ω-3 PUFA as Agents for Prevention and Treatment of Atherosclerosis

Instituto de Agroindustrias, Universidad Tecnológica de la Mixteca, México

Abstract
Atherosclerosis is a progressive disease that involves damage to the endothelium of the arteries, as well as the formation of atheroma plaques. The risk factors for atherosclerosis are unknown. However, high levels of LDL (Low Density Lipoprotein) and low levels of HDL (High Density Lipoprotein) cholesterol in the blood can encourage this disease. It has also been reported that patients with diabetes, hypertension and obesity may be more susceptible to atherosclerosis. The ω-3 polyunsaturated fatty acids (PUFA) are essential to human beings and are taken at concentrations of about 3 g day⁻¹ for atherosclerosis prevention and treatment. Meta-analysis studies have been used to evaluate the effect of PUFA in the prevention and treatment of atherosclerosis and have proven to be an effective treatment of the disease, whereas in the case of prevention the results are inconclusive. This has led to the development of in vivo methods to quantify the effect of PUFA in the treatment and prevention of atherosclerosis. The two most important groups of studies that are addressed in this review article are related to 1) the oxo-derivatives of ω-3 PUFA and specialized pro-resolving mediators, and 2) the balance of ω-3 PUFA versus ω-6 PUFA and saturated fatty acids.

ABBREVIATIONS
LDL: Low-Density Lipoprotein; PDGF: Platelet Aggregation and Release from the Growth Factor; SMC: Smooth Muscle Cell; STAT1: Signal Transducer and Transcription Activator 1; IFN-γ: Interferon Production Regulators; TLR4: Toll Receptors type 4; NF-Kb: Nuclear Factor of Activated B-Cell Light Chains; TF: Tissue Factor; MMPs: Matrix Metalloproteinases; IRAK4: Interleukin 1 is Associated with Receptor Kinase 4; OxlDL: Oxidized Low-Density Lipoprotein; PPAR-Gamma: Peroxisome Proliferator-Activated Receptors; ROS: Radical Oxygen Species; HDL: High Density Lipoprotein; PAH: Polyanromatic Hydrocarbons; Ω-3 PUFA: Omega-3 Polyunsaturated Fatty Acids; EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid; DPA: Docosapentaenoic Acid; ALA: Α-Linolenic Acid; Π-3 PUFA: Polynsaturated Fatty Acids; TAG: Triacylglycerides; LA: Linoleic Acid; COX-2: Cyclooxygenase-2; Nrf2: Erythroid-Derived 2-Like 2; LOX: Lipoxigenase; Rve: E-Series Resolvins; Rvd: D-Series Resolvins; Mar: Maresins; GPCR: G Protein-Coupled Receptors; GPR: G Protein Receptors; LTBH4: B4 Leukotrienes; FFA: Free Fatty Acids; LCFA: Long Chain Free Fatty Acid; SPA: Saturated Fatty Acid; WT: Wild-Type; KO: Knockout; FFA: Receptor-Free Fatty Acid.

Introduction
Pathogenesis of atherosclerosis
Atherosclerosis is the leading cause of coronary heart disease in people. The origin and evolution of atherosclerosis cannot be reduced to a single physiological process. Previously, two hypotheses dominated regarding the cause of this disease: infiltration of lipids into the subintimal space and endothelial damage. However, according to various studies, several points of interaction between the two hypotheses have been identified. The interactions between lipid infiltration and endothelial damage in the pathogenesis of atherosclerosis was reported by Wisssler and are: 1) hyperlipidemia by oxidized low-density lipoprotein (LDL); 2) platelet aggregation and release, from the growth factor (PDGF) due to high LDL levels; 3) constant damage of endothelium by autoimmune reactions to oxidized LDL; 4) smooth muscle cell (SMC) proliferation due to the presence of LDL; 5) SMC receptors of LDL are potentiated in the presence of growth factors; 6) SMC produces proteoglycans, which bind to LDL in the subintimal space [1]. The subintimal space occurs between atheromatous plaques and middle and adventitial layers of the arterial wall.

