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FINAL DEGREE PROJECT

NEUROPROTECTIVE EFFECT OF OLIVE OIL POLYPHENOLS IN ALZHEIMER'S DISEASE

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Acronyms

A β : β -amyloid peptide

AChE: Acetylcholinesterase

AD: Alzheimer's disease

APOE: Apolipoprotein E

APP: Amyloid precursor protein

BACE-1: β -secretase 1

BBB: Blood brain barrier

COX-1 and COX-2: Cyclooxygenase-1 and 2

EVOO: Extra virgin olive oil

HDL: High-density lipoproteins

HT: Hydroxytyrosol

LDL: Low-density lipoprotein

MCI: Mild cognitive impairment

MD: Mediterranean diet

mtDNA: mitochondrial DNA

MUFA: Monounsaturated fatty acids

NFTs: Neurofibrillary tangles

NMDAr: N-methyl-D-aspartate receptor

NRS: Nitrogen reactive species

OLE: Oleuropein

PUFA: Polyunsaturated fatty acids

ROS: Reactive oxygen species

TYR: Tyrosol

VOO: Virgin olive oil

1. ABSTRACT

The number of people suffering from age-related neurodegeneration has been estimated to quadruplicate by the year 2050; being Alzheimer's disease (AD) the most common dementia in the western world in elderly people. AD has a great impact not only in patients and in their families, but also for the society due to the enormous health-care costs. First diagnosed in 1907 by Alois Alzheimer, AD is characterized by the loss of short-term memory, the negation of this loss, language difficulties and behavioral changes; although these changes can be sometimes difficult to distinguish from the normal forgetfulness in aging. The etiology is heterogeneous, with both genetic and environmental factors. In the pathogenesis of AD neuropathological changes (abnormal accumulation of harmful proteins) and in neurotransmitters (producing synaptic failure), oxidative stress, mitochondrial dysfunction and inflammation are present. Up to now, neither biomarkers for an early diagnosis nor effective treatments are available; therefore, prevention is the best strategy to delay the onset of the disease. In the last years, accumulating evidence sustains that diet can be an important factor in maintaining brain health. In fact, the Mediterranean diet is associated with a reduced risk of age-related cognitive impairment, and olive oil, a staple food of this dietary pattern, could have a pivotal role in the prevention of AD. In this sense, the phenolic content present in olive oil has been proposed as the main responsible for the preventive effects of this food, since many *in vitro* and *in vivo* studies have indicated that they act in multiple steps of the pathogenesis of AD. The present work reviews the data on the bibliographic supporting this hypothesis.

RESUM

El nombre de persones que pateixen neurodegeneracions relacionades amb l'edat s'estima que es quadruplicarà l'any 2050; sent, la Malaltia de l'Alzheimer (MA) la forma més comuna de demència al món occidental en persones d'edat avançada. La MA té un gran impacte no només en els pacients i en les seves famílies, sinó també per a la societat a causa dels enormes costos d'atenció sanitària. Diagnosticada per primera vegada al 1907 per Alois Alzheimer, la MA es caracteritza per la pèrdua de memòria a curt termini, la negació d'aquesta pèrdua, les dificultats de llenguatge i canvis de comportament; tot i que aquests canvis poden ser de vegades difícils de distingir de la pèrdua habitual de memòria de la gent gran. L'etiologia és heterogènia, amb factors genètics i ambientals. En la patogènesi de la MA s'hi ha descrit canvis neuropatològics (acumulació anormal de proteïnes perjudicials) i en els neurotransmissors (produint fracàs sinàptic), estrès oxidatiu, disfunció mitocondrial i inflamació. Fins ara, no s'han descrit marcadors de diagnòstic precoç ni cap tractament eficaç; per això, la prevenció és la millor estratègia per a retardar l'inici de la malaltia. L'evidència acumulada dels darrers anys sosté que la dieta pot ser un factor important en el manteniment de la salut del cervell. De fet, la dieta mediterrània s'associa amb un menor risc de deteriorament cognitiu relacionat amb l'edat, i l'oli d'oliva, un aliment bàsic d'aquest model d'alimentació, podria tenir un paper fonamental en la prevenció de la MA. En aquest sentit, el contingut fenòlic present a l'oli d'oliva s'ha proposat com el principal responsable dels efectes preventius de l'oli d'oliva, ja que molts estudis *in vitro* i *in vivo* han indicat que actuen en múltiples etapes de la patogènesi de la MA. El present treball revisa les dades a la bibliografia que donen suport a aquesta hipòtesi.

2. INTRODUCTION

With the increase in life expectancy of human society, there is a greater incidence of age-associated diseases, including Alzheimer's disease (AD). Therefore, very strong efforts are being made to investigate the exact alterations that take place in this pathology with the purpose to find reliable markers for an early diagnosis and effective therapies or, at least, treatments suitable to delay the manifestations of clinical signs (1). Up to now, prevention is still the best strategy to combat AD (2); an increasing interest for alternative options in the prevention and treatment based in plants aiming to combine efficacy with safety is rising up (3).

AD was first described in a 55 year old woman in Germany in 1907 by professor Alois Alzheimer, psychiatrist and neuropathologist. At first, the illness was thought to be a relative strange way of presenile dementia because the patient suffered from psychiatric symptoms with cognitive impairment, but at necropsy there were found plaques, neurofibrillary tangles (NFTs) and arteriosclerotic changes which nowadays are clear signs of AD (4).

AD has an insidious onset, sometimes difficult to distinguish from the usual forgetfulness that takes place in aging. When the first clinical manifestations appear, the pathogenesis of the disease is already in an advanced stage. AD originates from the abnormal accumulation of harmful proteins in the nervous system. These consist of β -amyloid ($A\beta$) peptide, the lipid-carrier protein apolipoprotein E (apoE), the microtubule associated protein tau and the presynaptic protein α -synuclein (which is also involved in Parkinson's disease). $A\beta$ peptide, apoE, tau and α -synuclein interact with a lot of molecules that modulate diverse signaling cascades regulating neuronal activity and survival (5). These accumulations of harmful proteins in the nervous system produce failures in the neural-network activity and impair synapses between neurons that create and keep microcircuits supporting learning and memory. This eventually causes atrophy and death of neurons in a process that can include excitotoxicity (overstimulation of neurotransmitter receptors on neuronal surface membranes), collapse of calcium homeostasis producing synaptic failure, inflammation, mitochondrial dysfunction and oxidative damage (5). Microscopically, the protein abnormalities cause cerebral plaques laden with $A\beta$ peptide, dystrophic neurites in the neocortical terminal fields and prominent NFTs in the medial temporal lobe (6).

From a clinic point of view, AD usually appears with a subtle lack of memory that can be associated with normal aging followed by a slow but progressive dementia that lasts several years. Anatomopathologically, there is a diffuse and manifest atrophy of the cerebral cortex, with secondary enlargement of the ventricular system (6).

Although the etiology of AD is not very clear, multiple genetic and environmental factors had been identified, such as family background, advanced age, gender, diabetes and exposure to toxicants among others. As some modifiable lifestyle-related factors are associated with cognitive decline, diet has become an object of intense research in relation to cognitive aging and neurodegenerative diseases. It has been seen that a higher adherence to the Mediterranean Diet (MD) is not only associated with a lower prevalence and incidence of chronic diseases, as cardiovascular disease, cancer, metabolic syndrome and diabetes, but also is associated with a reduced cognitive

decline (7). As olive oil is an ingredient never absent in this diet, the beneficial effects towards the neuroprotective effect were associated during a lot of time to its major component: the unsaturated lipids, particularly oleic acid. However, now the antioxidant phenolic content in olive oil is thought to be the main component responsible for the reduced risk of age-related cognitive impairment (8).

