

The effects of Mediterranean diet and EVOO consumption in relation to human health

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Abstract

The Mediterranean basin is characterized by the presence and cultivation of olive trees since antiquity. All of the Mediterranean countries and especially Greece and Italy, are characterized by similar food patterns that are described as “Mediterranean diet”. The core of this diet is the daily intake of plant foods and as its main source of fat is olive oil and particularly in Greece EVOO (Extra Virgin Olive Oil). EVOO contains a large percentage of MUFA (monounsaturated fatty acids - approximately 80% of its total lipid concentration), as well as some other substances in smaller concentrations such as oleocanthal, hydroxytyrosol, oleuropein etc. The greater the adherence to the Mediterranean diet and to the intake of EVOO as the main fat source, the greater the health benefits encountered. Some of these benefits that have been reported are due to the antioxidant properties of EVOO, its anti-inflammatory effects, its ability to regulate the endothelial function, lipids, haemostasis, coagulation and fibrinolysis. Moreover, EVOO has a positive effect in chronic diseases such as obesity, metabolic and amyloid diseases, while it reduces the risk of cardiovascular and neurodegenerative diseases. Furthermore, it influences the aging process by reducing the genome and proteome damage that lead to aging. All the data collected, demonstrate that a daily intake of EVOO combined with a good percentile of adherence to the Mediterranean diet lead to a healthier lifestyle, longevity and a reduced morbidity rate.

Keywords: aging; anti-inflammatory; antioxidant; cardiovascular diseases; neurodegenerative diseases; oleocanthal; oleuropein

Abbreviations: AACD (age-associated cognitive decline), AAMI (age-associated memory impairment), AD (Alzheimer’s disease), ANS (8-anilino naphthalene-1-sulfonic acid), ARCD (age-related cognitive decline), CRP (C-reactive protein), CT (computer tomography), CVD (cardiovascular diseases), D76N β 2-microglobulin, DDR (DNA damage responses), DLS (dynamic light scattering), EVOO (extra virgin olive oil), FRET (fluorescence resonance energy transfer), gLMS (general labeled magnitude scale) HDL (high density lipoprotein), IL-6 (interleukin-6), ILSA (Italian longitudinal study of aging), LDL (low density lipoprotein), LMP (lysosomal membrane permeabilization), LPO (lipid peroxide), MCI (mild cognitive impairment), MOMP (mitochondrial outer membrane permeabilization), MRI (magnetic resonance imaging), MUFA (monounsaturated fatty acids), OleA (oleuropein aglycone), ox-LDL (oxidized low density lipoprotein), PAI-1 (plasminogen activator inhibitor-1), PD (Parkinson’s disease), PFL (phonemic verbal fluency), PN (proteostasis network), PUFA (polyunsaturated fatty acids), RCTs (randomized controlled trials), ROS

(reactive oxygen species), SFA (saturated fatty acids), TC (total cholesterol), TEM (transmission electron microscopy), TF (tissue factor), TFPI (tissue factor pathway inhibitor), TPF (total phenolic fraction), TRP (transient receptor potential), Trp (tryptophan), VOO (virgin olive oil), WML (white matter lesions).

Introduction

The Mediterranean basin is characterized by the cultivation of olive trees since antiquity (Therios, 2009). Inhabitants of the region exploited every part of olive tree in their daily lives even prior to their domestication and cultivation. Olives and olive oil played a major role in their everyday dietary patterns, while flowers and leaves of olive trees were used as treatments for various diseases; its wood was also used for heating, lighting and various crafts (Therios, 2009).

Although the production of olive oil and table olives is by far the most important reason for olive cultivation, the species serves other important functions as well. In several countries of the Mediterranean basin, olive trees have been closely associated -since ancient times- with the traditional and natural landscape of the region; and their presence, together with cypress, is typical of religious sites. In these areas, it is one of the few species that demonstrate high drought resistance while being suitable for cultivation in terrains with calcareous/rocky soils and sloping terrains (Therios, 2009). Finally, its wood is characterized by natural durability and beauty and is of high value in some parts of the Mediterranean basin, such as in southern Italy (Lambardi and Rugini, 2003).

The presence of olive tree in the Mediterranean region since antiquity -e.g. olive tree cultivation is referenced in an Egyptian scroll dated in the 16th century B.C. (Therios, 2009) - explains its important role in the dietary patterns of the people residing in the countries surrounding the Mediterranean Sea.

The term 'Mediterranean diet' reflects food patterns typical of Greece, Italy and other Mediterranean countries such as Lebanon, Morocco, Portugal, Spain, Tunisia, Turkey etc. (Willet *et al.*, 1995). The diet is closely tied to traditional areas of olive cultivation on the Mediterranean region and is presented with some differences amongst these olive oil producing countries (Willet *et al.*, 1995). In the traditional Mediterranean diet, plant foods constitute the core of daily intake whereas animal derived foods are not encountered as frequently or in large amounts (Willet *et al.*, 1995). These plant foods are minimally processed, seasonally fresh and locally grown. A few of these plant foods are fruits, vegetables, breads from wheat as well as from other cereals, beans, nuts and seeds. The typical daily desert in the Mediterranean diet consists of fresh fruits while sweets containing concentrated sugars or honey are also consumed a few times per week. The main source of fat in this diet is olive oil and particularly in Greece extra virgin olive oil (EVOO) (Buckland and Gonzalez, 2015). Dairy products, consisting mainly by cheese and yogurt, are consumed with moderation, while zero to four eggs are recommended for consumption on a weekly basis. Red meat is consumed in low amounts as well as wine, which usually accompanies the meals (Willet *et al.*, 1995; Yubero-Serrano *et al.*, 2018). The value and importance of the Mediterranean diet have been recognized by UNESCO which has led to the inclusion of the Mediterranean diet in the Intangible Cultural Heritage List (UNESCO, n.d.) (Nikou *et al.*, 2019).

These components of the Mediterranean diet partially explain its beneficial effects on human health. The minimal processing of seasonally fresh foods is considered to maximize the contents of dietary fiber, antioxidants, other micronutrients and nonnutritive substances found in foods from plants. Moreover, the balance between a high intake of vegetables and low intake of foods from animal sources, increases the amounts of vitamin B12 and available iron, while it keeps the contents of saturated fat low (Willet *et al.*, 1995). Nowadays, the Mediterranean diet is still considered to be a golden standard for healthy nutrition in spite its high fat content (monounsaturated fatty acids [MUFAs]), unlike the diets recommended by many nutrition experts in other geographical areas (Yubero-Serrano *et al.*, 2018).

In northern European countries the principal source of fat in the dietary patterns comes from animal fats, whereas in the Mediterranean region olive oil is the main source (Nikou *et al.*, 2019). For instance,

according to Mediterranean tradition, butter should be used only in small amounts or on special occasions while olive oil should replace –and not be added to- other sources of dietary fat such as butter, margarine, or vegetable oils and shortenings (Yubero-Serrano *et al.*, 2018).

