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Department of Molecular Sciences

# The role of omega-3 polyunsaturated fatty acids in improving the immune system – with a focus on inflammation and Multiple sclerosis

*Rollen av fleromättade omega-3 fettsyror vid förbättring av immunsystemet – med fokus på inflammation och multipel skleros*

Salam Kamalmaz

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## Abstract

Inflammation is part of the body's responses to infections and injuries, but an improper inflammatory response together with the production of proinflammatory molecules can cause serious diseases. Multiple sclerosis (MS) is an autoimmune inflammatory disease that affects the central nervous system. The disease is the primary cause of disability among young adults aged between 20-40 years. The reason of MS is unknown and no treatment can fully cure the outcomes of it. There are disease-modifying drugs used to slow down the progression of MS. Such drugs are not always effective, are of high cost and have side effects. Therefore, it can be discussed whether a complementary alternative in the form of a natural source may be more efficient in treating MS than the used drugs. An example of one such natural source is fish due to the anti-inflammatory properties of omega-3 polyunsaturated fatty acids that exist in fish and fish oil. The aim of this thesis was to make a review by extracting information from available literature as well as published scientific articles about the link between the dietary intake of omega-3 polyunsaturated fatty acids (particularly from fish) and an enhanced immune system. Another purpose was to shed light on the role such fatty acids play in decreasing inflammation and lowering the risk of MS and treating the outcomes of it.

There are not so many clinical trials involving the research about if omega-3 fatty acid intake helps to reduce the risk and treat the outcomes of MS. Most of the available studies state that omega-3 fatty acid intake improve the overall quality of life and boost the immune system. Studies have proven that omega-3 fatty acids reduce the expression and production of proinflammatory molecules. Several studies have also shown that the high intake of omega-3 fatty acids help to treat the outcomes of MS such as the physical disability, fatigue, and depression. Further information about the role of nutrigenomics in the immune system functionality is needed in order to know if there is a direct link between MS and the intake of omega-3 fatty acids.

*Keywords: Omega-3 PUFA, autoimmune disease, immune system, inflammation, metabolism and multiple sclerosis.*

## Sammanfattning

En inflammation är en del av det medfödda immunsystemets svar på skador och infektioner. Ett felaktigt inflammatoriskt svar kan dock orsaka en rad olika kroniska sjukdomar.

Multipel skleros (MS) är en autoimmun inflammatorisk kronisk sjukdom i centrala nervsystemet. MS är den främsta orsaken till funktionshinder bland unga vuxna. Orsaken till sjukdomen är okänd, och det finns ingen terapi som fullständigt kan behandla MS. Vissa sjukdomsmodifierande läkemedel används för att förhindra och fördröja sjukdomens progression, men dessa är kostsamma och ger biverkningar. Det kan därför diskuteras om ett komplementärt alternativ från en naturlig källa kan vara effektivare i behandlingen av MS än de använda läkemedlen. Ett exempel på en sån naturlig källa är fisk, vars fleromättade omega-3 fettsyror har antiinflammatoriska egenskaper. Syftet med denna avhandling var att sammanställa information från tillgänglig litteratur samt publicerade vetenskapliga artiklar om sambandet mellan intaget av fleromättade omega-3 fettsyror genom kosten (särskilt från fisk) och ett förstärkt immunförsvar. Syftet var också att granska vilken roll sådana fettsyror har i minskandet av inflammation och förbättrandet i behandlingen av MS.

Det finns endast ett fåtal kliniska prövningar som involverar forskning kring hur omega-3 intaget kan påverka MS. De flesta tillgängliga studier visar emellertid att omega-3 fettsyror förbättrar den totala livskvaliteten och ökar immunförsvarets effektivitet. Studier visar också att omega-3 fettsyror hämmar bildningen av proinflammatoriska molekyler vilket följaktligen minskar inflammationen. Det är också bevisat i vissa studier att ett högt intag av omega-3 fettsyror kan hjälpa vid behandling av symptom på MS såsom fysiska funktionshinder, trötthet och depression. Ytterligare information om nutrigenomikens roll för immunsystemets funktionalitet är nödvändig för att fastställa om det finns en direkt länk mellan intaget av omega-3 och förbättring av livskvaliteten för personer som är sjuka i MS.