Thanks to the continuing efforts of researchers, more and more natural compounds are being identified that can hit multiple targets implicated in AD thanks to the growing awareness of the pathological processes that take place in the disease. In fact, clinical and epidemiological studies have shown that nutritional components with antioxidant properties, such as polyphenols in olive oil, exert protective effects against neurodegenerative disorders (9).

3. OBJECTIVES

Alzheimer's disease has a great repercussion and will continue to have it in the next years as its incidence and prevalence will continue to grow. Moreover, it has an insidious onset and when clinical signs emerge the pathogenesis is already in an advanced stage; at the present, early diagnostic biomarkers and effective therapeutics are missing. For these reasons, the present review will aim to answer the following objectives:

- i. Describe Alzheimer's disease epidemiology, definition, differences with normal aging, clinical manifestations and etiology.
- ii. Analyze the pathogenesis, diagnostic and treatments for Alzheimer's disease.
- iii. Study the possibility of non-pharmacological approaches to combat Alzheimer's disease.
- iv. Assess the potential effect of the Mediterranean diet in the neuroprotection of Alzheimer's disease.
- v. Describe the composition of olive oil and study the major polyphenols present in it.
- vi. Establish the relation between Alzheimer's Disease pathogenesis and where do polyphenols act in this process.
- vii. Review the most important epidemiological studies in humans with olive oil phenols.
- viii. Draw conclusions from the studied objectives.

3.1. INTEGRATION OF THE FIELDS OF STUDY

This study has been developed in a way that integrates three different fields of study of the Pharmacy degree: physiology and physiopathology, as the main scope and, pharmacology and therapeutics and nutrition and bromatology as secondary disciplines.

The principal field of study is physiology and physiopathology as this work describes all the aspects related to the disease. Definition, first ever diagnosed case, multiple risk factors for developing the disease, clinical manifestations in the different stages, differences between "normal cognitive aging" and "memory and cognition disorders" and the pathologic mechanisms that occur during Alzheimer's disease in the brain are developed in this work.

Pharmacology and therapeutics has been integrated in the development of this dissertation because even though Alzheimer's disease does not have an effective treatment, there are some drugs capable to slow down its progress. Groups of treatment, mechanisms, pharmacokinetics, daily doses and adverse effects of drugs used nowadays are going to be considered in the present work.

Last but not least, nutrition and bromatology is also an important field of study in this work. With the support of the actual evidence, this work has considered the Mediterranean Diet as an approach to prevent Alzheimer's disease development. Olive oil composition is described and olive oil polyphenols beneficial properties in the pathogenesis of the disease are reviewed.

4. MATERIAL AND METHODS

To achieve the proposed objectives an extensive literature research has been conducted through primary and secondary bibliographical sources present in the *PubMed* and *Web of Science* databases and in books of pathological anatomy (10), of internal medicine (6,11) and drug catalogues (12).

A first general search was done through *PubMed* to focus in which neurodegenerative disease this study would be centered. The diseases considered were Alzheimer's disease, Parkinson's disease and Huntington's disease. According to the results of this first search, Alzheimer's disease is the most studied in relation to the neuroprotective effect of olive oil polyphenols; for this reason, it was the disease chosen to develop the present study.

Books have been used to understand the basis of Alzheimer's disease physiopathology, differences with normal aging, clinical manifestations in every stage and current diagnosis. The official webpage of Alzheimer's association (13) and other validated ones were also consulted.

After a first and general idea of the disease, journal articles obtained from *PubMed* and *Web of Science* were used to develop the study up to May 2017. The information obtained was used to complement the basis of Alzheimer's disease and to study the Mediterranean Diet and its one ingredient never absent: olive oil. Olive oil composition, its polyphenols and the steps of the pathogenesis where these compounds act have been treated in the present review.

The exact search dates were from 1997 to 2017 with no restriction of language using the terms in the title/abstract of: "hydroxytyrosol" OR "tyrosol" OR "oleuropein" OR "oleocanthal" OR "olive oil polyphenols" OR "olive oil" AND "Alzheimer's Disease" OR "neurodegenerative disease" OR "aging". The search done looked for experimental and human studies evaluating the effect of olive oil polyphenols in neurodegenerative diseases, especially Alzheimer's disease with the idea of providing evidence for health claims.

5. RESULTS AND DISCUSSION

5.1. ALZHEIMER'S DISEASE EPIDEMIOLOGY

Dementias are a progressive deterioration in several cognitive domains that are severe enough to interfere with daily functions (7). Alzheimer's disease (AD) is the most common way of dementia in the Western societies in elderly people, as its incidence increases with age (mostly through the 7th and 8th decades of life). The United Nations estimates that the number of people suffering from age-related neurodegeneration is as high as 25 million and is predicted to be quadrupled by the year 2050 (14). Approximately a 70% of these cases of neurodegeneration are attributed to AD. The highest prevalence (all the subjects suffering from the disease, independently of the contraction date) and incidence (number of new cases in a specific period of time) rates are in decreasing order for the populations from North America, Western Europe, Latin America, China, Western-Pacific regions and Africa (14,15).

Vascular dementia is the second most common cause of dementia in old people; it is a loss of cognitive function because of ischemic, hypoperfusive or hemorrhagic brain lesions due to cerebrovascular disease or cardiovascular pathology. The combination of AD and vascular dementia is called mixed dementia (7).

It must be taken into consideration that this disease does not only affect patients and their families, but also has a high repercussion on society, as the monthly overall mean cost of AD dementia in Spain is of 1,412.73 euros per patient every month (16) and only in the United States causes an estimated health-care cost of \$ 172 billion per year (14). In fact, AD is the third most costly disease (17).

5.2. DIFFERENCES BETWEEN “NORMAL COGNITIVE AGING” AND “MEMORY AND COGNITION DISORDERS”

Before starting with the description of the development of the disease, a concept that is important to clarify is the fact that people suffer through life a normal and slow but progressive deterioration of memory that is not considered dementia. For this reason, is important to distinguish between "normal cognitive aging" and "disorders of memory and cognition" (11).

Differentiate normal age-related changes with signs of AD can be difficult sometimes for two main reasons: first of all, because changes may be subtle sometimes and secondly, because as everyone is different so are the symptoms they may experience (18).

In normal cognitive aging, short-term memory is usually well preserved; in contrast, there are impairments related to long-term memory. Memory problems reflect a decrease in the efficiency of both the information being processed and the information being retrieved, with a bigger deterioration in the processes of recovering memory than in the coding ones (11).

Even though memory loss is a common symptom in all types of dementia, memory loss does not necessarily mean dementia. Dementia is diagnosed when two or more brain functions - such as memory and language skills – are deteriorated significantly without the loss of consciousness. There are some disorders that can cause dementia-like

symptoms without having dementia, such as: depression, drugs with anticholinergic activity, endocrine abnormalities (as hypothyroidism), nutritional deficiencies, brain tumors, anoxia or hypoxia (when the oxygen supply of the brain is reduced or cut entirely), decreased vision and hearing and heart and lung problems (11).

