affect platelet function and inflammation. Human diets rich in fish oils, largely composed of the n-3 PUFA, eicosapantaenoic acid (EPA) and docosahexaenoic acid (DHA), have repeatedly been associated with a low incidence of cardiovascular disease (3-7). Additionally, animal studies have supported those epidemiological findings; dietary manipulation to increase DHA and EPA intake resulted in a decrease of atherosclerotic disease symptoms (reviewed in (8)). The mechanism (s) of the antiatherogenic effects of those fatty acids has not been clarified. Because effects can be seen with relatively low dietary levels of the fish oil fatty acids, the mechanism probably does not simply involve displacement of dietary proatherogenic with n-3 fatty acids. Both DHA and EPA have been shown to alter serum lipid profiles *in vivo*, with increased consumption resulting in decreased serum triglycerides and very low-density lipoprotein (reviewed in 9, 10). These fatty acids along with other PUFA including conjugated linoleic acid (CLA), have also been shown to have anti-inflammatory effects on endothelial cells *in vitro* (11). The monounsaturated fatty acid, oleic acid (OA), has also received attention because diets high in olive oil have been associated with a lower incidence of cardiovascular disease (reviewed in 12). Oleic acid also appears to exert its positive effects both by regulation of cholesterol levels, and by interfering with the inflammatory response (12).

Peroxisome proliferation-activated receptors

The possible link between dietary fatty acids and alterations in expression of proteins involved in the inflammatory component of atherosclerosis may be a recently discovered family of ligand-activated transcription factors, peroxisome proliferator-activated receptors (PPAR). Ligands for peroxisome proliferators can cause an increase in the number and size of hepatic peroxisomes, a subcellular organelle that performs a diverse array of metabolic functions, including H_2O_2-based respiration, cholesterol metabolism, and β-oxidation of fatty acids. This group of ligands includes hypolipidemic agents, industrial chemicals, herbicides, fatty acids, prostaglandins and leukotrienes. It is currently believed that peroxisome proliferators elicit their effects via activation of the PPAR. They are a family of transcription factors belonging to the nuclear receptor superfamily. Functionally, PPAR are a group of ligand-activated transcription factors, which are involved in gene expression. Target genes for PPAR include lipid metabolizing enzymes, growth regulatory genes, and genes involved in the inflammatory process. For example, PPAR γ, plays a role in regulating proinflammatory genes such as inducible nitric oxide synthetase, tumor necrosis factor-alpha (TNF α), cyclooxygenase-2 (COX-2), interleukin (IL)-1β and IL-6 (13), which may be involved in atherosclerosis. A number of fatty acids as well as eicosanoid metabolites can serve as ligands for PPAR. They have been shown to regulate transcription in multiple ways. After ligand activation, PPAR α, PPAR γ, and PPARβ/δ can regulate transcription by dimerizing with the retinoid X receptor and binding to PPAR response elements (PPRE) within the regulatory re-

gions of target genes (14). Alternatively, PPAR can repress gene transcription by interfering with the transcription factors, nuclear factor-κB (NF-κB) or activating protein-1 (15). A schematic diagram of the possible set of events leading to regulation of gene expression by fatty acids through PPAR is shown in Fig. 2.

In this review, we will focus on the role of dietary fatty acids in regulating the inflammatory response known to be involved in the progression of atherosclerosis through PPAR. We hypothesize that specific dietary fatty acids exert an anti-inflammatory, anti-atherogenic effect in endothelial cells by acting as PPAR ligands and modulating gene transcription. We have reviewed the anti-atherogenic effects of PPAR activation in endothelial cells, as well as the evidence that unsaturated fatty acids act as PPAR ligands, and have compared the effects of unsaturated fatty acids on endothelial inflammation processes with those of PPAR activation.