*Nyckelord: Omega-3 fettsyror, autoimmuna sjukdomar, immunsystemet, inflammation, metabolism och multipel skleros.*

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## Abbreviations

AA	Arachidonic acid
ALA	$\alpha$ -Linolenic Acid
BBB	Blood-brain barrier
B cells	B lymphocytes
CNS	central nervous system
DHA	Docosahexaenoic acid
EFA	essential fatty acids
EPA	Eicosapentaenoic acid
FA	fatty acids
FCD	first clinical diagnosis
LA	Linoleic acid
LC PUFA	long chain polyunsaturated fatty acid
MS	Multiple sclerosis
MUFA	monounsaturated fatty acid
n-3	omega-3
n-6	omega-6
PUFA	polyunsaturated fatty acid
SFA	saturated fatty acid
T cells	T lymphocytes
TNF- $\alpha$	Tumor necrosis factor Alpha



# 1 Introduction

MS is an autoimmune inflammatory chronic disease that affects the central nervous system by causing serious damage to the myelin sheath, which encloses the nerve cells (Riccio & Rossano, 2015). The disease interferes with the ability of the different parts of the nervous system to communicate in order to function (Stauffer, 2006). It results in neuronal degradation and major life-long damage, both mentally and physically. The cause of the disease is unknown, but several factors that might influence and trigger it are suggested (Altowaijri *et al.*, 2017). Such factors include genetics, environmental factors, vascular disease risk factors, and lifestyle together with dietary habits. MS is one of the primary causes of disability within young-aged people (20-40 years) (da Cunha Matta & Orsini, 2017). Approximately 2.5 million people worldwide suffer from the disease and women are more affected than men with a ratio of 2-3:1. There is no known cure for MS, but some disease-modifying drugs help to reduce the severity of the outcome of certain symptoms (Riccio & Rossano, 2015). Each patient reacts to such drugs differently, depending on every patient's individual case. Therefore, it can be discussed whether a treatment from a natural source can be more efficient in the treatment of MS than the used drugs. One such natural source is fish, due to the anti-inflammatory properties of omega-3 polyunsaturated fatty acids that exist in fish and fish oil. It is proposed that such fatty acids can be efficient to improve or limit the outcomes of MS (Riccio & Rossano, 2015).

The aim of this thesis was, therefore, to make a literature review about the role of dietary intake specifically omega-3 polyunsaturated fatty acid intake originated from fish, in the improvement of the immune system's functionality. Another purpose was to shed light on the effects of omega-3 fatty acid intake in lowering the episode of inflammation, together with a focus on the effects of such fatty acids in lowering the risk of MS and treating the outcomes of it. Examples of this are downregulating the inflammation caused by MS at the molecular level, and slowing down progression rate and physical disability.

## 2 Methods

This thesis was literature-based, and to find suitable literature relevant published scientific papers and articles were searched on Web of Science, Scopus, and PubMed and categorized to review the required information. Examples of the keywords used were: “Omega-3 fatty acids” in a combination with “metabolism”, “autoimmune disease”, “autoimmunity”, “inflammation”, “multiple sclerosis” and “immune system. Further information was obtained from online books available at SLU’s library search device Primo.

## 3 Background

### 3.1 Fatty acids

Fatty acids (FA) are the primary components in lipid classes, they serve as structural components in cells and as energy substrates that store energy in adipose tissues (Tvrzicka *et al.*, 2011). Additionally, they play a role as ligands of nuclear receptors influencing gene expression (Kremmyda *et al.*, 2011).

FA are carboxylic acids (-COOH) that consist of a hydrocarbon chain bound to an alkyl unbranched end methyl tail -CH<sub>3</sub> (Mir *et al.*, 2016). The chain can be of variant lengths, in mammals it usually ranges from 12 to 24 carbon atoms and it can be saturated or unsaturated (Tvrzicka *et al.*, 2011). Saturated fatty acids (SFA) have straight chains without double bonds between the carbon atoms in the hydrocarbon chain (Mir *et al.*, 2016). SFA can be long, medium or short-chained (Tvrzicka *et al.*, 2011). Contrariwise, unsaturated fatty acids have one (monounsaturated fatty acids -MUFA-) or more double bonds (polyunsaturated fatty acids -PUFA-) between the carbon-carbon atoms in the hydrocarbon chain (Mir *et al.*, 2016). The position of the first double bond (from the -CH<sub>3</sub> tail) determines the structure and properties of the PUFA, when the double bond is at the third carbon the PUFA is called omega-3 (n-3 or ω-3), and when the first double bond is at the sixth carbon atom the PUFA is called omega-6 (n-6 or ω-6). The position of the last double bond is of great significance, it determines which biological functions the PUFA will possess. For instance, some PUFA possesses important regulating functions of the nervous system and the brain. The generated lipid mediators from long-chained (LC) PUFA have substantial functions in regulating immunity.