On the other hand, non-normative cognitive decline is caused by any disorder that permanently damages large areas of association in brain hemispheres or subcortical areas that affect memory and learning (11). According to the National Institute of Neurological Disorders and Stroke, dementia is not a specific disease: it is a descriptive term that includes a collection of symptoms that are caused by a series of disorders that affect the brain. People with dementia have a significant impairment in intellectual functioning that interferes with normal activities and relationships; they lose their ability to solve problems and maintain emotional control and may suffer personality changes and behavioral problems (as agitation, delusions and hallucinations). Therefore, although dementia is common in old people, it is not a normal part of the process of aging (19).

Another difference between these concepts is that even though in both normal brain aging and in AD dementia neuritic plaques and NFTs are present, in normal aging they accumulate in small amounts whereas in AD they do so in excess (6).

5.3. ALZHEIMER'S DISEASE CLINICAL MANIFESTATIONS

AD follows an insidious and progressive course with an average of survival from 8 to 10 years after the diagnosis. It is characterized by the loss of short-term memory and the negation of this loss, language difficulty and behavioral changes (such as loss of sense of humor and social isolation) (6,11).

AD develops in three general stages: early, middle and late (sometimes referred as mild, moderate and severe). However, not every person progresses through the same stages in the exact way (20).

The initial changes are subtle; sometimes difficult to distinguish from the habitual forgetfulness of adults, but in AD these cognitive problems interfere with the daily routine. There is loss of short termed memory (such as forgetting recent conversations or names) and slight personality changes (like apathy) (6,11,18,20).

As the disorder progresses, the disease gets to the moderate stage. It is typically the longest stage, as it last for several years, and it is characterized by a more general deterioration of the cognitive functioning; changes in the superior cortical functioning (that affect the resolution of problems and the spatial relations), extreme confusion, disorientation and lack of capacity to make daily activities. Depression also may appear in patients aware of their deficits (6,11,20).

In the last stage patients are unable to react to the environment: they have difficulties in speaking, swallowing and walking (18). They also may suffer hallucinations and delirium. Uninhibited and aggressive behavior is alternated with passivity and social isolation. They require total cares and spend most part of time in bed, as they turn rigid, mute and incontinent. Death finally occurs usually due to malnutrition, secondary infections as pneumonia or heart disease (6,11).

The reason why the initial symptoms are only related to memory and as the disease progresses, they expand to many others is due to the fact that the first neurons damaged are usually in brain regions involved in creating new memories; yet, as neurons in other parts of the brain are destroyed, individuals experience other complications (18).

5.4. ALZHEIMER'S DISEASE ETIOLOGY

AD is caused by multiple factors, both genetic and environmental; it is likely that AD predisposing genes interact with other disease genes and environmental factors leading to the development of the illness (5).

5.4.1. Family background

Even though AD can be inherited only a small number of patients (probably less than 1%) have early-onset AD (when signs and symptoms develop before 65 years old, it has a more rapid rate of progression). This is because they need to have inherited autosomal dominant mutations in genes whose protein products — amyloid precursor protein (APP), presenilin 1 or presenilin 2 — are involved in the production of A β peptides (5,14). In contrast, mutations on tau proteins do not occur in AD, but are present in other types of dementia (10).

The most powerful genetic risk factor for AD is the ApoE ϵ 4 allele, which encodes the ApoE4 lipid carrier that reduces the age of appearance of the illness by 6 to 7 years. However, the more common ApoE ϵ 3 and the rare ApoE ϵ 2 forms of ApoE are relatively protective against AD. The inheritance of the ApoE ϵ 4 allele is also associated with a lower cognitive performance and mild cognitive impairment (MCI, an intermediate stage of cognitive deterioration in which functional independence is preserved but there is impairment in one or more cognitive areas; it is a good label for people that are at high risk of developing AD). However, the presence of this allele is neither necessary nor sufficient for developing the disease (5,14).

5.4.2. Advanced age

Ageing is the most important risk factor for AD. Ageing itself affects the brain; it produces a decline in sensory and motor abilities and in higher cognitive functions (7). AD incidence increases with age and the prevalence doubles approximately every 5 years: from 1% in the population from 60 to 64 years old up to a 40% or more in the population between 85 to 89 years old (10), as it can be seen in Figure 1 that shows AD distribution in the population of the United States. Even aggressive autosomal dominant AD mutations do not lead to obvious deficits until the fourth or fifth decade of life (5).

Numerous mechanisms may protect the young brain against AD, as higher levels of growth factors, better energy metabolism and more efficient mechanisms for clearing misfolded proteins and repairing cells. The failure of these protective mechanisms can contribute to the development of AD. With ageing also increases the prevalence of obesity, diabetes and atherosclerosis; illnesses that promote AD through metabolic or vascular mechanisms. The inflammatory activity of immune cells, in special macrophages and microglia, and of astrocytes increases with ageing and contrary as it may seem can promote ageing-related disorders, as it will be seen (5).

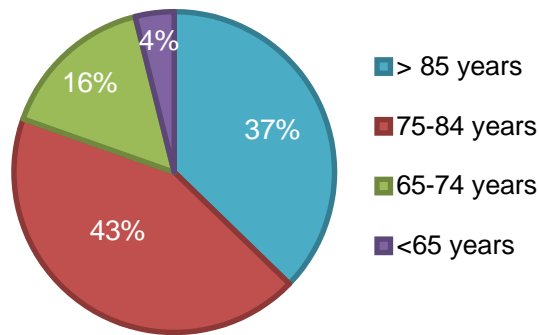


Figure 1. Distribution in percentages of people with AD in the United States depending on the age of the patient. Percentages do not total 100 because of rounding. Adapted from (18).

5.4.3. Gender

The female sex may also be a risk factor in developing AD regardless of their longevity, as two thirds of the patients are women. A large study exposed that the ApoE ϵ 4 genotype, the biggest genetic risk factor for AD, may have a stronger association with AD in women than in men. It is unknown why this may be the case, but some evidence suggests that it may be due to an interaction between the ApoE ϵ 4 genotype and the sex hormone estrogen (18).

Another factor, that may influence in the gender difference could be the fact that low education is a risk factor for dementia and as in the first half of the 20th century there was lower educational attainment in women than in men this could be the reason for a higher risk of AD and other dementias in women (18).

5.4.4. Diabetes

Brain insulin resistance, an alteration in prediabetes and diabetes mellitus 2, may be linked to AD. Patients suffering from diabetes are at a higher risk of developing AD as insulin resistance, increased inflammation and impaired metabolism are pathological features for both AD and diabetes. Even though the exact mechanism through which diabetes causes AD remains unknown, it may include both cerebrovascular and noncerebrovascular mechanisms. Such relation has led to some authors to name AD as type 3 brain diabetes (2,14).

Insulin and insulin-like growth factor are thought to regulate some biological processes related to memory and learning, in hyperinsulinemia insulin resistance causes the activation of glycogen synthase kinase 3 β , a key factor in cognitive decline producing brain injury as it phosphorylates tau (2,21).

5.4.5. Toxicants

The exposition to some organophosphate pesticides and metals (for example copper, zinc, iron and manganese) can cause neurotoxicity, neuroinflammation and/or neurodegeneration. The “aluminium hypothesis” maintains a relation between aluminum exposure (through drinking water, diet and occupation) and both A β plaques and NFTs (22).