According to Yubero-Serrano *et al.* (2018), there are plenty of data that show a strong correlation between the adherence to the Mediterranean diet and reduced mortality and morbidity, a lower incidence rate of cardiovascular complications (coronary events, stroke or hypertension), neurodegenerative diseases (Capurso *et al.*, 2018), certain types of cancer and other chronic and metabolic diseases (such as type 2 diabetes mellitus or metabolic syndrome).

Virgin olive oil (VOO) and EVOO have been established in the consciousness of people worldwide as superior nutritional and health promoting edible oils with exceptional organoleptic and sensory properties (Nikou *et al.*, 2019). In particular, consumption of EVOO as part of the Mediterranean diet has beneficial effects in cardiovascular risk factors such as inflammation, oxidative stress, coagulation, platelet aggregation, fibrinolysis, endothelial function or lipids (Yubero-Serrano *et al.*, 2018; Nikou *et al.*, 2019). Moreover, consuming EVOO while adhering to this diet leads to advanced life expectancy and reduced incidences of age-related diseases (Nikou *et al.*, 2019). The Mediterranean diet is responsible for a significant 8% reduction of deaths from any causes, a 10% reduction of deaths and/or incidences of cardiovascular and cerebrovascular diseases, a 6% reduction of the incidences of neurodegenerative diseases including Parkinson's disease and Alzheimer's disease (Capurso *et al.*, 2018).

Chemical constituents of EVOO

The main source of fat in the Mediterranean diet is olive oil, the constituents of which play a major role in the diet's health benefits (Willet *et al.*, 1995; Perez-Jimenez *et al.*, 2005; LeGendre *et al.*, 2015; Capurso *et al.*, 2018; Yubero-Serrano *et al.*, 2018). It is -chemically- an extremely multifaceted assembly of molecules mainly composed of lipophilic components (95-97%), rich in monounsaturated fatty acids (MUFA) (Nikou *et al.*, 2019). Olive oil consists of the polar fraction (Total Phenolic Fraction - TPF), also known as olive oil "polyphenols" or "biophenols", representing its antioxidant component. The chemical classes characterizing the olive oil polyphenols are very diverse and they include phenylalcohols, phenolic acids, secoiridoids, flavonoids and lignans (Kanakis *et al.*, 2013; Mitsopoulos *et al.*, 2016 (a)). The characteristics of olive oils (Table 1) are influenced by several endogenous and exogenous factors such as olive variety, cultivation practice, harvesting period, weather conditions, milling procedure itself etc. (Agiomyrgianaki *et al.*, 2012; Mitsopoulos *et al.*, 2016(b)).

EVOO contains a large proportion of MUFA which is considered to be the main reason for its healthy properties. Moreover, EVOO is low in saturated fat and a major source of the antioxidant vitamin E. Additionally, MUFA molecules have only one double bond, thus making EVOO more resistant to oxidation and hereby contributing to its antioxidant properties, high stability and long shelf life compared to PUFA-enriched oils (Yubero-Serrano *et al.*, 2018). Moreover, - EVOO contains oleic acid - accounting for up to 80% of its total lipid composition oils (Yubero-Serrano *et al.*, 2018) which is considered to be antithrombotic, especially when compared to saturated fatty acids (Willet *et al.*, 1995).

There is a plethora of other bioactive components in EVOO that have beneficial properties. Some of them are classified in unsaponifiable compounds -such as squalene, sitosterols, triterpenes, pigments etc.- and are defined as the fraction extracted with solvents after the saponification of the oil and soluble or hydrophilic compounds and other compounds with antioxidant properties (Yubero-Serrano *et al.*, 2018). These components contribute to the unique flavor and taste of EVOO, distinguishing it from other oils derived from seeds and fruits that need to be refined for human consumption, thus losing the vast majority of these minor and yet beneficial components (Yubero-Serrano *et al.*, 2018).

Table 1. Olive oils characteristics as presented by the official journal of the European Union (27/01/2011)

Category	Acidity (%)	Acid content		Peroxide index mEq O ₂ /kg	Waxes mg/kg	Sterols composition		Total sterols (mg/kg)	Organoleptic evaluation	
		Myristic (%)	Linolenic (%)			Cholesterol (%)	Brassicasterol (%)		Median defect (Md)	Fruity median (Mf)
Extra virgin olive oil	≤0,8	≤0,05	≤1,0	≤20	≤250	≤0,5	≤0,1	≥1.000	Md=0	Mf>0
Virgin olive oil	≤2,0	≤0,05	≤1,0	≤20	≤250	≤0,5	≤0,1	≥1.000	Md≤3,5	Mf>0
Lampante olive oil	>2,0	≤0,05	≤1,0	-	≤300	≤0,5	≤0,1	≥1.000	Md>3,5	-
Refined olive oil	≤0,3	≤0,05	≤1,0	≤5	≤350	≤0,5	≤0,1	≥1.000	-	-
Olive oil composed of refined and virgin olive oils	≤1,0	≤0,05	≤1,0	≤15	≤350	≤0,5	≤0,1	≥1.000	-	-
Crude olive-residue oil	-	≤0,05	≤1,0	-	>350	≤0,5	≤0,2	≥2.500	-	-
Refined olive-residue oil	≤0,3	≤0,05	≤1,0	≤5	>350	≤0,5	≤0,2	≥1.800	-	-
Olive-residue oil	≤1,0	≤0,05	≤1,0	≤15	>350	≤0,5	≤0,2	≥1.600	-	-

Another component of EVOO is (-)-Oleocanthal (OC), a dialdehydic form of ligostride aglycone. It is a potent antioxidant, a non-steroidal anti-inflammatory agent, a neuroprotectant that alters the structure and the function of the neurotoxins β -amyloid and Tau that are associated with the debilitating effects of Alzheimer's disease (Capurso *et al.*, 2018). Furthermore, it is an inhibitor of proliferation, migration and invasion of human breast, prostate and colon cancer cells and an inhibitor of macrophage inflammatory protein-1 α in multiple myeloma (LeGendre *et al.*, 2015).

All these chemical compounds encountered in EVOO have demonstrated multiple and beneficial impacts on human health. Consuming EVOO in combination with food sources rich in dietary fibers, antioxidants, other micronutrients and vitamin B12 increases the available iron in an organism while decreasing the amount of saturated fat. Consuming EVOO is considered to be preventing the occurrence of a variety of diseases (cardiovascular, neurodegenerative etc.), as many of its compounds are responsible for the interference with various mechanisms in order to protect the human health (Table 2).