### 3.2 Biosynthesis of long chain polyunsaturated fatty acids

Mammals including humans are unable to produce certain FA, such as n-3 PUFA (Mir *et al.*, 2016) since humans lack the enzymes desaturases that are necessary to replace hydrogen atoms from the hydrocarbon chain of a FA with a double bond. Desaturases establish the carbon-carbon double bond (C = C) and produce Linoleic acid (LA; 18:2n-6) and  $\alpha$ -Linolenic acid (ALA; 18:3n-3). Thus, both LA and ALA are considered essential FA (EFA) and should be supplied from the diet (Tvrzicka *et al.*, 2011). Additional elongation and desaturation processes of LA and ALA are needed to establish LC PUFA. The human body converts LA to  $\gamma$ -linolenic acid (18:3 n-6) and dihomo-  $\gamma$ - linolenic acid (20:3 n-6) for further synthesis of arachidonic acid (AA ; 20:4 n-6) (Schmitz & Ecker, 2008). The latter can be converted to docosapentaenoic acid (22:5 n-6) or eicosanoids. Moreover, ALA is also converted to stearidonic acid (18:4 n-3) and eicosatetraenoic acid (20:4 n-3) to synthesize eicosapentaenoic acid (EPA; 20:5 n-3) which in turn is metabolized to docosahexaenoic acid (DHA; 22:6 n-3). These processes are driven by a series of desaturase- and elongase-enzymes, which are the same for both n-3 and n-6. Due to that, there is a competition between those PUFA in the conversion process. Eicosanoids differ according to the PUFA they are converted from, the mediators derived from EPA and DHA possess anti-inflammatory properties, whereas the ones derived from AA have proinflammatory properties (Schmitz & Ecker, 2008). The aforementioned processes of producing LC PUFA are possible in humans but not always sufficient, therefore such FA are considered as conditionally essential (Tvrzicka *et al.*, 2011). The gene encoding the human desaturase enzymes varies depending on each person's genetics and affects the metabolism of FA and plasma lipid profiles (Kremmyda *et al.*, 2011).

The human body is unable to synthesize ALA, so it needs to be provided from the diet, whereas the body is able to synthesize EPA and DHA from ALA, only not in sufficient amounts (Tvrzicka *et al.*, 2011). ALA is found in flaxseed oil and walnuts. Both EPA and DHA are abundant in the flesh of oily fish such as sardines, trout, salmon, fresh tuna, and herring, and even in non-oily fish but in lower concentrations. The fish are able to synthesize such FA like mammals and humans, but not in sufficient amounts. Instead, the fish acquires most of such FA by marine bacteria that synthesize them (Mir *et al.*, 2016). Such bacterial species are for example *Shewanella spp.* and *Photobacterium* (Wall *et al.*, 2010). The content of n-3 PUFA in fish varies depending on several factors, such as the total fat content of the fish and the geographical location of the waters where the fish exist. Table 1.

Table 1. n-3 content in different fish species, developed by using Table 1 from the review by (Wall *et al.*, 2010).

Fish species	n-3 (EPA&DHA) content per 100 g fish
Atlantic herring	2.01
Atlantic salmon	1.28-2.15
Sardines	1.15-2
Rainbow trout	1.15
Tuna	0.28-1.51
Tuna (canned)	0.31
Cod	0.28
Scallop	0.2

### 3.3 Cellular effects of different fatty acids

n-3 PUFA affect the neuronal cell membrane health since both EPA and DHA are present in the neural membrane of phospholipids and are important for membrane fluidity (Rivera Jr & Watson, 2014). The profile of phospholipids influences the structure of the cell membrane and consequently the activity of the membrane-associated proteins (enzymes, receptors, and transporters) (Kremmyda *et al.*, 2011). Another factor that has an impact on membrane fluidity is the degree of FA unsaturation. Membrane-FA are usually *cis*-PUFA, and their double bonds conduct a folding of  $\sim 60^\circ$  of the hydrocarbon chain. Due to that, *cis*-PUFA chains occupy greater space and increase membrane fluidity. On the contrary, SFAs and *trans*-unsaturated FAs occupy less space, thus decreasing membrane fluidity. Therefore, increasing the number of phospholipids would not increase the membrane fluidity. The increase in the degree of unsaturation of the phospholipids is what has an effect on membrane fluidity (Kremmyda *et al.*, 2011).

PUFA are important in transporting fat-soluble vitamins such as vitamins A, D, E and K. Additionally, n-3 PUFA play an essential role in blood clotting (Wall *et al.*, 2010) and regulation of blood pressure, eicosanoids production, metabolism of minerals and affect the lipoprotein concentrations (Tvrzicka *et al.*, 2011). The latter covers the cell membrane and comprises a number of phospholipids (Kremmyda *et al.*, 2011). DHA is present in high concentrations in the eye retina and nervous tissues serving a fundamental function in the signal transduction between neurons (Tvrzicka *et al.*, 2011). The n-3 PUFA family share common anti-inflammatory properties (Rivera Jr & Watson, 2014), they also serve as anti-inflammatory agents and possess immunomodulatory properties by inhibiting the

T lymphocyte activation (Tvrzicka *et al.*, 2011) and inhibit the expression of pro-inflammatory molecules e.g. eicosanoids derived from AA (Calder, 2006). EPA influences the production of proinflammatory cytokines such as tumor necrosis factor TNF- $\alpha$  causing their production to decrease (Kremmyda *et al.*, 2011). Thus, n-3 PUFA are suggested to be used as a therapeutic alternative or complementary medicine for some diseases (Tvrzicka *et al.*, 2011).