These environmental factors can initiate or worsen the disease as they increase the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) with the subsequent oxidative damage that causes AD, as it will be seen later (23).

5.4.6. Other causes

The risk of AD may be increased by a low level of education, severe head injury, Down Syndrome and vascular risk factors (as shown in Figure 2). However, up to now, it remains uncertain whether avoiding life-style risk factors can significantly lower chances of suffering the disease, especially in people with genetic risk for AD. It is likely that AD predisposing genes interact with other disease genes and environmental factors (5,14,15).

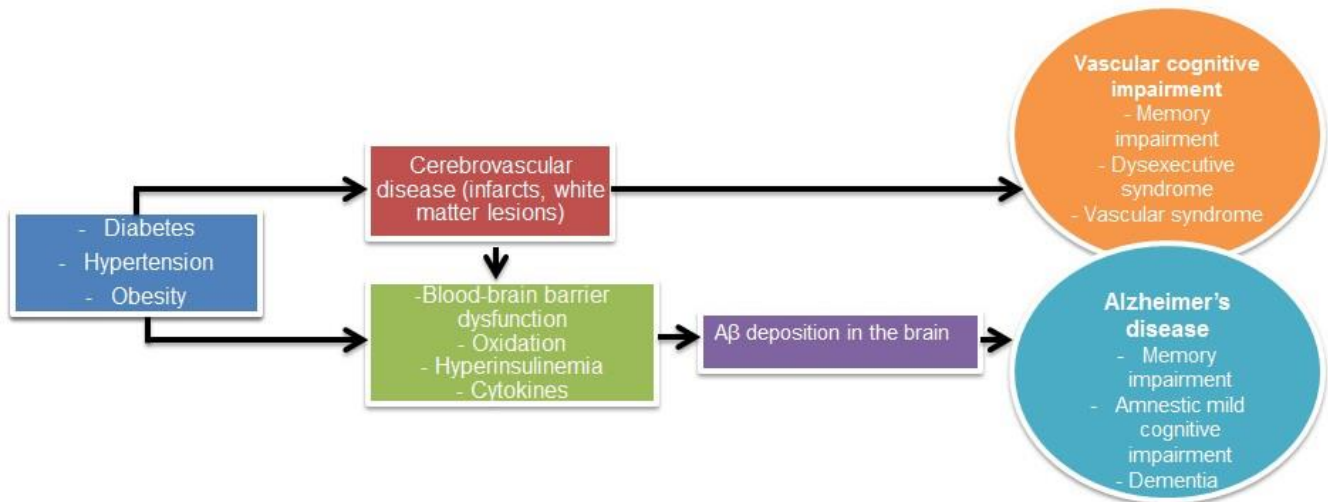


Figure 2. Potential mechanism linking vascular risk factors with vascular cognitive impairment and AD. Adapted from (14).

5.5. ALZHEIMER'S DISEASE PATHOGENESIS

Physiopathologically, AD implies changes both neuropathological and in the neurotransmitters. There is cortical atrophy, neuronal degeneration and synaptic loss, particularly in the parietal and temporal lobes (mainly in the hippocampal formation and the amygdala (15)). In case of significant atrophy ventricular growth occurs because of the loss of encephalic tissue.

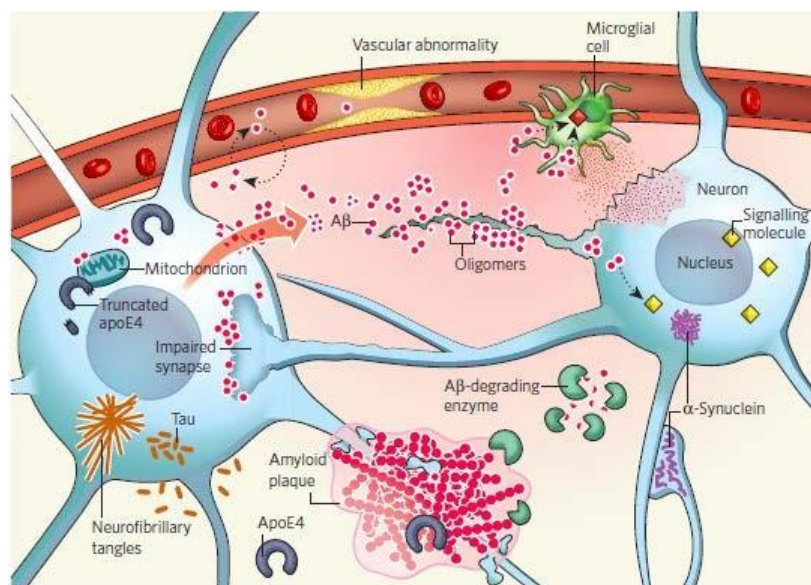


Figure 3. Key players in the pathogenesis of AD (5).

Microscopically, the main characteristics of AD are extracellular senile plaques of amyloid deposits, intracellular NFTs of hyperphosphorylated tau, ApoE accumulation and cerebral angiopathy (8,11). These changes begin before clinical symptoms emerge (18). Oxidative stress, mitochondrial dysfunction and inflammation are also present. The representation of all these elements is displayed in Figure 3.

The Positron Emission Tomography (PET) shows that the first metabolic changes occur in the parietal lobe. However, the most serious alterations take place in the hippocampus, the cerebral cortex and the basal ganglia as the disease progresses (6).

This description of the pathogenesis of AD does not pretend to be exhaustive, but aims to give an idea of the complexity of this pathology. As it will be seen there are multiple neurodegeneration-inducing factors that ultimately lead to the initiation of a common signaling cascade of neuronal cell death causing the disease (23).

5.5.1. Synaptic failure

AD could be principally a disorder of synaptic failure. In patients with MCI, hippocampal synapses begin to occur and the remaining synaptic profiles show compensatory increases in size. In mild AD, there is already a reduction of some of the presynaptic vesicles and as the disease progresses, synapses are disproportionately reduced relative to the number of neurons; this loss is the best correlation with dementia. However, in normal aging synaptic loss is also present. The dentate region of the hippocampus is particularly affected by the loss of synapses. Subsequent to this loss, some signaling molecules important to memory are inhibited (21); such as glutamate, acetylcholine and neurotrophins.

This damage is produced because at high concentrations A β oligomers may suppress basal synaptic transmission facilitating the endocytosis of the N-methyl-d-aspartate receptor (NMDAR) disrupting the release of presynaptic neurotransmitters (21). Consequently to this reduction, there is an abnormal prolonged release of glutamate that causes excitotoxicity and cell death because an increase in calcium ion influx via the NMDAR causes dysregulation of different calcium dependent processes (including learning and memory) (22). A β oligomers also bind to postsynaptic sites and cause synaptic and neuronal loss (24).

Acetylcholine is a neurotransmitter with an important role in memory and learning. The “cholinergic hypothesis” sustains that the loss of cholinergic function in the central nervous system contributes significantly to the cognitive decline present in people with AD. Acetylcholine is synthesized by choline acetyltransferase and degraded by cholinesterase (mainly acetylcholinesterase, AChE, in the brain) (22). A β peptide and tau bind to acetylcholine receptors impairing the release of acetylcholine from the presynaptic terminal (21). This reduction has a quantitative relationship with the number of neuritic plaques and the severity of the dementia (6). However, the concentrations of the enzymes responsible for the synthesis and degradation of ACh do not drop until the later stages of the disease (25).