Table 2. EVOO compounds and their beneficial function

EVOO compounds	Function	Mechanism	References
Oleic acid	Antithrombotic		Willet <i>et al.</i> , 1995
Vitamin E	Antioxidant		Willet <i>et al.</i> , 1995; Yubero-Serrano <i>et al.</i> , 2018
MUFA	Resistance to oxidation	Double bond	Yubero-Serrano <i>et al.</i> , 2018
Oleocanthal	Potent antioxidant		Capurso <i>et al.</i> , 2018
	Non-steroidal anti-inflammatory agent		Capurso <i>et al.</i> , 2018
	Neuroprotectant	Alters the structure and function of neurotoxins β -amyloid and Tau	Capurso <i>et al.</i> , 2018
	Human breast, prostate and colon cancer cells inhibitor	Inhibition of cancer cells proliferation, migration and invasion	LeGendre <i>et al.</i> , 2015
	Inhibitor of multiple myeloma	Inhibition of macrophage inflammatory protein-1 α	LeGendre <i>et al.</i> , 2015
	Responsible for the apoptotic and necrotic death of cancer cells	Altering the lysosomal membrane permeabilization	LeGendre <i>et al.</i> , 2015; Proskuryakov <i>et al.</i> , 2003
	Reduction of inflammation	Inhibition of cyclooxygenase-1 and cyclooxygenase-2 activity	Ryu <i>et al.</i> , 2015
Hydroxytyrosol	Inhibitor of protein expression in human umbilical vein endothelial cells stimulated by lipopolysaccharides or cytokines	Inhibition of expression of cell adhesion molecules VCAM-1 and ICAM-1	Scoditti <i>et al.</i> , 2012
	Increase of antioxidant activity and reduction of oxidative status	Increase of expression of the antioxidant glutathione peroxidase and reductase in the presence of hydrogen peroxide	Giordano <i>et al.</i> , 2014
	Decrease of inflammation and of oxidative stress	Increase of the nitric oxide production in endothelial cells	Marin <i>et al.</i> , 2011; Yubero-Serrano <i>et al.</i> , 2018
Hydroxytyrosol and oleuropein	Reduction of inflammation in endothelial cells during angiogenesis		Scoditti <i>et al.</i> , 2012
Oleuropein	Decrease of both systolic and diastolic blood pressure		Lockyer <i>et al.</i> , 2017
Oleuropein aglycon	Inhibition of amyloid diseases	Remodel and inactivate toxic amyloid oligomers	Ladiwala <i>et al.</i> , 2011
	Protection against A β 42 aggregation and plaque formation in tissue	Interference in the aggregates' binding to the cells' membrane	Diomedea <i>et al.</i> , 2013; Grossi <i>et al.</i> , 2013; Leri <i>et al.</i> , 2018

Oleocanthal (OC)

The beneficial effects of consuming EVOO within the limits of a balanced diet such as the Mediterranean one is numerous. EVOO contains a considerable amount of phenolic compounds that are responsible for its peculiar taste and high stability oils (Yubero-Serrano *et al.*, 2018) as well as its inhibiting function against reactive oxygen species (ROS) (LeGendre *et al.*, 2015; Nikou *et al.*, 2019). One major phenolic compound of EVOO is OC which is responsible for the pharyngeal pungency caused in humans (Andrews *et al.*, 2003; Beauchamp *et al.*, 2005). There are many compounds that are characterized as oral irritants, especially from chili peppers and horseradish, but none of them causes this unusually localized sensation. This localized sensation in the pharyngeal region arises from the stimulation of non-specialized nerve-endings in the epithelium. The free nerve-endings signal a wide range of endogenous and environmental stimuli such as protons, pressure, temperature and nociceptive agents (des Gachons *et al.*, 2011).

High quality EVOOs have several noteworthy sensory characteristics such as bitterness and pharyngeal pungency, often leading to coughing and throat cleaning (Carluccio *et al.*, 2003; des Gachons *et al.*, 2011) and are sometimes referred to as 'one cough' or 'two coughs' oils (the latter being more highly prized) because of this peculiar pungency (des Gachons *et al.*, 2011). The only other compound known to trigger this restricted pharyngeal irritation -apart from OC- is the anti-inflammatory drug ibuprofen and its congeners (Breslin *et al.*, 2001).

According to Clapham (2003), the transient receptor potential (TRP) family of ion channels plays a prominent role in the signaling of free nerve-endings. In particular, the thermos TRP channels (TRPV1-TRPV4, TRPV8 and TRPA1) are expressed in keratinocytes and primary sensory neurons of the nociceptive pathway (which is responsible for sensations of irritation) and have been shown to participate in the transduction of pain induced by thermal mechanical and chemical stimuli (Levine and Alessandri-Haber, 2007). Furthermore, eight individuals agreed to an immunochemical analysis of TRPA1 expression in sensory neurons which led to the identification of tissues that are OC-sensitive and OC-insensitive. The complete loss of sensitivity to OC implicates TRPA1 as the requisite native target in sensory neurons (des Gachons *et al.*, 2011).

The phenylpropanoic, non-steroidal, anti-inflammatory drugs (NSAIDs) such as ibuprofen (IBU), trigger a pharyngeal irritation remarkably similar in quality which is restricted to the same location as that elicited by OC (Breskin *et al.*, 2001). The robust responses to IBU have only been observed in neurons that respond to OC, suggesting that OC and IBU act on the same receptor(s). According to des Gachons *et al.* (2011), the OC's neuronal signal is transduced by TRPA1 and no other thermos TRP channel. After testing a total of 20 sensory neurons, the results confirmed that IBU selectively activates TRPA1 (directly or indirectly) and no other receptor of the sensory neurons (des Gachons *et al.*, 2011). TRPA1 is well positioned to protect the lungs by triggering defensive cough responses to reactive agents (Bessac and Jordt, 2008; Taylor-Clark *et al.*, 2008).

Most pungent food products elicit strong sensation throughout the oral cavity; these include the plant-derived oral irritants such as isothiocyanates (mustard, winter cress), allicin (garlic), cinnamaldehyde (cinnamon bark), carvacrol (oregano) or thymol (thyme), all of which trigger TRPA1 (Bandell *et al.*, 2004; Jordt *et al.*, 2004; Bautista *et al.*, 2005; Macpherson *et al.*, 2005; Xu *et al.*, 2006; Lee *et al.*, 2008). However, these irritating compounds are experienced in foods at higher concentrations (mM) than the OC concentrations found in EVOO (100-700 μ M) (des Gachons *et al.*, 2011).

Under protocols approved by the Office of Regulatory Affairs at the University of Pennsylvania, volunteers at that institution were asked to evaluate the irritation elicited by the test compounds using a computerized general Labeled Magnitude Scale (gLMS) (Green *et al.*, 1996). These tests examined the irritation of the tongue, the throat as well as the irritation of the nose. In 2011 des Gachons *et al.* recorded that both EVOO and horseradish trigger pharyngeal irritation with matching intensity. However, EVOO elicited very little pungency on the anterior tongue -where the horseradish was strongly sensed- but it triggered

irritation to the throat in contrast to horseradish. The same test was conducted for IBU. Both OC and IBU irritated the throat and the nose with the latter irritation increasing accordingly to their concentration; thus, demonstrating that they clearly activate human trigeminal nerve *in situ* (Breslin *et al.*, 2001; des Gachons *et al.*, 2011).