Discussion
Atherosclerosis is manifested by the formation of fatty arteries that are defined by the presence of plaques, flat or slightly elevated, that do not narrow the lumen of blood vessels. Subsequently, there is an endothelial accumulation of cells with a very rich lipid cytoplasm, called “foam cells”. Foam cells are an indication of plaque buildup and are associated with an increased
risk of heart attacks and cerebrovascular damage. Foam cells are formed when the body, in its attempt to remove damaged endothelial cells, sends macrophages to a fat deposit in the walls of blood vessels. Macrophages try to eliminate fat-rich endothelial cells by surrounding them. Lipids surrounded by macrophages have a “foamy” appearance, hence the term “foam cells”. Later the formation of atheromatous plaques occurs, which are vascular lesions consisting of foam cells, fibrous connective tissue and T lymphocytes. Atheroma plaques are made up of a fibrous tissue covering or cap representing 80% of the plaque, called the sclerotic, while the remaining 20% is termed the atheromatous nucleus. The latter, when its size increases, causes the atheroma plaque to soften and be susceptible to rupture. The probability of an atherosclerotic plaque rupturing is increased primarily by a rise in lipid accumulation.

The development of atheroma plaque involves multiple extra and intracellular signals, compromising the cells of the immune and vascular systems. Cross-communication between the types of cells that interact with the atheroma results in the activation of the transcription factor called signal transducer and transcription activator 1 (STAT1). It also stimulates an increase in the expression of proinflammatory mediators [2]. Proteins called interferon production regulators (IFNγ) and toll receptors type 4 (TLR4) are key in the development of atheroma plaque. The exchange of signals between IFNγ and TLR4 of vascular and immune cells, mediated by STAT1, interacting with the atheroma result in increased leukocyte recruitment, adhesion, proliferation and migration of smooth muscle cells. This process leads to endothelial dysfunction.

In addition, Monaco et al., found that endothelial cells, expression of adhesion molecules and trans-endothelial migration are regulated by the enhanced nuclear factor of activated B-cell light chains (NF-κB) [3]. The normal NF-κB activation pathway regulates the thrombotic potential of human atherosclerotic plaques, including tissue factor (TF), matrix metalloproteinases (MMPs) and inflammatory cytokines. Activation of NF-κB in endothelial cells also occurs from the altered blood flow and leads to arterial inflammation [4].

Normal blood circulation prevents monocytes from entering the endothelium. This behavior changes when a dysfunctional endothelium is present, favoring the secretion of adhesion factors. Interleukin 1 is a cytokine produced by activated macrophages, which mediates the inflammatory response, and may induce the secretion of an adhesion factor. Kim et al., found in a study of atherosclerotic mice that interleukin 1 is associated with receptor kinase 4 (IRAK4) [5]. Interleukin 1 is also involved in the activation of NF-κB by the presence of oxidized LDL (oxLDL), a chemo-attractant for monocytes, with consequent expression of pro-inflammatory genes [6]. After adhesion, monocytes enter the subintimal space attracted by secreted chemo-attractants from endothelial cells and SMC. Saturated fatty acids can increase this adhesiveness.

LDL does not cause lipid accumulation, however oxLDL has been shown to promote lipid accumulation in the subintimal space, triggering atherogenesis [7]. Atherosclerotic patients have three types of oxLDL in their bloodstream [8,9]. In advanced atherosclerosis, oxLDL causes apoptosis of the surrounding cells, which in turn leads to rupture of the atherosclerotic plaque. In response to this, peroxisome proliferator-activated receptors (PPAR-gamma) in SCM increase the expression of endothelial growth factor to produce new blood vessels. These new blood vessels are susceptible to bleeding. It should be recalled that red blood cells, present in hemorrhages, are rich in hemoglobin, iron, non-esterified cholesterol and glycoprotein A. These constituents are involved in the production of radical oxygen species (ROS) and accumulation of cholesterol, and consequently, attract monocytes [10]. These processes occur repeatedly, inducing a thinning of atherosclerotic plaque to produce an acute coronary syndrome [11].

Risk factors and diet

The risk factors for atherosclerosis are unknown. However, it is speculated that high levels of cholesterol, LDL and low levels of HDL (High Density Lipoprotein) in the blood can foster this disease. It has also been reported that patients with diabetes, hypertension and obesity may be more susceptible to this disease. In addition, it has been documented that mutagenic substances present in the environment can lead to atherosclerosis [12]. Among these substances are polyaromatic hydrocarbons (PAH) resulting from the partial combustion of organic compounds, as well as arsenic in the form of As₂O₃, which is present in air, tobacco, meat, soil, water and food, and lastly, cadmium in cigar smoke [12].