Neurotrophins are secreted signaling proteins that promote proliferation, differentiation and survival of neurons and glia mediating learning, memory and behavior (3,21). The binding of neurotrophins to their receptors protect neurons from damage and reverse neurodegeneration. The normal high levels of neurotrophin and their receptors in cholinergic neurons in brain are greatly reduced in the late stage of AD (21).

5.5.2. Abnormal accumulation of harmful proteins

A lot of evidence suggests that neurodegenerative diseases, including AD, stem from the abnormal accumulation of harmful proteins in the nervous system. In AD, these include A β , ApoE, the microtubule associated protein tau and the presynaptic protein α -synuclein (which is also involved in Parkinson's disease). It is still a debated issue how abnormal accumulation of harmful proteins in nervous system may cause cognitive decline (5).

5.5.2.1. β -amyloid peptide

All of us make A β peptide in the brain: A β peptides are natural products of the metabolism consisting of 36 to 43 amino acids, with unknown function. Monomers of A β 40 are much more prevalent than the aggregation-prone and damaging A β 42 species. A β is highly amyloidogenic (prone to aggregation); it accumulates in senile plaques as amyloid fibrils, they originate from proteolysis of the amyloid precursor protein (APP, a big sized transmembrane protein with neurotrophic and neuroprotective properties) by the sequential enzymatic actions of beta-site APP-cleaving enzyme 1 (BACE-1), a β -secretase, and a γ -secretase, a protein complex with presenilin 1 at its catalytic core. This process also generates AICD, which is a short tail (approximately 50 amino acids) that is released into the cytoplasm and targeted to the nucleus signaling transcription activation. On the other hand, there is a non-amyloidogenic pathway in which APP cleavage is initiated by α -secretase originating a carboxy-terminal fragment that is then digested by γ -secretase. This means that amyloid plaques result from the equilibrium between A β deposition and clearance (21).

When A β concentration is increased by overproduction, defective clearance or alterations in transport of the processes that shuttle A β across the blood-brain barrier (BBB), A β self-aggregates into multiple coexisting physical forms: ranging from oligomers (2 to 6 peptides soluble) to protofibrils, fibrils and amyloid plaques (insoluble) (21). Autophagy, a lysosome-mediated catabolic pathway responsible for turnover of long-lived proteins and organelles, seems to have a key role at this point; it protects neurons against A β and tau induced cytotoxicity (26).

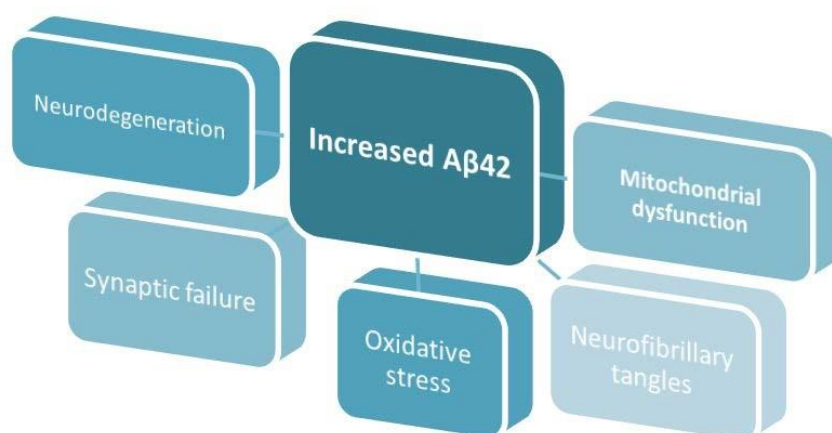


Figure 4. The amyloid cascade hypothesis. Adapted from (27).

The “amyloid cascade hypothesis” (represented in Figure 4) considers the soluble A β peptides as the primary toxic species in AD. It maintains that A β peptide depositions in the brain initiate a sequence of events that lead to AD, as: mitochondrial dysfunction, aggregation of tau causing NFTs, oxidative stress and synaptic failure.

There is another theory that explains the neurodegeneration produced in AD. This hypothesis came from the idea that soluble oligomers and intermediate amyloids are the most neurotoxic forms of A β (dimers and trimers of A β are toxic to synapses). In fact, the severity of the cognitive deficit in AD correlates with the levels of oligomers in the brain, not the total A β burden. This led to the “toxic oligomer hypothesis” because oligomers are known to be the most toxic species probably causing neurodegeneration by affecting mitochondrial and synaptic function, both early signs of AD (21).

5.5.2.2. *Apolipoprotein E*

The lipid-carrier protein ApoE4 increases A β peptide production and impairs A β clearance. When produced within stressed neurons, ApoE4 is cleaved into neurotoxic fragments that destabilize the cytoskeleton and, like intracellular A β , impair mitochondrial functions as they accumulate there (5,27).

5.5.2.3. *Microtubule associated protein tau*

Tau and α -synuclein can also self-aggregate into pathogenic oligomers and into larger inclusions in neurons displacing vital intracellular organelles, known as NFTs and Lewy bodies, respectively. By definition, all patients with AD have many plaques and tangles; most patients also have Lewy bodies (5).

NFTs, which are filamentous inclusions in pyramidal neurons, are resistant to chemical and enzymatic degradation and can persist in the encephalic tissue long after the neuron in which they originated has died and disappeared extracellularly (10,11). The misfolding and fibrillation of tau to NFTs can be generated by genetic mutations, posttranslational modifications or intracellular environmental changes (28). The major component of the tangles is an abnormally hyperphosphorylated and aggregated form of tau. NFTs of tau occur in AD and other neurodegenerative disorders termed tauopathies. The number of NFTs is a pathologic marker of the severity of AD (21).

Normally tau (hydrophilic and cationic) is an abundant soluble microtubule-associated protein predominantly expressed in the axons of neurons of the central nervous system that promotes assembly and stability to the microtubules and vesicle transport. The binding of tau to microtubules is mediated by three or four microtubule binding domains at the C-terminus of the protein. It has six different isoforms created by alternative splicing and is unfolded in native conditions; its function is regulated by posttranslational modifications as phosphorylation (mainly), glycosylation, ubiquitination, truncation and nitration. The ability of the protein to join the microtubules and perform its action depends on the number of phosphates joined to it. In AD, abnormal phosphorylation happens on determinate tau residues (Ser202, Thr205, Ser235 and Ser404) by kinases in post-translational modifications. Hyperphosphorylated tau is insoluble, lacks affinity for microtubules and self-associates into paired helical filament structures heading to abnormal structural changes, disruption of cellular traffic and favoring synapse dysfunction and loss. Consequently, enzymes that add and those that remove phosphate residues regulate the extent of tau phosphorylation (6,8,11,29).

Like A β oligomers, intermediate aggregates of abnormal tau molecules are cytotoxic and impair cognition but, A β oligomers impair synaptic functions whereas fibrillary amyloid plaques displace and distort neuronal processes (5). Experimental evidence indicates that A β accumulation precedes and drives tau aggregation (21).