According to LeGendre *et al.*'s (2015) research, OC mediated cancer cell death which was promoted by destabilization of the lysosomal membrane; thus, leading to the induction of lysosomal membrane permeabilization (LMP). In particular, OC induced loss of viability in cancer cells within 30 minutes, in dose-dependent manner via different mechanisms depending on whether serum was absent or present. Under serum withdrawal, OC promoted primary necrotic cell death in cancer cells, while in the presence of serum, a combination of apoptosis and secondary necrosis was observed (LeGendre *et al.*, 2015).

The signaling pathways that regulate cell death, participate in both apoptosis and necrosis thus making it possible for apoptotic and secondary necrotic cell death to occur within the same cell population (Proskuryakov *et al.*, 2003; Fink and Cookson, 2005). The data from LeGendre *et al.*'s (2015) research, suggest that OC induces cell death of cancer cells through both apoptotic and necrotic mechanisms depending on the presence or absence of the serum. The primary necrosis is caused by a severe attack, either extracellular or intracellular and it can be identified by rapid permeabilization of the plasma membrane (Proskuryakov *et al.*, 2003). Recently, the implication of lysosomes in cell death through the release of lysosomal hydrolytic enzymes into the cytosol -which leads to apoptosis (through mitochondrial outer membrane permeabilization [MOMP] and caspase activation) or necrosis (via cytosolic acidification) - has been reported (Boya and Kroemer, 2008).

On the other hand, OC induced a reversible cell cycle arrest in non-cancerous cells without affecting their viability -due to their high lysosomal membrane integrity- and through the suppression of RB phosphorylation -which serves to protect healthy cells against the adverse effects of OC (LeGendre *et al.*, 2015). The lysosomal membrane integrity is regulated by the activity of ASM, a lysosomal lipase responsible for the hydrolysis of sphingomyelin (SM) to ceramide (Kirkegaard *et al.*, 2010).

Anti-inflammatory properties

The Mediterranean diet has been shown to reduce the inflammatory responses which are usually present in chronic diseases. This decrease has been partially attributed to the high intake of EVOO accompanying the Mediterranean diet (Lucas *et al.*, 2011). Many studies have shown the importance of minor EVOO components (such as phenolic compounds) on the inflammatory response (Lucas *et al.*, 2011; Yubero-Serrano *et al.*, 2018). According to Yubero-Serrano *et al.* (2018), these fewer inflammatory responses are accompanied by a downregulation of the expression of pro-inflammatory genes, low levels of pro-inflammatory proteins as well as a lower total plasma/serum concentration of pro-inflammatory markers in both chronic and post prandial levels.

Consuming EVOO while adhering to the Mediterranean diet has beneficial effects for the human health. The benefits of EVOO are detectable only after a few weeks of the beginning of regularly consuming it. These benefits are greater and more evident during chronic consumption of EVOO, on daily basis. Specifically, after 12 weeks of consuming EVOO, an increase of the anti-inflammatory effect of HDL (High Density Lipoprotein) was reported, alongside with a reduction of the anti-atherogenic activity in healthy subjects (Loued *et al.*, 2013). The chronic consumption of a Mediterranean diet -rich in EVOO- from subjects with a high cardiovascular risk, lead to the reduction of C-reactive protein (CRP), interleukin-6 (IL-6) as well as other pro-inflammatory interleukin levels (Mena *et al.*, 2009; Estruch, 2010). According to Esposito *et al.* (2004), after two years of Mediterranean style diet, the endothelial function and insulin sensitivity have been enhanced in patients with metabolic syndrome.

An important polyphenolic compound of EVOO, hydroxytyrosol, has the ability to inhibit the expression of cell adhesion molecules (VCAM-1 and ICAM-1) in human umbilical vein endothelial cells

stimulated by lipopolysaccharides or cytokines. Furthermore, hydroxytyrosol reduces the inflammatory process during angiogenesis in endothelial cells in combination with oleuropein -another EVOO component (Scoditti *et al.*, 2012). Ryu *et al.* (2015), used the phenolic compound oleocanthal of EVOO as an anti-inflammatory drug and it was able to induce dose-dependent inhibition of substances involved in the inflammatory process (cyclooxygenase-1 and cyclooxygenase-2).

Antioxidant properties

Most of the minor components of EVOO, that are not found in other oils, are powerful antioxidants and are considered to be an effective treatment against the oxidative stress associated with several diseases (Perez-Jimenez *et al.*, 2007; Capurso *et al.*, 2018; Yubero-Serrano *et al.*, 2018). According to de la Torre-Carbot *et al.* (2010), EVOO consumption protects LDLs (low density lipoproteins) from being oxidized and turning to ox-LDLs (oxidized LDLs) due to its capacity in phenolic compounds which bind to LDL particles thus inhibiting oxidation.

Plasma LPO (lipid peroxide) and nitrotyrosine levels were reduced in elderly men and women when they followed a diet rich in EVOO in comparison to other diets with different fat composition (PUFA and SFA). This decrease suggests that the composition of EVOO has a protective effect against free radicals as its consumption has been reported to lower postprandial stress during the aging process (Yubero-Serrano *et al.*, 2011; Capurso *et al.*, 2018). One important component of EVOO, hydroxytyrosol, is believed to be capable of reducing the oxidative status when in the presence of hydrogen peroxide. Hydroxytyrosol in combination with hydrogen peroxide protect humans against oxidization by increasing the antioxidant activity and expression of both antioxidant glutathione peroxidase and reductase (Giordano *et al.*, 2014).

EVOO regulates the endothelial function

The endothelial damage is the first step preceding the development of atherosclerosis. The vascular endothelium is essential for maintaining a proper vasodilation and regulating the metabolism of different molecules involved in the endothelium damage. The endothelium damage can lead to various dysfunctions to the coagulation system, the platelets and even the circulating inflammatory mediators (ICAM-1 and VCAM-1) (Borissoff *et al.*, 2011).

Some environmental factors (such as diet patterns) impair the vascular endothelium function, by exerting their effects in both the vascular vasomotor capacity or influencing cellular regulators. These dysfunctions lead to the production of an imbalance in the redox system, due to the increase of oxidative stress and a decrease in nitric oxide availability that is caused at the vascular site (Brunner *et al.*, 2005). In this context, Yubero-Serrano *et al.* (2011) examined the effects of the Mediterranean diet on the endothelial function. According to them, meals rich in EVOO (the main source of MUFA in this diet) have favorable effects on the postprandial vasomotor function of the endothelium, which enhance the vasodilator capacity during this phase, when compared to meals rich in SFA (Trichopoulou *et al.*, 2003; Nikou *et al.*, 2019).