POSSIBLE MECHANISMS FOR PPAR ACTIVATIONAL EFFECTS

PPAR activation requires the binding of a specific ligand to PPAR, forming a complex that heterodimerizes with the retinoid X receptor-alpha. That in turn binds to PPRE, specific DNA sequences in the promoter region of particular genes. The PPRE consists of a direct repeat of the nuclear receptor hexameric recognition sequence separated by one or two nucleotides. PPAR activation can also effect the transcription of inflammatory factors indirectly by way of transrepressive activities. For example, ligand-bound PPAR-α may act to interrupt the signal transduction pathway initiated by an inducer, thereby inhibiting the resultant transcription of the endothelial activation factor without direct interaction with the DNA promoter region. One possible target of such activity is NF-κB, a family of transcription factors known to regulate cytokine expression in response to inflammatory stimulation. In fact, several studies have shown PPAR activators to have an inactivating effect on nuclear receptor NF-κB (16-19). The precise mechanism by which PPAR activators inactivate NF-κB has not yet been determined.

The participation of activated PPAR-α in the regulatory activity of NF-κB on the VCAM-1 promoter region has been examined (18). The use of deletional VCAM-1 promoter constructs provides evidence that PPAR-α activation was effective in reducing TNF-α induced VCAM-1 promoter activity only when the promoter contained its NF-κB response element (18).

EFFECTS OF PPAR ACTIVATION ON ENDOTHELIAL CELLS

Vascular endothelial cells are the first line of defense against the progression of atherosclerosis and thus are an important focus. It is hypothesized that the expression of adhesion molecules could be an early predictor of coronary artery disease. The expression of VCAM-1 and MCP-1 can be regulated by inflammatory mediators. Thus, the development of therapeutic agents focused on the reduction of those chronic inflammatory genes represents an important area of cardiovascular research. Vascular endothelial cells also express PPAR (18,20,21) with activation of both PPAR-α and PPAR-γ leading to an inhibition of processes important for inflammation and thus, atherogenesis (1,18, 22). VCAM-1 is normally increased after endothelial cells

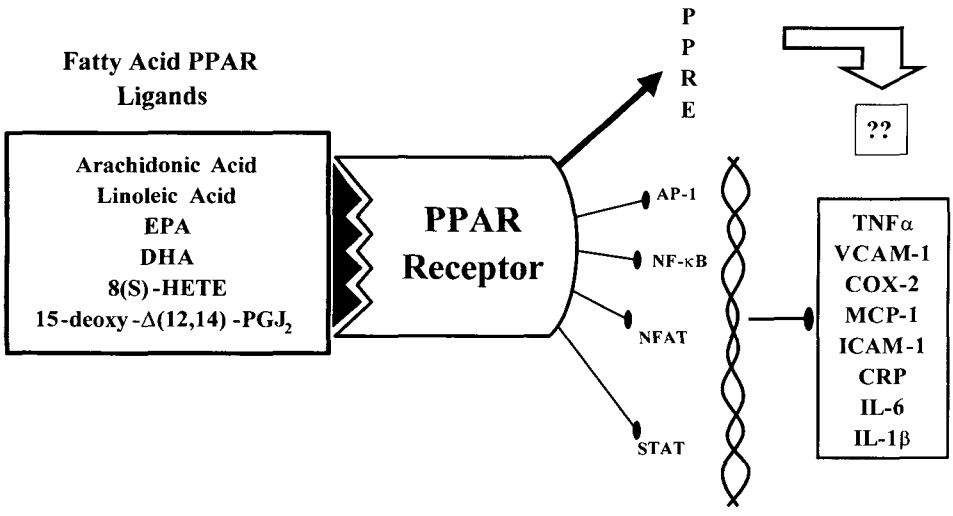

Fig. 2. Schematic diagram of possible fatty acid effects on gene regulation through PPAR. Binding of fatty acid to PPAR may activate the nuclear receptor for binding to PPRE or for trans-repressive activity through AP-1, NF-κB, NFAT, or STAT. The genes listed are candidates for regulation by fatty acids through PPAR. CRP, C-reactive protein.