On the other hand, there are foods that contain nutrients which can help prevent and treat atherosclerosis. Torres et al., performed a number of nutrients in foods that have attracted attention because they help prevent or delay the effects of atherosclerosis [13]. Among these nutrients are resveratrol, taurine, lycopene, B-complex vitamins, vitamin C, zinc, potassium, magnesium and omega-3 polyunsaturated fatty acids (ω-3 PUFA).

This review article describes and analyzes the beneficial effects of ω-3 PUFA in the prevention and treatment of atherosclerosis.

Sources of ω-3 PUFA

Fish is the main source of ω-3 PUFA, providing eicosapentaenoic acid (EPA, C₂₀:₅n-₃, 20:5Δ11,14,17) and docosahexaenoic acid (DHA, C₂₂:₆n-₃, 22:6Δ4,7,10,13,16,19), while docosapentaenoic acid (DPA, C₂₂:₅n-₃, 22:5Δ7,10,13,16,19) occurs in meat and is also a product of EPA enzymatic elongation (Figure 1). Concentrations of EPA and DHA depend on fish species. To cite a few examples, it has been found that salmon and tuna have concentrations up to 2150 and 1510 mg • 100 g⁻¹, respectively; while cod has concentrations of 280 mg • 100 g⁻¹ [14]. These amounts represent the sum of EPA + DHA. In addition, α-linolenic acid (ALA, C₁₈:₃n-3, 18:3Δ9,12,15) is an ω-3 PUFA found mainly in seed oils from plants.

General characteristics of ω-3 PUFAs and their importance in biochemical processes related to atherosclerosis

Polysaturated fatty acids (PUFA) consist of C₁₈, C₂₀ and C₂₂ hydrocarbon chains and are essential in the human diet. These compounds are the main constituents of the cell membrane phospholipids, and are also part of the triacylglycerides (TAG) [15]. The latter compounds are the energy reserve [16]. PUFAs in humans are biosynthesized from linoleic acid (LA, C₁₈:₂n-6, 18:2Δ9,12).
18:2\(\Delta^{6,12}\)), and \(\alpha\)-linolenic acid (ALA, \(C_{18}H_{30}O_2\), 18:3\(\Delta^{9,12,15}\)), by enzymatic processes of elongation and desaturation of hydrocarbon chains [17]. The most representative \(\omega-3\) PUFAs are ALA, EPA, and DHA (Figure 2).

In humans, ALA conversions to EPA and, EPA to DHA are limited. Only between 0.2 and 8% of ALA is converted to EPA and between 0 and 4% of the initial ALA is converted to DHA [18]. Goyens et al., suggest that the conversion of ALA to EPA functions as a temporary substitute for external sources of EPA and DHA [19]. The tissues and circulating levels of EPA and DHA are mainly related to dietary intake. Harper et al., suggest that LA possibly induces a decrease in the conversion of ALA to EPA and DHA because it competes for the enzyme desaturase \(\omega-6\) [20]. Truong et al., found that genetic variations of the \(\omega-6\) desaturase enzyme in humans could be related to cardiovascular diseases [21]. Therefore, it is important to have a balanced diet of ALA and LA, or consume EPA and DHA [22].

It is important to note that \(\omega-3\) and \(\omega-6\) PUFAs compete for the same enzymes to carry out metabolic processes such as the production of eicosanoids [23]. For this reason, it is important to maintain a balance of these groups of compounds. An imbalance between \(\omega-3\) and \(\omega-6\) PUFAs, where \(\omega-6\) PUFA predominates, may lead to pro-thrombotic and pro-aggregatory physiological states [24].