Marin *et al.* (2011), demonstrated that chronic consumption of EVOO from elderly people reduces the microparticles concentration as well as it increases the level of endothelial progenitor cells in comparison to a SFA-rich diet or a low-fat-carbohydrate diet. Moreover, EVOO consumption leads to the decrease of the levels of nitric oxide degradation products that are detected in urine. These data suggest that a lower level of oxidative stress and a higher DNA stability in the endothelial cells, result in their protection.

The Mediterranean diet is capable of leading to the reduction of inflammation as well as oxidative stress and to the increase of nitric oxide's bioavailability. These effects are attributed to EVOO –which was the main source of MUFA- regardless of the presence or absence of the antioxidant enzyme Q10 in the frames of this diet (Marin *et al.*, 2011). Hydroxytyrosol, another compound encountered in EVOO, has been found to be responsible for the high levels of nitric oxide in endothelial cultures (Yubero-Serrano *et al.*, 2018). In 2017 a

research conducted by Lockyer *et al.* (2017) attributed the decrease of systolic and diastolic blood pressure of healthy subjects to the consumption of oleuropein extracts (an EVOO component) for 6 weeks.

Lipids, haemostasis, platelet coagulation and fibrinolysis

The total cholesterol (TC) concentration in plasma –including its fractions: HDL and LDL- is related to cardiovascular risk (Yubero-Serrano *et al.*, 2018). The HDL cholesterol plays an important role in decreasing the process of atherosclerosis. In particular, it is responsible for removing the TC, inhibiting the oxidation of LDL as well as for reducing the inflammatory response of atherosclerosis (Rader *et al.*, 2009). When EVOO is used to replace saturated fats, it can reduce the concentration of TC and LDL as well as the ratio of TC/HDL (Yubero-Serrano *et al.*, 2018). Moreover, several studies have reported a correlation between polyphenol concentrations in EVOO and a favorable plasma lipid response, after chronic substitution of saturated fats with MUFA (EVOO) (Lockyer *et al.*, 2017).

The triglycerides are involved in the increase of the risk of cardiovascular events independently of the cholesterol content (Langsted *et al.*, 2011). A substance that may lead to a low postprandial hypertriglyceridemia is omega-3. The amounts of omega-3 needed for this purpose though cannot be easily consumed through a regular meal (Sanders *et al.*, 2000). A more effective way to regulate the levels of triglycerides, is to follow the MUFA and phenolic rich Mediterranean diet. These traits of the Mediterranean diet (high levels of MUFA and phenolic compounds) derive from EVOO which leads to an early and high peak of the triglyceride level, followed by a fast lipid clearance. On the other hand, SFA-rich meals produce a smaller increase in triglycerides in comparison to the balanced meals of this diet (Sanders *et al.*, 2000). Buchholz and Melzig (2015) investigated potential mechanisms with anti-hyperlipidaemic effects caused by EVOO phenolics. These potential mechanisms probably inhibit pancreatic lipases in the small intestine, thus causing the delay of postprandial lipaemia (Buchholz and Melzig, 2015).

According to Yubero-Serrano *et al.* (2018), patients with a high risk of developing cardiovascular diseases demonstrate a strongly pro-thrombotic environment. Specifically, the primary and secondary mechanisms of haemostasis as well as the mechanism of fibrinolysis are involved in such events. The primary haemostasis mechanism is the platelets activation, while the second one that of their coagulation. Many researchers agree that all these mechanisms can be regulated by EVOO (Yubero-Serrano *et al.*, 2018). In particular, a high adherence to the Mediterranean diet from patients with atrial fibrillation, resulted in the decrease of the urinary excretion of the pro-thrombotic molecule 11-dehydrothromboxane A2 (TXA2). TXA2 is involved in the progression of cardiovascular disease because of its effects on platelet aggregation, vasoconstriction and cellular proliferation. However, the effects of MUFA-rich diets and others rich in PUFA on platelet activation were controversial (Pignatelli *et al.*, 2015). In the studies in which EVOO was used as the main source of MUFA, its consumption was associated with reduced platelet aggregation (Karantonis *et al.*, 2002).

Factor VII (FVII), a coagulation protease, is responsible for a number of proteolytic events resulting in thrombin generation, fibrin deposition and platelet activation (Yubero-Serrano *et al.*, 2018). An EVOO-rich diet that lasts for 3 weeks can reduce the non-fasting levels of FVIIa in comparison to a diet that is rich in sunflower oil (Larsen *et al.*, 1999). Other studies, reported an increase of FVII's concentration and the presence of a pro-coagulant environment when a diet rich in SFA was followed, in comparison to a MUFA diet which is rich in EVOO. During the fasting state, FVIIc is correlated with the amount of fat in the diet, plasma TC and triglycerides (Kelly *et al.*, 2001). Although FVII is dependent upon the amount of fat in the diet, there is evidence correlating the effect of the type of fat consumed with its dietary variations (Sanders *et al.*, 2003). The quantity and quality of fatty acid in a diet modulate the expression of TF (Tissue Factor) – a transmembrane glycoprotein that activates FVII by adhering to it- in monocytes as a response to lipopolysaccharide thus confirming the evidence of Sanders *et al.* (2003). Moreover, SFA-rich diets cause an increase in the levels of TF while its expression is inhibited by PUFA. On the other hand, MUFA-rich diets (based on EVOO) decrease

the activity of TF in circulating monocytes (Bravo-Herrera *et al.*, 2004). Specifically, replacing the calories from a palm-oil enriched diet or a low-fat diet by an EVOO-rich diet decreases the levels of TFPI (Tissue Factor Pathway Inhibitor), a natural inhibitor of TF (Yubero-Serrano *et al.*, 2018).

Contradicting data have been presented from Poppitt *et al.* (2004) who did not detect any alterations in the levels of fibrinogen (a final factor in the thrombus formation) analogous to the different types of fatty acids consumed (MUFA, PUFA and SFA) or the different sources of fat (rapeseed, sunflower and butter). The results were inconclusive because of the differentiated levels of fat that were administered in each study (Yubero-Serrano *et al.*, 2018). The stabilization and progress of thrombus in fibrinolysis is controlled by PAI-1 (Plasminogen Activator Inhibitor -1); which is known to be reduced after a meal rich in MUFA from EVOO or n3 from vegetable origin (Delgado-Lista *et al.*, 2008).