5.5.3. Oxidative stress

Oxidative stress is the result of an imbalance between oxidative systems and antioxidant defenses (which can be non-enzymatic, especially dietary antioxidants, or enzymatic), in favor of the first ones producing reactive species. This process conduces to the oxidation of biomolecules with the consequent loss of their biological functions (30). The chronicity of this process leads to important implications in the development of chronic illnesses (30): “the free radical theory of aging” maintains that aging is a result of the oxidative damage we suffer through life that leads to cell death and organism malfunction (31). However, ROS and RNS are produced during normal physiological processes; in fact, they are important in the immune response and in cell signaling when in the right proportion (22). There are two main compounds that keep free radical levels within the physiologically beneficial: antioxidant enzymes (that neutralize reactions against free radicals and ROS) and nutrients (that act as co-factors in catalytic activities) (7,22).

Several biological agents are found to be responsible for the accumulated damage oxidative stress produces in ageing, some come from environmental sources (as the ones from the exposition to ultraviolet light) and some are produced endogenously (as by-products of normal living) (32).

The effects of ROS are linked with elevated levels of free divalent transition metal ions (like iron, copper and zinc) and aluminum, causing neurodegeneration in several ways, for instance promoting aggregation of tau and changes in its conformation or phosphorylation (21). Oxidative damage can also be a predisposing factor and/or a consequence of A β deposition, as oxidative stress can induce the production of the A β producing enzymes: BACE and γ -secretase. Moreover, reactive species can also be generated in dysfunctional mitochondria and by A β in the presence of metal ions (22).

5.5.4. Mitochondrial dysfunction

Oxidative stress is closely related to mitochondrial dysfunction. Mitochondria's are primary sites of ATP production, they maintain calcium homeostasis, participate in calcium signaling and regulate intrinsic apoptosis (22). Even though all cellular structures can be targets for free radicals, the structures that suffer the higher amount of oxidative effects are cell membranes (32) and mitochondria is particularly susceptible to this changes. Its dysfunction plays an important role not only in brain ageing but also in the pathogenesis of neurodegenerative diseases (33).

A β peptide, a potent generator of ROS and NRS, is a key initiator of the mitochondrial damage. The dysfunctional mitochondria release oxidizing free radicals that cause oxidative stress as they peroxidate membrane lipids and produce toxic aldehydes (21). In AD, reduced activities of three mitochondrial enzymes (pyruvate dehydrogenase complex, alpha ketoglutarate dehydrogenase complex and cytochrome oxidase) are present (27). Carbonyl and nitrated derivatives are also generated because other essential proteins are directly oxidized by free radicals. This leads to an increase of

membrane permeability to calcium and reduced glucose transport, aggravating the energy imbalance (21).

Due to the mitochondrial dysfunction, there is impaired autophagy that could potentiate A β deposition. Therefore, early mitochondrial dysfunction and oxidative stress may proceed to A β overproduction and deposition (22).

“The mitochondrial theory of aging” maintains that cellular aging is a consequence of mutations in the mitochondrial DNA (mtDNA) genome as a result of oxidative damage (31). The mitochondria is the principal site of ROS production in cells as the electron transfer system constantly generates it and it is kept in balance by different defense mechanisms such as antioxidative molecules (as glutathione and vitamin E) and antioxidant enzymes (as superoxide dismutase and catalase) (33). A β peptide is a powerful mitochondrial poison; it especially affects the synaptic pool. In AD, exposure to A β peptide inhibits important mitochondrial enzymes in the brain and in isolated mitochondria: cytochrome c oxidase and alcohol dehydrogenase are specifically attacked. Thus, electron transport, ATP production, oxygen consumption and mitochondrial membrane potential all become damaged. Due to the accumulation of A β peptide within structurally damaged mitochondria, mtDNA sustains high levels of oxidative damage. This instability and the irreparability of the brain’s mitochondrial genome allow the gradual accumulation of mtDNA mutations, producing fragmentation of mitochondria and synaptic loss in AD (21).

5.5.5. Inflammation

In AD, vascular injury and parenchymal inflammation seem to play an important role in the pathogenesis as they perpetuate the cycle of protein aggregation and oxidation in the brain (21).

When A β accumulates in the cerebral arterioles it is called cerebral angiopathy and the activation of microglia and astrocytes to fight it, may seem a positive response to clear protein aggregates through phagocytosis and intracellular degradation; however, chronically activated becomes dysfunctional and leads to the production of nitric oxide, ROS, pro-inflammatory cytokines (as tumor necrosis factor- α , interleukin-1 β and interleukin-6) and prostaglandin-E₂, which finally cause neuronal death. Furthermore, this proinflammatory cytokines produce an increase in tau phosphorylation, APP generation and A β peptide synthesis through BACE-1 transcription (8).

Vascular abnormalities impair the supply of nutrients, the removal of metabolic by-products and cause microinfarcts. Ischemic disease affects 60 to 90% of patients with AD and many cases of dementia are mixed (8,34) therefore it is an important pathogenic point to consider.

5.6. ALZHEIMER’S DISEASE DIAGNOSIS

The interest in finding a diagnosis in early phases is growing more and more due to the increasing impact of this disease as the population continues to live longer.

A reference neuropathological criterion is being searched for the definitive diagnosis, because amyloid plaques and NFTs are present in people without dementias. This means that AD exists in absence of dementia during a period of time, a period that can

be called preclinic where there is presence of Alzheimer's histopathologic changes before clinical changes emerge (35).

In order to standardize the medical practice, it is necessary to follow some criteria. Basically, there are two main criteria: the ones from Dubois et al. (36) and the updated ones from the National Institute of Neurological and Communicative Disorders which reached an agreement with the Alzheimer's Disease and Related Disorders Association and developed the criteria NINCDS-ADRDA for AD diagnosis, now called NIA-AA criteria (35).

In one hand, the ones from Dubois et al. (36) are exclusive for the diagnosis of AD. They evaluate the gradual affectation in episodic memory by neuropsychological tests and analyze the correspondent biomarkers placing subjects in probable AD. The diagnosis is only definitive when there is clinical and anatomopathological evidence (at biopsy or necropsy) or, when there is clinical and genetic evidence of AD. However, this criteria need technical and financial resources to obtain a greater probability of certain, something that is not available in every single center (35).

Table 1. Diagnostic criteria for AD from Dubois et al. (36), it requires A criteria plus at least one of B, C, D or E. Adapted from (35).

MAIN CRITERIA	A. Initial and significant episodic memory disorder that includes the following characteristics: <ol style="list-style-type: none"> 1. Progressive and gradual memory loss for at least six months communicated by the patient or by a reliable informant. 2. Objectify by neuropsychological tests the episodic memory loss. 3. The episodic memory defect can be isolated or associated with other cognitive alterations.
ADDITIONAL CHARACTERISTICS	B. Presence of atrophy in the medial temporal lobe: Volume loss of the hippocampus, amygdala and entorhinal cortex, evidenced by magnetic resonance.
	C. Alterations of biomarkers in cerebrospinal fluid: Decreased A β 42 or increased concentration of total tau or phosphorylated tau, or combined.
	D. Characteristic alterations with positron emission tomography: Hypometabolism of bilateral glucose in temporal and parietal regions or other alterations with radioligands.
	E. Evidence of an autosomal dominant mutation in a first-degree relative.

On the other hand, NIA-AA criteria are for diagnosing the level of impairment (dementia, MCI or normal cognitive functions for the age of the patient). They combine clinical and neuropathological patterns and assign diagnoses of "possible", "probable" and "Probable or possible AD with evidence of the Alzheimer's pathophysiological process" (14,35). Actually, these criteria are again under review (14).