### 3.4 Immunity and autoimmune diseases

The immune system is comprised of the mechanisms that protect the human body from foreign and pathogenic microorganisms such as bacteria, fungi, and virus (Devereux, 2002). It consists of organs, cells, and molecules that are distributed in the whole body (Esser, 2016). The immune system is divided into two systems: the innate immune system (natural/non-specific) and the adaptive immune system (acquired through lifespan/specific). The latter comprises B lymphocytes (B-cells) and T lymphocytes (T-cells) as the main cells in this system (Esser, 2016). Such cells originate in the bone marrow and possess immunoglobulin proteins (antibodies) on their surface (Stauffer, 2006). Those antibodies recognize strange and harmful substances (antigens) and consequently produce a secretion of soluble antibodies that bind and immobilize the antigens and target them for further decay.

An inflammation is part of the innate immune system's responses and is the body's direct reaction to infections caused by harmful pathogens e.g., viruses, bacteria, and injuries e.g. damaged cells (Calder, 2006). The response is characterized by redness and swollenness caused by the increased blood flow and cellular permeability to the infection site in order to repair infected/damaged cells (Stauffer, 2006). Inflammation is initiated when leukocytes are drawn to an infection site due to the enhanced blood supply and vascular permeability. Phagocytes contribute by migrating to the infection site and secrete cytokines, which in turn regulate the leukocyte number and send more of them to the infection location. Cytokines are small proteins that affect the activity of other cells. An example of cytokines is t TNF- $\alpha$ . The inflammation is terminated when the site is eradicated properly and programmed cell death of the immune cells is achieved. However, some of the immune cells remain in a rest condition and monitor for another infection. Nevertheless, an improper inflammatory response together with the production of cytokines and inflammatory molecules such as eicosanoids derived from arachidonic acid can cause serious chronic diseases (Calder, 2006). Accordingly, inflammation is considered to play a role in the pathogenicity and occurrence of certain diseases.

Besides the defense against harmful microorganisms, the immune system has another essential task of immunosuppression (Stauffer, 2006), which retains the balance inside the body and prevents immune cells from attacking and damaging the body's own cells and tissues. The immune system thus possesses a fundamental role in survival by distinguishing between the body's own cells and the foreign cells and substances. A dysfunction or disorder in the immune system can cause autoimmune diseases to arise (Devereux, 2002). In the case of autoimmunity, the immune system senses the body's own cells as foreign and attacks them (Stauffer, 2006). The recognition of the body's own cells is programmed into developing populations of B and T cells, such self-reactive cells are activated in the presence of self-antigen.

Autoimmune diseases can occur depending on several genetic and environmental factors including the exposure to environmental toxins and some dietary components (Ceribelli, Generali & Selmi 2016).

### 3.5 The central nervous system (CNS)

CNS consists of the brain and spinal cord and comprises two types of cells: neurons and glial cells (Stauffer, 2006). Glial cells provide support and are classified into three subtypes of cells: astrocytes, oligodendrocytes, and microglia. A neuron's main function is to transmit electrical and chemical signals to other neurons via synapses to, from and within the CNS. An insulating substance known as *myelin* surrounds neurons. Myelin is produced by oligodendrocytes and comprises lipids and proteins that enable it to attach to the surface of neurons (Stauffer, 2006). There are gaps in the myelin sheath covering an axon, leaving space for protein channels for ion transport in and out the axon. Neurons can be stimulated due to various factors, including temperature or mechanical force (Brodal, 2010). When they are stimulated, an electrical cascade is created, also called an *action potential*. Besides the insulating function, myelin also provides an enhanced speed of the axon potentials.

The CNS is isolated from the rest of the body by a barrier that is composed of endothelial cells surrounding astrocytes. The barrier is called Blood Brain Barrier (BBB) and has a function to protect the CNS from foreign substances. When an inflammation manifests within the CNS, astrocytes are capable of producing the cytokine TNF- $\alpha$ . Which in turn regulates the migration of leukocytes into the BBB (Stauffer, 2006). In addition to that, astrocytes are able to produce other cytokines that induce and stimulate the migration of T cells to the location of the inflammation.

### 3.6 Multiple sclerosis (MS)

MS is an autoimmune inflammatory chronic disease that affects the CNS by destroying the myelin sheath (demyelination) (Stauffer, 2006). This leads to the formation of plaques in the myelin-missing areas. An activated T-cell migrates across BBB due to disruptions in the barrier and recognizes the myelin sheath as a foreign substance. It is not completely clear why this happens, perhaps the T-cell recognizes the component proteins of myelin as an analogous antigen and thus attacks it. T cells promote the production of anti-myelin antibodies by B cells. This, in turn, promotes an inflammatory response, which in turn is responsible for the migration of phagocytes to the site of inflammation and degradation of oligodendrocytes. The sites of inflammation are visible as lesions, causing a reduced or inhibited distribution of the electrical impulses and axon potentials along the nerves and inhibiting the signal from transportation between neurons. The dysfunction in transmitting the signals affects the body's different affecting organs, as they do not receive a signal telling them what to do, leading to abnormal functions. Furthermore, and as the disease progresses, different parts of the body lose their normal functionality, in addition to perivascular inflammation and BBB breakdown (Rivera Jr & Watson, 2014).