Obesity and chronic metabolic diseases

Obesity and the metabolic syndrome are inversely associated with the adherence to the Mediterranean diet (Lutsey *et al.*, 2008; Capurso *et al.*, 2018). The high composition of this diet in fruits, vegetables, grains, fish and low-fat products is the main reason for its beneficial effects on this type of patients. These patients are benefited by EVOO that inhibits the redistribution of body fat and decreases the disturbances of the postprandial lipid levels. These data have led scientists to believe that the Mediterranean diet could be used as a therapeutic and healthy dietary model for such patients (Lutsey *et al.*, 2008; Yubero-Serrano *et al.*, 2018). According to Elhayany *et al.* (2010), replacing a SFA-rich diet or a low-fat diet with a diet rich in EVOO in patients with type 2 diabetes mellitus, leads to the reduction of glycated hemoglobin (0.3-2%). This decrease achieved by a substitution in the dietary patterns of these patients has almost the same efficacy of some antidiabetic drugs administered to them (Elhayany *et al.*, 2010; Yubero-Serrano *et al.*, 2018). Esposito *et al.* (2004), demonstrated that adhering to the Mediterranean diet enhances the control of diabetes independently of the patient's weight. This beneficial effect of the Mediterranean diet is believed to derive from the increase of the circulating adiponectin levels which improve the mediated insulin sensitivity. This type of dietary modifications can effectively be used for treating insulin resistance as well as cardiovascular-renal consequences of the metabolic syndrome and/or obesity. Finally, Hodge *et al.* (2011) determined that patients with type-2 diabetes are benefited by the Mediterranean diet. Adhering to this diet reduces the incidences of cardiovascular diseases (CVD) as well as the CVD mortality in these patients (Hodge *et al.*, 2011).

Neurodegenerative diseases

The Mediterranean diet has been reported to reduce the risk of neurodegenerative diseases. During randomized controlled trials (RCTs), in a population of over 12,800,000 subjects a great adherence was noted between the Mediterranean diet and a reduced risk of overall mortality, cardiovascular disease, coronary heart disease, myocardial infarction, overall cancer incidence, diabetes and neurodegenerative diseases (Capurso *et al.*, 2018; Yubero-Serrano *et al.*, 2018). A higher adherence to the Mediterranean diet has been found to be associated with low levels of C-reactive interleukin (Fung *et al.*, 2005). Therefore, a possible underlying mechanism for the neuroprotective effects of the Mediterranean diet could be its vascular protective properties and its ability to reduce inflammation and oxidative stress. Inflammation and oxidative stress, are both associated with the pathophysiology of many degenerative diseases (Frisardi *et al.*, 2010). Furthermore, adhering to the Mediterranean diet leads to the decreased risk of another pre-dementia syndrome, MCI (Mild Cognitive Impairment) (Capurso *et al.*, 2018).

The MCI, also known as age related cognitive decline (ARCD), age associated cognitive decline (AACD), or age associated memory impairment (AAMI), is an intermediate stage between the expected cognitive decline of normal aging and the more serious decline of dementia (Solfrizzi *et al.*, 2011). This syndrome is characterized by the objective decline in cognitive functioning associated with the aging process

but within normal limits given the person's age. The cognitive areas include memory and learning, attention and concentration, thinking, language and visuospatial functioning (Singh *et al.*, 2014). The exact causes of mild cognitive decline are unknown; however cardiovascular and other chronic diseases (hypertension, diabetes mellitus, depression and low levels of physical activity) have been identified as risk factors for MCI (Blazer *et al.*, 1991). According to White *et al.* (1997) some of factors that have been associated with a protective effect against MCI are a flexible personality in the middle age, a high socioeconomic status as well as the maintenance of vision and hearing.

Elder people often exhibit deficiency in various micronutrients such as vitamins B1, B2, B6, B12, C and folate. These deficiencies have been associated with cognitive impairment (Capurso *et al.*, 2018). Solfrizzi *et al.* (2003) examined the correlation between dietary macronutrient intakes and age-related changes in cognitive functions in Southern Italy within a sample of 5,632 subjects aged between 65 and 84 years that were either free living or institutionalized. The results demonstrated an inverse relationship between the energy intake from MUFA and the cognitive decline. In particular, the risk of having a compromised cognitive function was very high in the lowest percentile of daily MUFA intake (<800kJ per day) but it decreased exponentially with the increase of daily MUFA intake (Solfrizzi *et al.*, 2003; Capurso *et al.*, 2018).

This protective effect of MUFA could be related to the role of fatty acids in maintaining the structural integrity of neuronal membranes. In fact, dietary fatty acids can modify neuronal membrane fluidity. PUFAs in particular regulate the fluidity of synaptosomal membranes and thereby regulate neuronal transmission. Moreover, essential fatty acids have the ability to modify the function of the neurotransmitters' receptors such as cholinergic receptors, nicotinic receptors etc. (Capurso *et al.*, 2018). Essential fatty acids interfere with the function of membrane proteins as they can affect the calcium, chloride and potassium ion channels (Yehuda *et al.*, 1999). In particular, unsaturated fatty acids have demonstrated the ability to influence cell proliferation (e.g. in hematopoietic cells) (Capurso *et al.*, 2018).

The protective effect of high adherence to the Mediterranean diet towards MCI incidences was confirmed by Scarmeas *et al.* (2009) in a study that was conducted in a multiethnic community in New York. From 1,393 cognitively normal participants, 275 developed MCI during a mean follow-up of 4.5 years. Subjects of the middle Mediterranean diet adherence tertile when compared to subjects of the lowest tertile, had a no significant (17%) less risk of developing MCI; while those in the highest tertile had a significant (28%) less risk of developing MCI (Scarmeas *et al.*, 2009; Capurso *et al.*, 2018). Singh *et al.* (2014) conducted a meta-analysis of six studies that had been conducted in the United States, Australia and France with more than 3,000 participants. This meta-analysis evaluated the association between Mediterranean diet and cognitive impairment; yet again the higher the adherence to Mediterranean diet the lower the levels MCI risk and incidences (Singh *et al.*, 2014; Capurso *et al.*, 2018).

Solfrizzi *et al.* (2004) correlated the vascular risk factors with the prevalence, incidence and rate of progression of MCI to dementia (Capurso *et al.*, 2018). Approximately 3,000 individuals, aged between 65 and 84 from Italy (ILSA), were evaluated for this purpose, while a 3.5-year follow-up was also conducted. At the baseline survey, 139 MCI patients were diagnosed and 113 new MCI incidences were added to that number during the following 3.5 years (Solfrizzi *et al.*, 2004; Capurso *et al.*, 2018). The rate of MCI events was calculated as 21.5 per 1,000 persons-year, while its progression rate to dementia was 3.8 per 100 persons-year. Moreover, the progression rate for AD, vascular dementia (VaD) and other types of dementia were calculated at 2.43, 1.3 and 0.3 per 100 persons-year, respectively (Solfrizzi *et al.*, 2004). Amongst the subjects whose MCI incidence progressed to dementia, 60% progressed to AD and 33% to VaD (Solfrizzi *et al.*, 2004; Capurso *et al.*, 2018).