PUFAs are indispensable for the biosynthesis of eicosanoids and hormone-like signaling molecules, including thromboxanes, prostaglandins and leukotrienes (Figure 1) [25]. Cells in the presence of significant concentrations of \(\omega-3\) PUFA lead to the formation of corresponding eicosanoids, which are less inflammatory and promotes lower growth than eicosanoids derived from \(\omega-6\) PUFA [26]. The \(\omega-3\) acids that are precursors of eicosanoids and hormone-like derivatives inhibit the formation of new blood vessels (angiogenesis), which is an important stage in the expression of atherosclerosis. The decrease in the angiogenic activity of \(\omega-3\) PUFA-derived eicosanoids is consistent with the presence of prostaglandin \(\text{PGE}_3\). \(\omega-6\) PUFA-derived eicosanoid, whereas \(\text{PGE}_3\), derived from \(\omega-6\) PUFA, is considered a promoter of angiogenesis (Figure 3) [27]. These same authors suggest that PUFAs undergo a biotransformation, induced by COX-2 (cyclooxygenase-2, a 72kDa protein) to lipids that modulate angiogenesis. COX-2 is a mediating enzyme in inflammation processes and in prostanoid signaling. Expression of this enzyme occurs in monocytes, macrophages, and endothelial cells, among others.

In addition to prostaglandins and leukotrienes which are classical mediators derived from PUFA, two new classes of structurally distinct mediators have also been identified, which are called oxo-derivatives of PUFA [28] and "specialized pro-resolving mediators" (SPM) [29].
The oxo-derivatives of ω-3 PUFAs are potent mediators of pleiotropic anti-inflammatory signaling and act through the activation of gene expression dependent on nuclear factor (erythroid-derived 2)-like 2 Nrf2 and the suppression of gene expression promoted by NF-kB. Nrf2 is a transcription factor that regulates the gene expression of detoxifying and antioxidants enzymes. The production of 7-oxo-DPA and 7-oxo-DHA compounds (Figure 4), is catalyzed by COX-2 in human macrophages [28].

SPM are a class of self-resolving molecules with anti-inflammatory properties and are produced through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. EPA produces E-series resolvins (RvE), while DHA produces protectins, D-series resolvins (RvD) and maresins (MaR), (Figure 5) [30]. Protectins, resolvins, and maresins for DPA have also been reported, analogous to DHA derivatives [31].

Fredman et al., demonstrated that SPM levels promoted by the 5-LOX enzyme, especially RvD1, and the RvD1 ratio versus pro-inflammatory B4 leukotrienes (LTB4) decreased in vulnerable regions of advanced atherosclerotic plaques in humans and mice [32]. This study implied a supply of RvD1 to knockout mice Ldr−/−. The Ldr−/− mice are genetically modified, such that the gene expressing the LDL receptor has been deleted. The results of this study showed a progressive restoration of the RvD1/LTB4 ratio,
reducing injuries and stabilizing atherosclerotic plaques. These results are likely to be the starting point for the development of new therapies to promote the stability of SPM-based plaques such as RvD1.

**Protective effects of PUFA**

Deficiency of ω-3 PUFA in human beings can lead to changes in the physicochemical properties of cell membranes, activation of eicosanoids syntheses, which are proinflammatory and vasoconstricting, as well as the induction of a systemic inflammatory syndrome [33]. These conditions promote the onset of atherosclerosis and its progression [33]. Hence, the intake of ω-3 PUFA may reduce the risk of this condition.

Meta-analysis is a set of statistical methods focused on locating, selecting, evaluating and combining relevant results from different studies using statistical methods like exact tendency, non-normal mixtures and multiple endpoints [34]. Meta-analysis has been used to evaluate the effect of PUFA on different studies in humans, for example in the prevention of allergies [35], cardiovascular diseases, breast and colorectal cancer, and type 2 diabetes [36-38]. Also, meta-analysis has been used to evaluate PUFA influence on platelet aggregation [39]. This last meta-analysis, conducted from studies available in the PubMed, Embase and Cochrane libraries, on humans of both sexes, aged 25 to 77 years, demonstrates that ω-3 PUFA supplementation is associated with a significant reduction in platelet aggregation in patients with cardiovascular diseases, thus avoiding atherothrombotic events. In contrast, healthy people do not experience a significant reduction in platelets aggregation. From these results, Gao et al., reported that ω-3 PUFAs are not effective in the primary prevention of platelet aggregation [39]. This result is concurrent with a report by Rizos et al. [40]. They conducted a meta-analysis to determine the effect of ω-3 PUFA on cardiovascular disease prevention from available studies in Medline, Embase and Cochrane libraries, and concluded that ω-3 PUFA supplementation is not associated with a decreased risk of sudden death, cardiac death, myocardial infarction or fulminant attack. These results generated controversies among the scientific community, to the extent that Sethi et al. [41], sent a letter to the JAMA journal editor, remarking that Rizos et al. [40], considered two factors inadequately. The first factor was an ingestion of ω-3 PUFA lower than that recommended by the FDA (3 g•day⁻¹ of EPA + DHA) in most of the groups studied. The second factor was the ω-3 PUFA source. Sethi et al. [41], claimed that fish oil contains triacylglycerides (TAG) with ω-3 PUFA at the sn-2 position, whereas marine mammalian oil such as the Greenland seal has TAG with ω-3 PUFA at sn-1,3 positions, which can make them more bioavailable. These structural variations could eventually alter the metabolism of ω-3 PUFA in humans, and thus produce different results. Undoubtedly, it is important to undertake new experimental studies to determine the effect of dosages and the chemical structure of the TAG on the protection of healthy people against cardiovascular diseases. TAG are tri-substituted glycerol esters derivatives of fatty acid including PUFA.