Researchers aim to find early biomarkers for the diagnosis of AD. The use of them must consider that biomarkers are different in function of the phase of the disease, for instance: at the beginning they can only be found amyloid accumulation biomarkers, as the illness develops synaptic dysfunction biomarkers are the ones searched and lastly, when the disease is advanced, biomarkers of neuronal loss are the ones examined (35). However, there are still problems related with their clinical use, such as insufficient

standardization of measurement methods and where the cut points are to separate normal levels from pathologic ones. In addition, there are not very sensitive in the early phases of the illness and the margin of error is still very big (35).

Actually, the most common way of diagnose is for exclusion; the diagnosis can only be confirmed by microscopical analysis of the tissue obtained by cerebral biopsies or necropsies. Therefore, diagnose is based on clinic data through an algorism established on the combination of clinical evaluation and radiologic methods. Firstly, it is necessary to establish dementia with a clinical exam alterations of consciousness must be absent and there must be a beginning between 40 and 90 years old; it is also important to evaluate the lack of systemic or encephalic disorders that could explain the deficits of memory or cognition (through neuroimaging tests as computed tomography or magnetic resonance imaging). This means, that in the first stage of the disorder it is common to exclude other causes of dementia by metabolic studies: such as vitamin B₁₂ deficit, thyroid dysfunction and electrolyte imbalance (6,11). This exclusion method is capable of giving an accurate diagnosis in the 80-90% of the cases then confirmed in the autopsy (10).

5.7. ALZHEIMER'S DISEASE TREATMENT

AD treatment pursues to improve quality of life and maximize the functional performance by enhancing cognition, mood and behavior. Treatment includes both pharmacological and non-pharmacological approaches (37).

As the exact pathophysiological mechanism of AD is not clear, it hinders the process of finding effective treatments (22). In fact, nowadays AD does not have a curative treatment, but there are some drugs capable to slow down its progress and control the comorbidities associated, especially in early stages. As A β peptide and tau aggregation are implicated in AD pathogenesis, the therapeutic strategies interfere in this point (38,39). There are three groups: cholinesterase inhibitors, glutamate regulators and a combination of a cholinesterase inhibitor and a glutamate regulator approved by the FDA (20).

5.7.1. Acetylcholinesterase inhibitors

They are prescribed to treat symptoms related to memory, thinking and other thought processes. There are three different medications from this group: donepezil, galantamine and rivastigmine. The first one is approved to treat all stages of AD while the other two are only approved for mild-to-moderate stages (38).

Its pharmacological action consists in the inhibition of the enzyme cholinesterase (AChE), increasing levels of acetylcholine (a neurotransmitter involved in learning and memory). The function of AChE is to break acetylcholine down so it can be recycled. But in AD cells that produce and use acetylcholine are destroyed, therefore an inhibitor of this enzyme will promote an increase in acetylcholine concentration and duration of action, contributing benefits to the patients (22,38) on cognitive, functional and behavioral symptoms of the disease (25). Although they improve neurotransmission and relieve symptoms, they lose efficacy over time (21).

They are usually well tolerated and their side effects include: nausea, vomiting, loss of appetite and increased frequency of bowel movements (38).

Clinical trials have shown that cholinesterase inhibitors may delay or slow worsening of symptoms. However, the combination of the three drugs hasn't shown any advantage respect the administration of one alone, but side effects result in greater frequency (38).

In the figure below (Table 2) mechanisms, pharmacokinetics and daily doses of the drugs approved in this group are described.

Table 2. Pharmacology, pharmacokinetics and daily doses of acetylcholinesterase inhibitors (12,25).

GENERIC	MECHANISM	PHARMACOKINETICS	DAILY DOSIS
Donepezil	Non-competitively reversibly inhibits AChE. It is highly selective for it.	Metabolized in the liver by the CYP450 (CYP2D6 and CYP3A4) and by glucuronosyl transferase with extensive first-pass metabolism. It binds to plasma proteins.	Once daily before going to bed. The initial dose is 5 mg/day with an increase in the fourth week to 10 mg/day.
Galantamine	Selective reversible AChE inhibitor and positive allosteric modulator of nicotinic receptors.	Metabolized by CYP450 isoenzymes (mainly CYP2D6 and CYP3A4) in the liver, poorly bound to plasma-proteins and has a terminal elimination of 5 hours.	Once daily in the mornings for the extended released capsules and two daily for the oral solution: in the first month 8 mg/day, 16 mg/day on the second and 24 mg/day on the third if there is good tolerability.
Rivastigmine	Is a pseudoirreversible inhibitor of AChE and butyrylcholinesterase.	As it does not have hepatic metabolism by CYP450 it has low pharmacologic interactions, it is hydrolyzed by esterases and has low plasma-protein binding.	Two doses are required daily: initially 3 mg/day increasing gradually until 12 mg/day.

5.7.2. Glutamate regulators

Actually, there is only one drug approved in this group: memantine, which is used to treat moderate to severe AD (38).

Glutamate is a neurotransmitter that normally participates in learning and memory processes by triggering NMDAr to let calcium into cells (this ion is important for information storage). Nevertheless, in AD the abnormal accumulation of harmful proteins produces excitotoxicity (overstimulation of NMDAr) causing glutamate excess and allowing too much calcium into the nerve cells. Memantine is a drug that acts like a non-competitive NMDA antagonist inhibiting the glutamate action (25,38).

It is administered orally and has an approximate absorption of 100%; moderately bound to plasma-proteins suffers a limited metabolism, which leads to inactive metabolites (12).

This drug can produce headache, constipation, confusion and dizziness (38). Initially 5 mg/day are given to the patient; the dose is gradually increased weekly in 5 mg/day, until 20 mg/day (12).

5.7.3. Namzaric®

There is one FDA approved drug (Namzaric®) that combines the cholinesterase inhibitor rivastigmine with the glutamate inhibitor memantine. It is approved for moderate-to-severe stages in patients taking donepezil hydrochloride 10 mg. Namzaric® both prevents the breakdown of acetylcholine in the brain and protects the brain's nerve cells against excess amounts of glutamate (38).

The recommended dose of Namzaric® is 28 mg/10 mg once daily. It has low binding to plasma proteins and suffers from partial hepatic metabolism (12).

Namzaric® can cause serious side effects, as: increased stomach acidity (raising the chance of suffering ulcers and bleeding; being even higher in patients taking aspirins or other anti-inflammatories), slow heartbeat, fainting and muscle problems, among others (38).

5.7.4. Behavioral symptoms

Often patients with AD also suffer from other disorders such as depression, insomnia, hallucinations, agitation and aggressiveness. As they have a great impact in the quality of life, these disorders can also be treated in a complementary way (20).

In addition, it is also very important to maintain the patient socialization and to give support to the family. These tasks can be facilitated by self-help groups and day care centers (6).

5.7.5. Future developments

Even though the great efforts of researchers to find early and approachable markers of AD, it is still diagnosed very late, when neurological symptoms emerge and neuropathology is already in an advanced point. Consequently, two important factors in the actual research of AD are early diagnose and prevention (8).

As we have seen, in AD there are implicated multiple pathogenic factors and the medication actually approved does not stop the destruction of nerve cells; therefore the ability of these drugs to improve symptoms sooner or later declines as brain cell damage progresses (38).