Maioli *et al.* (2007) detected 15 cases of dementia (28%), from 52 elderly -with a mean age of 73 years- that were evaluated for subtypes of MCI (amnesic MCI, non-amnesic MCI and memory and other cognitive domains of MCI) and their progression to dementia. The progression to AD accounted for 53% of all recorded cases. Moreover, a 33.4% of the cases progresses to AD with cerebrovascular disease and finally a 13.3% of all cases developed frontotemporal dementia. During the follow-up period (approximately 2 years), 53.8% of the

participants remained stable and 17.3% reverted to normal. The converters were generally older, demonstrated a higher prevalence of atrophy at neuroimaging, high serum of HDL levels and lower serum folate levels (Maioli *et al.*, 2007; Capurso *et al.*, 2018).

The conversion of MCI to AD type dementia was investigated by Rozzini *et al.* (2007). For this purpose, 119 subjects diagnosed with amnesic MCI went through a multidimensional assessment and a neuropsychological battery assessment, also repeated 1 year later. The demented MCI subjects (33.6%) were older than stable MCI subjects. Moreover, the WML (White Matter Lesions) on CT scan or MRI were easier detectable in the demented group of subjects. The data from the follow up (a year later) demonstrated a deterioration in the demented patients especially on PFL (phonemic verbal fluency) (Rozzini *et al.*, 2007). However, the conversion from MCI to AD was not accompanied by important levels of memory decline (Capurso *et al.*, 2018). According to Rozzini *et al.* (2007), patients suffering from amnesic MCI have poor global cognitive performance and their levels of executive functioning are decreased. Their levels of memory functioning are not decreased, thus supporting their independent association with the conversion to AD type dementia (Capurso *et al.*, 2018).

Singh *et al.* (2014), collected the data from 5 studies for a total of more than 7,500 individuals and conducted a meta-analysis. Although the number of studies was considered small, the data collected were enough to present accurate results regarding the evaluation of the effects of the Mediterranean diet concerning the progression of MCI to AD, as well as the risk of developing either one. The results showed that the greater the adherence was towards the Mediterranean diet, the lower the risk of developing either MCI or AD was. They also demonstrated that a single point of increase in the Mediterranean diet score -in cognitively normal individuals- leads to an 8% risk reduction of them developing AD. Specifically, individuals of the middle tertile (according to their Mediterranean diet scores) had a 13% reduced risk of developing AD, while subjects of the higher tertile had a 36% risk reduction in comparison to the lower tertile (Singh *et al.*, 2014; Capurso *et al.*, 2018).

The importance of environmental factors over genetics was investigated in elderly African-Americans and Japanese residing in the United States as well as their homelands. The findings demonstrated a much higher prevalence of AD in the US residents in comparison to those residing in their ethnic homelands. It is apparent that the prevalence of AD is more influenced by diet and nutrition, the environment as well as the lifestyle of the individual rather than genetics. Other studies indicated that cerebral infarcts as well as strokes are responsible for the prevalence of AD. They also confirmed that the diet is a key factor in the development of AD. According to data collected from 11 countries, from 18 community-wide studies for a population with an average age over 65; concluded that fat as well as the total calories consumed are associated with the AD prevalence rates (Capurso *et al.*, 2018).

There are in particular some diet components that have been reported to increase and some to decrease the prevalence of AD. Fat and high total calories tend to increase the prevalence of AD, while in European and North American countries fish consumption has been found to decrease it. Apart from fish, other dietary components and supplements that delay the development of AD are cereals, red wine, possibly green tea and other naturally-rich in polyphenols foods, EVOO and other phenol-rich foods, antioxidants and nonsteroidal anti-inflammatory drugs.

The second most common neurodegenerative disease after AD, which affects approximately 1% of individuals over 60 in North America and Europe, is Parkinson's disease (PD). PD is characterized by the progressive loss of specific neuronal subpopulations leading in the end to movement disorders. The progression of PD depends upon impaired protein degradation and their subsequent aggregation within neuronal cells, as well as impaired mitochondrial function which leads to energy depletion due to oxidative stress and finally to cell death.

An increasing number of scientists support the correlation between high consumption of dairy products and the increased risk of PD. This association of dietary patterns and the development of PD, was examined within a population consisting of 49,692 men and 81,676 women free of PD and during the 16-year follow up

examination, 508 new PD cases were reported. Within this population, two different types of dietary patterns were identified, the “prudent” and the Western. The “prudent” one was characterized by high intakes of fruit, vegetables and fish (quite similar to the Mediterranean diet) and was inversely associated with PD risk, while the Western pattern was not (Capurso *et al.*, 2018).

The research regarding the dietary patterns of individuals in association with the development of PD became more specific. The adherence to the Mediterranean diet was a major topic for investigation thus leading to the data collection from 257 PD patients and 198 PD-free individuals. The dietary habits of the participants were collected over the course of one year. Following that one year, the diet score was obtained by applying the method described by Trichopoulou *et al.* (2003). According to the results, the higher the adherence to the Mediterranean diet, the lower the risk of PD, and vice versa. Moreover, low scores of adherences to the Mediterranean diet were correlated to the development of PD at younger ages.

Aging

Aging is a multifactorial process that is accompanied by cellular damage and is promoted by both genetic and environmental factors. One definition of ageing describes it as a decline of both functional capacity and stress resistance, while it is correlated with higher odds of morbidity and mortality (Argyropoulou *et al.*, 2013). Age-related genome damage as well as proteome damage are considered to be interconnected. Non-functional or damaged proteins may influence the function of crucial cellular processes, such as DNA Damage Responses (DDR), resulting in genomic instability. The genome damage affects the structure and/or the function of proteins leading to disturbances of the proteome. The proteostasis network (PN) is responsible for maintaining the proteome quality control (Trogakos *et al.*, 2013). An extensive analytical screening of more than 130 EVOOs originating from diverse areas of Greece (harvested in the period of 2014-2015) revealed different levels of oleocanthal and oleacein. The bioactivity of three TPFs (low, intermediate and high levels of both compounds) as well as pure oleocanthal and oleacein, were evaluated for their potential to activate cytoprotective PN modules in normal human foreskin BJ fibroblasts and mouse highly differentiated C5N keratinocytes, as well as at the *in vitro* experimental model of *Drosophila melanogaster*. Both compounds were found to activate ageing-promoting cytoprotective pathways while suppressing the oxidative stress in both mammalian cells and in flies (Nikou *et al.*, 2019).

Amyloid diseases and oleuropein aglycone

Systemic amyloidosis is characterized by multiorgan deposition of insoluble fibrillar polymeric material grown from misfolded proteins (Leri *et al.*, 2018). A plethora of data support that the concurrent presence of amyloid deposits leads to the manifestation of the pathological features of systemic amyloidosis ((Stefani, 2012; Stefani and Rigacci, 2014). According to Scarmeas *et al.* (2006), the Mediterranean diet which is naturally enriched in plant polyphenols such as resveratrol (contained in red wine) and oleuropein (the main polyphenol in the olive leaves, drupes and oil) and many others; is an effective means against age-related deterioration and with the ability to alleviate symptoms or delay the development of aging-associated degenerative diseases and neurological deficits. Amyloid cytotoxicity is considered to be the result from the complex interplay of biochemical and biophysical properties of both amyloid aggregates and the cell membrane they interact with (Canale *et al.*, 2017).