**Effects of ω-3 PUFA supplementation combined with SFA and PUFA ω-6 on human health**

Dias et al., carried out a clinical study with healthy subjects to evaluate the effect of saturated fatty acid (SFA) or ω-6 PUFA on plasma lipid levels and lipoprotein profiles in subjects with a suitable ω-3 PUFA index [42]. The test subjects were initially supplemented during four weeks with 4 x 1 g of oil capsules, where each capsule contained 100 mg of EPA and 500 mg of DHA. This period was called pre-supplementation. Subsequently, subjects were given, in addition to ω-3 PUFAs, either SFA or ω-6 PUFA for ten days. The amount of SFA was 20.9 g and was supplied daily to subjects from 24 g butter and 40 g white chocolate. At the same time, subjects supplemented with ω-6 PUFA consumed 20 g of...
these fats daily from 20 g margarine and 42 g of sunflower seeds. The results showed that ω-3 PUFA in the pre-supplementation period modulated the effect of SFA and ω-6 PUFA on most lipoprotein profiles. However, a diet enriched with ω-6 PUFA caused a decrease in LDL concentration. This led to an increase in ω-3 PUFA in blood plasma and lipid tissue, leading to a potential reduction of inflammation and clot formation. These results appear confusing, however it is clear that there are synergistic effects between ω-3 PUFAs and ω-6 PUFAs that induce beneficial effects in healthy humans.

**Synergistic effect between DHA and EPA**

Takashima et al. evaluated the effect of DHA and DHA/EPA combination on apoE knockout mice (Apoe−/−) [43]. The Apoe−/− mice are genetically modified, so that the gene coding for apolipoprotein E, a constituent of different types of lipoproteins, has been deleted. Apoe−/− mice develop hyperlipidemia and atherosclerosis under any type of diet. The results obtained by these authors show that the combination of EPA and DHA, at concentrations of 3.7 and 3.0 g• (kg body weight day)−1, respectively, causes a greater inactivation of macrophages, by reducing expression of the toll-like receptor 4 (TLR4) in lipid rafts, than mice which were only supplemented with DHA at concentrations of 3.0 g• (kg body weight day)−1. TLR4 is a type 1 transmembrane glycoprotein that activates the expression of immuno reaction genes and is part of innate immunity in humans and mice. This study demonstrated that lipid rafts in macrophages could also serve as a potential therapeutic target for atherosclerosis study.

**EPA/DHA versus LA/ALA**

Liu et al. evaluated the effect of various DHA/EPA ratios (2:1, 1:1 and 1:2) and/or LA and ALA enriched oils on atherosclerosis in Apoe−/− mice studies with a ω-6/ω-3 ratio of 4:1 [44]. The results indicate that supplementation with PUFA significantly reduces atherosclerotic plaque, lipid profile in blood serum, inflammatory response, production of ROS in the aorta, and HDL levels, among other parameters. The results showed a greater effect in the groups of mice supplemented with EPA- and DHA-rich ω-3 PUFA than in those supplemented with a high content of LA and ALA.