The word Sclerosis means scars, and multiple is attributed to many scars formed since the inflammation results in a damage of the myelin sheath in several sites (Stauffer, 2006). There are two forms of the disease, the relapsing-remitting (RRMS) which comprises relapse and remission episodes and is the dominant form in most MS cases (Riccio & Rossano, 2015). The other form is the primary progressive (PPMS) which features progressive neurological damages and a decay upon the beginning of the disease.

Approximately 2.5 million people worldwide suffer from the disease with a ratio of 2-3:1 of women being more affected than men (da Cunha Matta & Orsini, 2017).

## 4 Linking disease with lifestyle and dietary habits

### 4.1 The diet as a complementary medicine

Diet and lifestyle play a fundamental role in the human health and have a substantial impact on the metabolism of human cells and the intestinal microbiome (Riccio & Rossano, 2015). The diet participates in the regulation of proinflammatory molecules and keeps the intestinal microbiota at balance (da Cunha Matta & Orsini, 2017). Immunodeficiency and the likelihood of developing an infection are both associated with malnutrition (Calder *et al.*, 2002). The western-style diets with high sugar, salt, red meat, SFA and low fiber intake have been shown to contribute to an increased inflammatory response in humans (da Cunha Matta & Orsini, 2017). Diseases characterized by the inadequate production of proinflammatory eicosanoids derived from AA are shown to have a link with SFA intake (Kremmyda *et al.*, 2011).

The dietary composition is suggested to have an effect on human cells by interacting with enzymes, transcription factors and nuclear receptors (Riccio & Rossano, 2015). This can evoke inflammatory and autoimmune responses by affecting the metabolism of certain amendments of cellular metabolism toward anabolism or catabolism. The microbiota of the gut is influenced by different kinds and amounts of dietary factors, which in turn influences the diversity of the constituents of the microbiota (Calder *et al.*, 2002). Figure 1. Any alterations in the balance of microbiota can result in a change in the mucosal immune system. Because of that, any increase in the number of pathogenic microorganisms in the gut can lead to a weakened immune system, resulting in a higher inflammation, immune-metabolic and degenerative diseases (Riccio & Rossano, 2015). Furthermore, the microbiota has an important functionality in producing neuro-active molecules that can influ-

ence both the CNS and the peripheral nervous system (Altowaijri *et al.*, 2017); hence, a balanced microbiota is very important.

## 4.2 How saturated fatty acids and *trans*-unsaturated fatty acids influence human cell metabolism and modulate inflammation

The high consumption of SFA can be a leading cause of human chronic diseases because SFA alters the balance of the gut microbiota (Ricchio & Rossano, 2015). Which affects the balance between the beneficial and harmful bacteria and results in a dysfunction in the intestinal immunity and a low-grade systemic inflammation. The consumption of SFA particularly of long chains can also have a major impact on increasing the levels of cholesterol, which can lead to an increased inflammation (Tvrzicka *et al.*, 2011).

*trans*-unsaturated FA also have a considerable effect on increasing the gut inflammation, which in turn contributes to increasing inflammation (Altowaijri *et al.*, 2017). Additionally, they affect the metabolism of *cis*-unsaturated FA and prevent the body from utilizing them. *Trans*-unsaturated FA cause capillary obstruction and decreased vascular wall elasticity and cell membrane fluidity. In several studies, the intake of SFA was associated with an increased metabolic syndrome (Kremmyda *et al.*, 2011). The intake of n-6 PUFA have been shown to result in an increased insulin sensitivity and alteration in the fat allocation along the body (Tvrzicka *et al.*, 2011). The metabolic effects of n-6 PUFA include increased mRNA for LDL receptors leading to increased cholesterol synthesis and enhanced activity of LDL receptors. The relationship between inflammation and dietary intake of PUFA is attributed to the fact that n-6 PUFA is the precursor to the proinflammatory eicosanoids production (Calder, 2006). Such molecules are generated by arachidonic acid, which is an n-6 PUFA.

## 4.3 The molecular mechanisms of fatty acids in inflammation and Multiple sclerosis

In order to understand the link between dietary interventions and MS, it is important to understand the molecular mechanisms of how dietary molecules, in this case, n-3 PUFA interact with enzymes and transcription factors in human cells

(Riccio & Rossano, 2015). There are factors that trigger an inflammation and play a role in the neuronal and axonal damage in the brain and spinal cord (Altowajri *et al.*, 2017). Such factors include the autoreactive T cells and B cells, mitochondrial dysfunction, and oxidative stress, in addition to lipid dysmetabolism. Both n-3 PUFA and n-6 PUFA affect the nuclear receptors (NR) NF $\kappa$ B, PPAR, and SREBP-1c (Schmitz & Ecker, 2008). Such receptors are involved in a series of inflammatory signalling and in lipid metabolism. n-3 PUFA play a role in regulating the genes encoding inflammatory response and in lipid synthesis and impact the FA degradation. NR are ligand-activated transcription factors that control genes encoding lipid metabolism and inflammatory signalling (Schmitz & Ecker, 2008). NF $\kappa$ B is a transcription factor belonging to the NR family and is essential for the production of inflammatory cytokines such as TNF- $\alpha$ .