ε ·e stimulated with proinflammatory agents such as TNF α ε ıd lipopolysaccharide (LPS). Endothelial cells incubated ν ith inflammatory agents have been shown to upregulate ' CAM-1 expression, which is decreased in the presence (f PPAR-α agonists such as fenofibrate and Wy-14643 (1,] 8,22). That effect was not due to an alteration in the ı ıRNA half-life, but the use of VCAM-1 promoter con- s ructs revealed that VCAM-1 promoter activity was de- (reased by the agonists (18). Those studies do not confirm t ıe binding of activated PPAR-α to PPRE, but strongly s ıggests that PPAR-α activation down-regulates the tran- s ɔription of VCAM-1. That effect was not seen in the ab- s ɔnce of inflammatory stimulation (18), indicating that the (ffect may be unique to inflammatory circumstances or in- j ıry, and possibly dependent on a transrepressive effect (f PPAR-α. That is, the agonists may inhibit the inflam- ı ıatory mediator-induced increase in VCAM-1 by inter-] ɔring with the signal transduction pathway initiated by t ıe inflammatory stimulant. Such mechanisms may in- ' olve the inactivation of NF-κB, a transcription factor com- ı ıon to inflammatory responses that regulates cytokine ex-] ression. Indeed, PPAR activators such as Wy-14643, fen- (fibrate, fenofibric acid, and 15-deoxy-$\Delta^{12,14}$-prostaglandin . ɔ, which were shown to impair VCAM-1 expression in] EAhy926 endothelial cells, significantly reduced the ɔ mount of nuclear p65 (23). The NF-κB components, p65 ɔ nd p50, hetero- or homodimerize and form the activated NF-κB.

MCP-1 is a potent chemoattractant for monocytes and] as recently been shown to directly activate smooth muscle (ells to proliferate and produce IL-6 (24). Like VCAM-1, MCP-1 protein can be regulated through PPAR. While sev- (ral PPAR agonists were successful in decreasing the ' ytokine-induced expression of MCP-1 in endothelial cells, he PPAR-δ activator, L-165041 had the greatest potency ɔ reduce MCP-1 secretion in EAhy926 endothelial cells 23). That effect was not limited to cytokine induction as he PPAR-α activators fenofibrate and Wy-14649 almost ompletely abolished the C-reactive protein (CRP)-induced xpression of MCP-1 in cultured human umbilical vein ndothelial cells (25).

E-selectin is another adhesion molecule whose increased xpression can signal inflammation. Recent studies have ıggested that a soluble form of E-selectin (sE-selectin), s elevated in the blood of subjects with risk factors for ıeart disease (26). In studies with vascular endothelial cells *n vitro*, E-selectin expression was not altered when PPAR ıgonists were used during activation with inflammatory ɔtimulants (27). However, *in vivo* levels of sE-selectin and ɔther adhesion molecules were significantly decreased ıfter 1-year treatment with the 3-hydroxy-3-methylglutaryl HMG)-CoA reductase inhibitor, simvastatin (28). Since that drug has recently been shown to induce the expression of PPAR in endothelial cells (29), a role for other PPAR ligands, including fatty acids may be to modulate sE-selectin expression and thus, inflammation.

Endothelial cells also possess the ability to secrete chemokines that can modulate macrophage activity. This paracrine regulation could further alter the inflammatory process in the vessel wall. Moreover, not only macrophage function but also the expression of chemokines may be susceptible to regulation by PPAR activity. Interferon (IFN)-inducible protein of 10 kD (IP-10), monocyte induced by IFN-γ (Mig), and IFN-inducible T cell-α-chemoattractant (I-TAC), all a part of the CXC cytokine group, are known to be expressed by activated endothelial cells (30). Coincubation of endothelial cells with PPAR-γ activators and the inflammatory stimulatant IFN-γ resulted in reduced mRNA and protein expression of IP-10, Mig, and I-TAC, with mRNA half-life unaffected by the treatment (30). Since chemokines are chemotactic proteins that facilitate the translocation of lymphocytes and monocytes into sites of Th1-type inflammation, decreased expression of these proteins would be expected to reduce the accumulation of monocytes into the injured endothelium, thereby attenuating lesion formation. Therefore, under circumstances which cause the activation of PPAR, pro-inflammatory events of the atherosclerotic process can be attenuated and thus an important focus for development of therapy for atherosclerosis.