**New methods to evaluate the effects of PUFA on atherosclerosis**

A few years ago, a molecular mechanism with the potential to effectively measure the impact of ω-3 PUFA on the prevention and treatment of atherosclerosis was identified. This molecular mechanism is related to orphan G protein-coupled receptors (GPCR). G protein receptors (GPR) show a structure with seven trans-membrane domains, with the amino group of the terminal peptide on the outside of the cell, and the carboxyl group inside. G protein receptors can form complexes with free fatty acids (FFA) [45,46]. Specifically, FFA1 (formerly called GPR40) and FFA4 (formerly GPR120) are identified as long chain free fatty acid (LCFA) receptors in G proteins [47,48]. FFA1, in the presence of saturated and unsaturated medium and long chain fatty acids, is involved in the stimulation of acute insulin secretion with a corresponding lipo-toxicity and a decrease in β-cell functions and resistance to peripheral insulin [49]. FFA4 is activated by saturated fatty acid chains (C14 to C18) and unsaturated fatty acids (C16 to C22). FFA4 is expressed in macrophages [50].

One of the more recent studies related to GPCR was reported by Shewale et al., and evaluated whether the in vivo activation of FFA4 leukocytes by ω-3 PUFA versus ω-6 PUFA is athero-protective [51]. The study was performed in wild-type (WT) and knockout (KO) mice. The results indicate that there is no distinction between ω-3 PUFA versus ω-6 in the activation of FFA4 leukocyte anti-inflammatory effects. A supplementation study of ω-3 PUFA in WT and KO mice, deficient in the FFA4 expressing gene, revealed that WT mice showed an inhibition of inflammation and increased sensitivity to insulin, while KO mice did not show these behaviors [52].

Vangaveti et al., evaluated the effect of oxidation products of linoleic acid, the hydroxydecalenoic 9-HODE and 13-HODE [53]. These acids are produced non-enzymatically in macrophages by 15-lipoxygenase-1 and its receptor, GPR132, which can be considered a sensor of lipid overload and oxidative stress. These processes are involved in atherosclerosis [53].

The previous studies have motivated molecular analyses to evaluate the interactions involved in the receptor-free fatty acid (FFA) complex. An example of this is the work done by Tikhonova and Poerio who carried out a study that identified an interaction site of the receptor FFA1 with linoleic acid. This acid is considered a potent FFA1 receptor agonist [54].

To obtain more conclusive results, molecular studies related to interaction of FFA1 and FFA4 receptors with PUFA need to be carried out, as well as studies that may associate the GPR132 and GPR30 receptors with partially oxidized derivatives of ω-3 PUFA, like oxo-PUFA and PMS.

**Clinical implications and upcoming researches**

Atherosclerosis is a multifactorial disease that is progressive and can lead to very severe affections to patient. However, studies have confirmed that balanced supplementation of ω-3 PUFA (ALA, EPA and DHA) relative to ω-6 PUFA in patients may help to slow the rate of atheroma growth. Additionally, the use of resolwins derived from ω-3 PUFA, such as RvD1, may help reverse atheroma growth. These results suggest that it is possible to find new treatments based on use of self-resolving molecules derived of ω-3 PUFA, such as protectins, resolwins and maresins; as well as the use of oxo-derivatives from ω-3 PUFA. These compounds are product of ω-3 PUFA enzymatic transformations through corresponding COX and LOX pathways, but could be also supplied to patients with atherosclerosis. This may give rise to new studies with atherosclerosis diseases patients.

**CONCLUSIONS**

The effect of ω-3 PUFA on the prevention and treatment of atherosclerosis remains open to new theoretical and experimental studies. Meta-analysis studies have questioned the effect of PUFA in preventing atherosclerosis in healthy people. Thus, new studies have focused on identifying possible molecular mechanisms associated with biological activities attributed to PUFA. These studies have led to the discovery of two new classes of structurally distinct mediators, which are called...
PUFA oxo-derivatives and SPM, and are responsible for the anti-inflammatory effects of ω-3 PUFA. Also, FFAR1 and FFAR4 receptors coupled to G protein have been identified. At present, studies are aimed at evaluating the ω-3 PUFA anti-atherosclerotic effect in vivo with KO mice where genes expressing specific proteins such as apolipoprotein E and G protein receptors have been deleted. Finally, some human studies have shown that consuming EPA and DHA may help reduce inflammation and blood clot formation in patients with cardiovascular disease.

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REFERENCES

Salas-Coronado et al. (2017)


49. Ichimura A, Hirasawa A, Hara T, Tsujimoto G. Free fatty acid receptors act as nutrient sensors to regulate energy homeostasis. Prostaglandins Other Lipid Mediat. 2009; 89: 82-88.