Oxidative metabolism is regulated by the enzymes AMP-activated kinase, Sirtuins (SIRT) and histone deacetylating enzymes, in addition to a nuclear receptor featured by the isotypes of the peroxisome proliferator-activated receptors PPARs (Riccio & Rossano, 2015). The beta-oxidation of FA in mitochondria and peroxisomes are influenced by the PPARs isotypes. Which in turn regulate the gene transcription that is engaged in the oxidation process, and form a network with AMPK and Sirtuins pathways. Such pathways are activated by low-calorie diets and physical exercise, besides the n-3 PUFA anti-inflammatory bioactive molecules.

To understand the link between inflammation and the dietary intake of FA, it is essential to know which factors are involved in inflammation and autoimmunity (Riccio & Rossano, 2015). Such factors include the activation of the nuclear transcription factor NF $\kappa$ B and the activator protein AP-1. Both of the aforementioned are activated in the case of MS and evoke the expression of proinflammatory genes and the production of proinflammatory molecules. It is unknown what is the underlying cause of their activation in the case of MS, NF $\kappa$ B can be activated due to several factors, such as viruses, oxidative stress and the intake of SFA. Contrariwise, EPA and DHA are activators of PPAR $\alpha$ , which in turn inhibits NF $\kappa$ B and hence reduce inflammation (Schmitz & Ecker, 2008). EPA inhibits the activity of NF $\kappa$ B more efficiently in comparison to DHA.

PPAR isotopes can make complexes with the retinoid X-receptor (RXR), the latter is activated by 9--cis-retinoic acid (RA) (Riccio & Rossano, 2015). The sterol regulatory element-binding proteins (SREBP-1c and SREBP-2) are activated by diets such as the western-style diets, including high SFA intake. Furthermore, such diets also activate the carbohydrate responsive element-binding protein ChREBP.

Both SREBP-1c and SREBP-2 are controlled by the “liver X” nuclear receptors (LXR), the latter is activated by cholesterol derivative oxysterols and glucose. LXR has a role in lipid-synthesis by activating SREBP-1c and inhibiting SREBP-2.

Oxidative stress is increased by the intake of n-3 PUFA compared to the intake of n-6 PUFA (Kremmyda *et al.*, 2011). The transcription of antioxidant enzymes (uncoupling protein 2, glutathione transferase 2 $\tau$ , superoxide dismutase) is also increased by the dietary intake of n-3 PUFA. The repressed transcription of enzymes that establish the production of oxygen and nitrogen species (RONS) is also suppressed by the intake of n-3 PUFA.

The course of relapse in MS that is characterized by new attacks of the autoreactive T cell to the myelin is a biosynthetic process, so dietary components that favour anabolism would promote the inflammatory status of MS and dietary components that favour catabolism, will have the opposing effect (Riccio & Rossano, 2015).

#### 4.4 Omega-3 anti-inflammatory molecules in treating Multiple sclerosis

There are various disease-modifying drugs and therapies used to slow down or to prevent the progression of MS (Riccio & Rossano, 2015). Such therapies rely on targeting the inflammatory status of MS (Altowaijri *et al.*, 2017), besides being merely effective in diminishing short-term morbidity (Riccio & Rossano, 2015). They can alleviate the progression and certain MS outcomes in some patients because each patient responds differently to such drugs. Such drugs and treatments do not cure the outcomes of MS, are of high costs and have serious side effects (Altowaijri *et al.*, 2017). Therefore, it can be discussed whether an alternative therapy from a natural source could be more efficient in MS treatment than the used drugs (da Cunha Matta & Orsini, 2017). Several studies showed that the prevalence of MS in populations with higher intake of fish is less than in those with lower intake of fish, thus linking n-3 PUFA intake with an enhanced immunity and prevention of the onset of disease (Altowaijri *et al.*, 2017). Besides the anti-arrhythmic and antithrombotic effects, LC n-3 PUFA possess anti-inflammatory effects (Kremmyda *et al.*, 2011). Such effects are typically characterized by a decreased production of the proinflammatory eicosanoids lipid-mediators derived from AA and inflammatory cytokines.

Postprandial inflammation and the expression of proinflammatory molecules are reduced by the intake of n-3 PUFA (Riccio & Rossano, 2015). n-3 PUFA particularly derived from fish suppress the production of cholesterol and induce the oxidation of FA. The intake of EPA and DHA can reduce the production of inflammatory cytokines and antagonizes the excessive presence of eicosanoids (Calder, 2006). There is a competition between n-3 PUFA and n-6 PUFA for the en-

zymes to produce further eicosanoids. An excess of one of the PUFA families causes a decrease in the conversion of the other (Schmitz & Ecker, 2008). If the conversion is favoured for the n-3 family, there will be a significant decrease in the proinflammatory eicosanoids derived from n-6 family, resulting in a lowered inflammation.