UNSATURATED FATTY ACIDS CAN ACT AS PPAR ACTIVATORS

The different subtypes of the PPAR superfamily were initially classified based on the ligands that bound them. However, some ligands have recently been shown to bind more than one member of the PPAR superfamily. Lipid-lowering fibric acids and some fatty acids are well-established PPAR-α ligands. Insulin-sensitizing drugs (thiazolidinediones) are selective PPAR-γ ligands, and 15Δ-prostaglandin-J_2 (15Δ-PGJ_2) is generally accepted as its natural ligand. In recent years, several studies have attempted to discover other endogenous ligands for both PPAR-α and PPAR-γ. Unsaturated fatty acids are likely candidates. Although unsaturated fatty acids will bind to all three members of the PPAR family, their affinity for PPAR α is much greater than for β or γ.

PPAR-α

To determine the PPAR-α activating capacity of unsaturated fatty acids, work has focused on receptor-ligand complex formation. Those experiments determined that arachidonic acid (AA), its metabolites, 8(S)-hydroxyeicosatrienoic acid (HETE), leukotriene B_4 (LTB_4), prostaglan-

dins (PG), as well as linoleic acid, conjugated linoleic acid (CLA), linolenic acid, EPA, and DHA were all effective in binding to PPAR-α (31-33). However, relative binding affinities have been tested. One study further tested the activation capacity of the unsaturated fatty acids by determining whether the PPAR-α-fatty acid complex was able to promote PPRE binding at the promoter region. Receptor-ligand complexes that bound to the PPRE sequence include AA, 8(S)-HETE, LTB_4, linoleic acid, linolenic acid, EPA, and DHA (31). Other studies did not examine the physical interactions between PPAR-α and unsaturated fatty acids, but compared the effects of unsaturated fatty acid to those of known PPAR-α activators, and found that some unsaturated fatty acids were effective in mimicking the results of the PPAR-α. These include linoleic acid, CLA, linolenic acid, EPA, DHA and OA (33,34). Converging evidence suggests that unsaturated fatty acids are at least capable of binding to PPAR-α, and may be capable of inducing the effects of PPAR-α activation. The extent to which a PPAR-α ligand induces PPRE binding may or may not predict its activating capacity, depending on the mechanism by which ligand-bound PPAR-α exerts its effect.

PPAR-γ

15Δ-PGJ_2 is synthesized from AA and is an endogenous ligand for PPARγ (17,22,35). Studies aimed at determining the possibility of unsaturated fatty acids acting as PPARγ activators compared the effects of unsaturated fatty acid treatment to those of known PPAR-γ activators. DHA, EPA, oxidized linoleic acid, and its metabolites, 9-HODE and 13-HODE, have been shown to be effective in mimicking the effects of known PPAR-γ activators *in vitro*. The expression of only two genes known to be modulated by PPARγ activation has been studied for the purpose of determining the efficacy of unsaturated fatty acids as PPARγ activators, and direct examination of the receptor-ligand binding capacity of unsaturated fatty acids to PPARγ has not yet been accomplished.

EFFECTS OF UNSATURATED FATTY ACIDS ON ENDOTHELIAL CELLS

Dietary fatty acids may exert an anti-inflammatory and anti-atherogenic effect in endothelial cells by acting as PPAR ligands. Unsaturated fatty acids of particular interest are AA, linoleic acid, linolenic acid, EPA and DHA, as the current literature provides significant evidence of the PPAR activating potential of these molecules in vascular endothelial cells. OA, AA, EPA and DHA, have been tested *in vitro* in experimental conditions similar to those in the PPAR activation studies summarized above. In nearly all of these studies, the consensus was that VCAM-1 expression was lowered by OA (12,19), AA, EPA, and DHA (36). Those conclusions strongly support our hypothesis that the anti-inflammatory effects induced by unsaturated fatty acids may be achieved by PPAR activation. Pretreatment with PPARα and/or PPARγ activators likewise resulted in the inhibition of VCAM-1 expression, and each of the above fatty acids has been indicated as having PPARα activation potential. PPARγ activating potential has also been suggested for AA, EPA and DHA. *In vivo* studies testing the effects of EPA and DHA on the vascular inflammatory response provide evidence of anti-atherogenic effects, including the inhibition of VCAM-1 expression. Patients with coronary heart disease who consumed fish oil supplements as a source of DHA and EPA, had significantly lower levels of soluble VCAM-1, as compared to control coronary heart disease patients who were consuming corn oil (37). Consumption of DHA alone was found to decrease levels of circulating VCAM-1, E-Selectin, IL-6 and IL-8 in response to inflammatory stimulation with IL-1, IL-4, TNFα or LPS (36). EPA alone also resulted in reduction of soluble VCAM-1 and sE-selectin in elderly male subjects (38).