Oleuropein aglycone (OleA) is a metabolic product deriving from the endogenous β -glucosidases. The research of Ladiwala *et al.* (2011), on OleA demonstrated its ability to remodel and inactivate toxic amyloid oligomers. *In vivo* studies conducted in transgenic animal models of A β 42 aggregation and plaque deposition such as *C. elegans* (Diomedea *et al.*, 2013) and the TgCRND8 mouse model (Grossi *et al.*, 2013); confirmed that OleA protects against A β 42 aggregation and plaque formation in tissue. Moreover, OleA can be located at the surface of the cell membrane interacting specifically with negatively charged phospholipids of synthetic

bilayers (Galiano and Villalain, 2015). This finding confirms the interaction of OleA with cell membrane, as well as its ability to interfere with bilayer-binding properties of amyloid species and/or increase the local concentration of the polyphenol itself. The latter characteristic is important considering the low amounts of polyphenol intake of a normal diet and its limited bioavailability (Leri *et al.*, 2018).

It has been reported that amyloid aggregates of D76N β 2m (β 2-microglobulin variant) interact with the plasma membrane of the exposed cells at the level of GM1, a major lipid component (Leri *et al.*, 2016). Findings showed that OleA reduces D76N b2m cytotoxicity by favoring the appearance of non-toxic aggregates, or by reducing aggregate interaction with the plasma membrane at GM1-enriched sites. OleA can also reduce D76N b2m cytotoxicity by enhancing cell resistance against the toxic effects of the aggregates (Leri *et al.*, 2018).

Leri *et al.* (2018) DLS (dynamic light scattering) analysis and negatively stained TEM (transmission electron microscopy) showed that the general size and morphology of D76N b2m species were affected by the presence of OleA (until 144h of incubation under aggregation conditions). The 144h incubation was selected due to the knowledge that up to that time the sample is populated almost exclusively by amyloid fibrils without any significant presence of precursors. TEM images taken at 24h and 96h showed the presence of short fibrils distinct from the densely packed globular clusters. Moreover, during the absence of OleA the presence of the short fibrils was evident. The length of the lag-phase was increased in the presence of OleA, thus delaying the aggregation (Habchi *et al.*, 2017).

The ANS (8-anilino-1-naphthalene-sulfonic acid) assay on D76N b2m grown in the presence of polyphenol OleA, demonstrated a significant reduction of ANS fluorescence. This indicates that the aggregates surfaces were exposed to a minor hydrophobic solvent (Leri *et al.*, 2018). Furthermore, the possibility of interaction between OleA and D76N b2m was also investigated by monitoring the modifications of tryptophan (Trp) exposure during D76N b2m aggregation in the presence or the absence of the polyphenol. The aggregation of D76N b2m resulted in a time-dependent increase in the emission intensity of 335nm (λ_{max}) (until 50h aggregation), while longer times of aggregation were accompanied by a decrease of the emission's intensity. The changes in the intensity at 335nm is indicative of an increase in solvent accessibility of Trp over time. The assemblies obtained in the presence of OleA showed a slight reduction of the intensity when the OleA concentration was increased thus indicating an intrinsic fluorescence quenching due to the presence of the polyphenol. The quenching effect along with the different surface hydrophobicity observed in the presence of OleA; indicate a polyphenol induced surface modification produced by the growing protein aggregates. No other alterations were observed through the analyses conducted (e.g. kinetic or structural differences) (Leri *et al.*, 2018).

The presence of OleA reduces the toxicity of D76N b2m aggregates even in different experimental settings such as when it is added during the process of protein aggregation, or when it is used during cell pre-treatment prior to aggregate exposure, or even when the cell treatment includes cell exposure to both the polyphenol and D76N b2m aggregates simultaneously. These data suggest that the polyphenol affects both the surface aggregates' properties and the cytotoxic effects of pre-formed aggregates. Moreover, the data indicate that the OleA-treated cells are more resistant to the aggregates toxic attack (Leri *et al.*, 2018).

The findings of the FRET (Fluorescence Resonance Energy Transfer) analysis indicate that OleA interferes with the binding of aggregates to the membrane causing modifications on the aggregates' surface properties or/and the cell membrane. This decrease in the interaction between aggregates and the cell membrane in the presence of OleA, is most likely the way the polyphenol functions in order to protect the cell (Leri *et al.*, 2018).

Conclusions

To conclude, all evidence supports that the consumption of EVOO, especially when combined with a good adherence to the Mediterranean diet, has beneficial effects in human health. The people living around the

Mediterranean basin have been using EVOO as their main source of fat in their diet for a few thousand years, without the report of any harmful effect in their health. The Mediterranean diet maximizes the effect of EVOO consumption as it enriches the diet with fibers, antioxidants and low saturated fats. EVOO is rich in MUFA, vitamin E and a plethora of minor components that have been proven to have beneficial biological effects. These beneficial effects result from their antimicrobial, antioxidant and anti-inflammatory properties. These properties result in many health benefits such as the creation of a less prothrombotic environment, the reduction of oxidative stress and inflammation, the regulation of the endothelia function etc. The phenolic compounds of EVOO have a higher degree of influence in chronic and/or hereditary diseases. In particular, consuming EVOO reduces the risk of fibrinolysis, cardiovascular diseases and platelet aggregation. EVOO's vascular protective properties as well as its ability to reduce inflammation and oxidative stress, result in a set of neuroprotective properties. Higher adherence to the Mediterranean diet and a daily intake of EVOO lead to reduced incidences of MCI and a great decline in its progression to AD or PD. This neuroprotective effect is a result of the high MUFA levels of EVOO and its high contents of polyphenols that demonstrate antioxidant and nutrigenomic effects that regulate -in cerebral level- the expression of genes and miRNAs involved in neuronal function and synaptic plasticity. Even the pungency of EVOO—which is attributed to OC- is associated with beneficial effects such as decreased risk of cancer, degenerative and cardiovascular diseases. Equally important is the polyphenol oleuropein that is effective against age-related deterioration while it improves conditions associated with neurological deficits and aging-associated degenerative diseases. One of its byproducts, OleA, is another important molecule with protective effects against protein amyloid aggregation. Its protective properties include the modification of the structural and biophysical features of the aggregates grown in its presence as well as its interaction with cell membrane and hindering amyloid binding. Finally, every content of EVOO that has been studied has demonstrated an important, health-beneficial effect that confirms that a moderate daily consumption of EVOO enhances human health.

Authors' Contributions

Conceptualization: MH and EB; Investigation: EB and CS; Supervision: MH. Visualization: MH, EB and CS; Writing original draft EB and CS; Writing-review and editing: MH, EB and CS. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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