Both DHA and EPA possess anti-inflammatory properties and immunomodulatory activities (Riccio & Rossano, 2015). DHA exists in the human brain and the concentrations of it are reduced in MS patients. It can, therefore, be discussed whether there is a link between n-3 PUFA and MS. Dietary components that contain arachidonic acid such as meat, or dietary components containing more n-6 than n-3 PUFA can be a contributing risk factor for MS patients. Therefore, the intake of them should be limited in MS patients to reduce the proinflammatory production of eicosanoids (Calder, 2006). The inflammatory processes in MS are suggested to be owing to the low ratio of n-3 PUFA in comparison to n-6 PUFA. This is logical since n-6 families are converted to proinflammatory eicosanoids that can affect the inflammatory status of MS.

## 5 Discussion

Unfortunately, it is unclear whether there is a direct link between dietary habits and the case of MS since clinical trials involved in the research of this topic are not so many (Ricchio & Rossano, 2015). There is no clear evidence that an imbalanced intestinal microbiota is a direct cause of MS. However, the anti-inflammatory properties of n-3 PUFA are shown to help improve the overall quality of life (Altowaijri *et al.*, 2017). Healthy dietary habits have a positive impact on MS outcomes such as reducing fatigue, relapse rate, physical disability, slowing down the progression of the disease and downregulating the production of proinflammatory molecules.

A review by Calder that has been done at 2015 on the effects of marine n-3 FA on inflammatory processes concluded that the intake of n-3 PUFA reduces the amount of AA in the membrane of cells involved in inflammation (Calder, 2015). It was also concluded that some of the effects of n-3 PUFA particularly from fish on the inflammatory cells are mediated by alterations in the FA composition in the cell membrane.

Experimental studies in a review by Simopoulos about the role of n-3 PUFA in inflammation and autoimmune diseases showed evidence that incorporating n-3 PUFA amended inflammatory and immune reactions (Simopoulos, 2002). Consequently, it can be argued that n-3 PUFA can be used as a therapeutic alternative in inflammatory and autoimmune disease such as MS. Further findings of the review were that there was a link between the pathophysiology of major depression and the alterations of FA composition and the production of proinflammatory eicosanoids and cytokines. This can also be implied to the MS-related depression.

In MS, the expression of various proinflammatory genes besides the production of proinflammatory molecules is promoted (Ricchio & Rossano, 2015). This can be triggered by some proinflammatory dietary components like SFA and *trans*-unsaturated FA. In contrast to low-calorie diets, calorie-rich diets including the intake of SFA are considered to induce the production of proinflammatory molecules. A review of the nutrition facts in MS by Ricchio and Rossano reviewed stud-

ies from the 1950s by Swank and Goodwin. The studies demonstrated that the development of MS is related to the intake of SFA of animal origin and that the consumption of such FA results in the synthesis of storage lipids and cholesterol leading to an increased inflammation (reviewed by Riccio & Rossano, 2015). SFA decreases membrane fluidity and obstructs CNS capillaries consequently causing inflammation and demyelination (Altowaijri *et al.*, 2017). A diet based on the low consumption of SFA was designed by Swank to emphasize the link between the prevalence of MS and high intake of SFA, it was shown that following this diet could decrease the physical disability and progression of MS (Altowaijri *et al.*, 2017).

In a review by Wall *et al.* 2010, it was concluded that the high intake of n-3 PUFA alters the production of proinflammatory eicosanoids derived from AA and regulates the inflammation and immune responses (Wall *et al.* 2010). Consuming n-3 PUFA was linked with decreased prevalence of chronic diseases and an improved mental health.

In an Australian case-control study done for the Ausimmune Investigator Group, the role of total FA intake in the risk of first clinical diagnosis (FCD) of CNS demyelination was investigated (Hoare *et al.*, 2016). In four regions of Australia, 267 FCD cases and 517 matching controls included. The dietary data were obtained by utilizing a validated food frequency questionnaire within four years. Main findings were that the high intake of n-3 PUFA specifically from fish was connected to a reduced risk of FCD. The study also compared different other studies such as a Swedish population-based case study of newly diagnosed MS patients, which connected n-3 PUFA intake to a lowered risk of developing MS. Two other studies were also reviewed, performed in France and Poland, both suggested that there was no link between the total consumption of FA and MS. It is not clear what type of FA was reviewed in both of the studies, therefore they are not considered reliable. The studies were designed differently and the data collection methodology was obscure and unclear in the French and Polish ones (Hoare *et al.*, 2016). Additionally, poor information was provided about the overall dietary habits in relation to the diagnosis of MS. The studies were performed on a different form and stage of the disease (Hoare *et al.*, 2016), in addition to that each patient has his own individual case, which is not similar to others (Riccio & Rossano, 2015). Nine regional Human Research Ethics Committees approved the study by Hoare. The conclusion of the Ausimmune study was that there was a significant decrease in the risk of FCD of the CNS demyelination associated with the high intake of n-3 PUFA particularly from fish (Hoare *et al.*, 2016).