One PPAR-independent mechanism by which EPA and DHA may influence inflammatory processes is by interfering with the lipid metabolizing cyclooxygenase and lipoxygenase pathways. These pathways result in the formation of prostaglandins and prostanoids, respectively, which are both families of pro-inflammatory compounds. EPA and DHA may alter these pathways by displacing the substrate arachidonate in membrane phospholipids at the Sn-2 position. Dietary DHA (36) and EPA (38) can result in increased incorporation into cellular phospholipids at the Sn-2 position, which in turn reduces the availability of AA.

The possibility that DHA and EPA exerts anti-inflammatory effects by PPAR-independent pathways does not discount the possibility that they also decrease inflammation by PPAR activation. Again, the PPAR activating capacity of DHA and EPA has been well established. Both fatty acids have been shown to mimic the effects of PPARα and PPARγ activators, because they can bind to PPARα, induce PPRE binding of the receptor-ligand complex, and inhibit VCAM-1 expression *in vivo* and *in vitro*. However, one problem in interpretation of many studies of PPARγ and inflammation is that the ligands thought to be specific for PPARγ, have regulatory effects on inflammatory parameters that are PPARγ independent.

In several epidemiological studies, OA has been shown to be inversely associated with atherosclerosis. Oleic acid, a monounsaturated fatty acid, may be capable of mimicking the effects of PPARα activators (34), like DHA and EPA. *In vivo* those fatty acids were also found to inhibit inflammation as well as VCAM-1 expression. OA reduced

E-selectin and intercellular adhesion molecule-1 (ICAM-1) expression (19). Other fatty acids, including linoleic and linolenic acid have been noted for their PPAR activation-like effects. Both were also capable of binding to PPAR α (31,32), and inducing receptor-ligand complex binding to PPRE (31). However, a lack of studies examining the effects of those two unsaturated fatty acids on vascular endothelial cells prevents any conclusion comparing their effects to those of PPAR activators in endothelial cells.

CONCLUSION

We have reviewed studies in support of the hypothesis that unsaturated fatty acids can exert an anti-inflammatory and anti-atherogenic effect on vascular endothelial cells by acting as PPAR activators. At this time, that hypothesis can neither be proven or disproven. However, strong evidence indicates that AA, EPA and DHA and some of their metabolites act as PPAR α activators. These dietary unsaturated fatty acids as well as established PPAR activators consistently have the same anti-inflammatory effect on vascular endothelium, notably inhibition of VCAM-1 expression. However, to date, the PPAR α binding activity and VCAM-1 inhibitory effects have not been directly linked. Nevertheless, we conclude that AA, EPA and DHA may inhibit VCAM-1 by a PPAR-dependent mechanism. OA, linoleic acid, CLA and linolenic acid may also act in a similar manner; however, there is presently a lack of evidence to compare their nuclear and physiological activities to those of known PPAR activators. The association of dietary n-3 fatty acids in fish oil, such as DHA and EPA, as well as OA with improved cardiovascular health offers an opportunity for preventing or reducing atherosclerosis. An improved understanding of the mechanisms by which these unsaturated fatty acids exert their effect will be useful in maximizing their antiatherogenic potential. PPAR activation induction of anti-inflammatory effects have been reported by a number of investigators; however, the exact mechanism remains unknown. The effect may be dependent on the presence of both an inflammatory stimulant, as well as NF-κB. It is, therefore, likely that activated PPAR interfere with the pro-inflammatory effects of NF-κB that can result in decreased expression of VCAM-1.

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