It should be taken into account that there are other factors influencing the case of each individual patient, like the overall dietary habits and lifestyle (Altowaijri *et al.*, 2017). The overall eating habits play a considerable role in reducing the out-

comes of MS such as fatigue, depression, relapsing rate, and physical disability. This is attributed to that a healthy diet improves the overall quality of life and enhances the immune system. It is suggested that another contributing factor that might trigger the onset of the disease is smoking (Riccio & Rossano, 2015). It might not be a direct cause of MS but may definitely reduce the health-related quality of life of smoking individuals compared to non-smokers. Another assisting factor that might lead to MS is hyper-caloric diets since the consumption of refined carbohydrates and sugar triggers the insulin levels to increase and result in the production of proinflammatory molecules and free radicals.

An international survey assessed the connection between dietary factors and health-related quality of life (Altowaijri *et al.*, 2017). It showed that the high intake of n-3 PUFA was associated with a better quality of life and less physical disability and fatigue in MS patients. Evidence from a number of studies shows that the intake of n-3 PUFA is associated with lower inflammatory cytokine production (Kremmyda *et al.*, 2011). The intake of EPA has been shown to reduce the degradation of the skeletal muscles and reduce the expression of proteasome activity.

## 6 Conclusion

In summary, incorporating dietary interventions as an alternative or complementary medicine in MS can be beneficial, by controlling and reducing the inflammatory status of MS, through reducing the postprandial inflammation. In addition to retaining the intestinal microbiota in balance to suppress intestinal- and systemic-inflammation and enhance immunity.

The consumption of n-3 PUFA is shown to help to improve the physical and mental health-related quality of life. n-3 derived eicosanoids possess anti-inflammatory properties that might help in the case of MS, by reducing the production of proinflammatory molecules. DHA and EPA possess anti-inflammatory properties and immune-modulatory activities.

Studies showed that the high intake of n-3 PUFA specifically from fish was linked with a lowered progression, fatigue, depression and physical disability in MS patients.

More information and data should be provided regarding the diet-gene interactions. As well as the role of nutrigenomics and the environmental modifications to gain a boosted immune system functionality and prevention against disease.

## 7 Tables & Figures

7.1 *Table 1. n-3 content in different fish species, developed by using Table 1 from the review by (Wall et al., 2010).*

Fish species	n-3 (EPA&DHA) content per 100 g fish
Atlantic herring	2.01
Atlantic salmon	1.28-2.15
Sardines	1.15-2
Rainbow trout	1.15
Tuna	0.28-1.51
Tuna (canned)	0.31
Cod	0.28
Scallop	0.2

## 7.2 Figure 1.

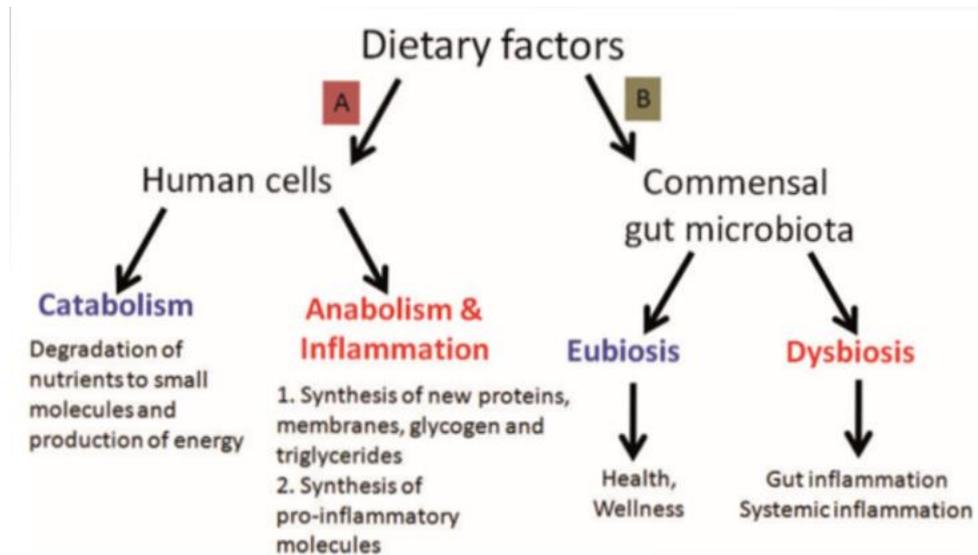


Figure 1. How the diet affects the human health in two ways: (A) the metabolism of human cells and (B) the population of the human gut microbiota (reviewed by Riccio & Rossano, 2015)

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