Right now, there are several clinical trials in progress. They are centered in patients on early stages of the disease and use several strategies to remove A β from the brain, like: immunological approaches (A β vaccination), interruption of A β formation with drugs against Y-secretase or BACE and also approaches to prevent tau alterations (fibrillation, misfolding and aggregation) (5,10,28).

The actual anti-AD strategy is based on hitting-one-target drugs. Because of its ineffectiveness, more attention is being given in finding multiple-targeted agents to hit more than one target involved in AD mechanism. Moreover, it is also interesting to find multiple-AD targets in one single structure, as it reduces the risk of drug-drug interactions. For this reason, finding natural multi-targeting agents to treat AD is catching

increasingly consideration (9) and plant polyphenols are increasingly being considered as the starting point to develop this (1).

5.8. FINDING POTENTIAL THERAPEUTIC TOOLS IN THE PREVENTION OF ALZHEIMER'S DISEASE

As AD does not have cure a lot of research is being done to find treatments to delay and relieve its symptoms (26). In order to develop prevention, a lot of attention has been placed in different lifestyles and dietary regimens that are associated with a reduced cognitive decline. Epidemiological data suggests that certain nutrients and foods may confer protection against AD, in particular adherence to the Mediterranean Diet (MD) shows a lower incidence of MCI and its progression to AD; which means that sufficient intake of certain micronutrients and secondary plant metabolites continuously during all life-span may prevent disease's onset (26,33).

Finding prevention to AD is particularly relevant because the amyloid disease is developed during a very long time but its symptoms manifest late in life, when tissue damage and functional derangement are already severe and often irreversible (40). Different therapeutic approaches are being studied and lately an increased interest has been placed into the neuroprotective power of olive oil polyphenols that are present in the MD (1).

5.8.1. The Mediterranean diet

The term MD refers to the traditional dietary patterns found in some of the Mediterranean countries (as Italy and Greece) in the fifties of the twentieth century (3,41). It is associated with lower mortality rates and a reduced risk of cardiovascular diseases and cancer, but in the last few decades it has also been studied its relation with a reduced incidence of neurodegenerative diseases (28).

The MD is characterized by a high intake of cereals, vegetables, legumes and fruit; relevant consumption of seafood; low-to moderate intake of dairy products; low intake of red meat and saturated fatty acids; and a regular but moderate intake of wine during meals (8,33,42). As olive oil is one ingredient never absent in this diet it was considered that it could be one of the elements that could help in the prevention of cognitive decline (8). In fact, the European Food Safety Authority and the American Food and Drugs Administration have recognized the beneficial effects of polyphenols in olive oil and recommend a daily intake of two table spoons (23 g) to prevent the onset of CVD, inflammation and to counteract the oxidative stress produced by free radicals (3).

5.8.2. Olive Oil

Olive oil is one ingredient never absent of the MD (8) it comes from the olive tree (*Olea europaea*) and is the main source of fat in the MD. Mainly consists of two groups of compounds: saponifiable lipids or glycerides (about the 98% of the total composition), most of them monounsaturated fatty acids (MUFA) as the oleic acid but also polyunsaturated fatty acids (PUFA) mostly linoleic acid and α -linolenic acid. And the "minor compounds" (unsaponifiable fraction) that consist from 1 to 2% of the total content of an olive oil (43) containing a variety of compounds which despite of their proportion, must be taken into consideration because of their activity. These last ones are also the responsible of the stability and the organoleptic properties of olive oil and consist of α -

tocopherol, several specific phenolic compounds (including phenolic acids, phenolic alcohols, lignans, flavones and secoiridoids; being the latter the most abundant and typical phenolic components of olive oil) (3,8) and phytoalexins. Phytoalexins are secondary plant metabolites that give leaves an unpleasant taste that discourages insects of eating them and also gives them defenses against microbial and fungal invasions (1).

Generally, the intake of olive oil ranges from 25 to 50 mL per day in the MD (24,29,42,44), consisting of a significant fraction of dietary polyphenols. The main problem related to the daily consumption of olive oil is its high caloric value (45). This review will be centered in the neuroprotective effects of polyphenols in olive oil, however, to understand completely the health benefits of olive oil in AD is necessary to consider all their components and not only the phenolic ones (43).

For a long period, the beneficial neuroprotective effects of olive oil were associated to the unsaturated lipids, particularly oleic acid. Nevertheless, it is now known that contrary to what was supposed, the phenolic content in olive oil may be the main ingredient responsible for the reduced risk of age-related cognitive impairment because if the effect of olive oil was attributed to its MUFA content, any type of olive oil or MUFA-rich foods would provide the same health benefits (1,29,46).

Total phenols in olive oil range between 100 (43) and 1000 mg/kg (42). This discrepancies are due that their concentration depends on different factors, such as olive cultivar (being the Coratina one of the richest olive tree varieties in content of polyphenols and the Arbequina one of the poorest), level of maturation at harvest time (early or late: early harvests drupes have a longer shelf life as they have higher polyphenol content), environmental factors (as altitude or amount of irrigation), extraction conditions and systems, oil storage modalities (when exposed to air and light, olive oil phenols oxidize and degrade easily) and the processing system employed to produce the olive oil (2,8,41). Moreover, there is inaccuracy in the methods that determinate total phenol content (47).

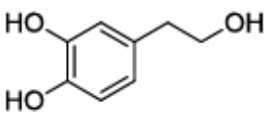
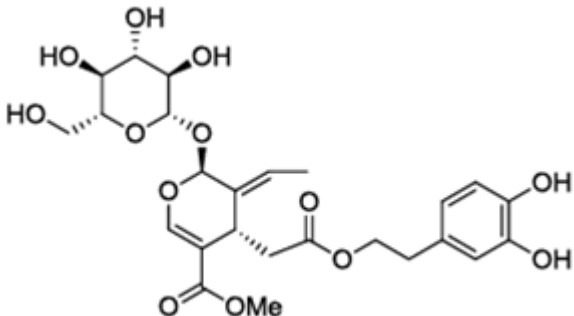
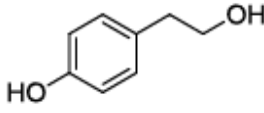
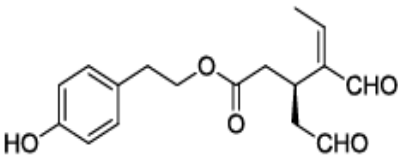
Olive oils can be classified in function of the processing system used to produce them in extra virgin olive oil (EVOO), virgin olive oil (VOO), olive oil or pomace oil. VOO is obtained from the fruit of the tree exclusively by mechanical or other physical methods that do not lead to the alteration of the oil. They do not suffer any treatment other than washing, decantation, centrifugation or filtration. If a VOO has a free acidity, expressed as g of oleic acid/100 g of olive oil, lower than 0, 8 g is an EVOO (3,41). VOOs with acidity superior or equal to 2 in Europe (European Regulation N.1513/0) are submitted to a refining process in which some components are lost, mainly phenolic compounds. If VOO and refined olive oil are mixed, an ordinary olive oil is produced. The rest of the olive drupe and seed after VOO production is processed and submitted to a refining process, resulting in pomace olive oil, to which a certain quantity of VOO is added (41,46). Consequently, the different types of olive oil do not have the same health benefits; being EVOO and VOO the ones associated with a more reduced cognitive decline because of their higher polyphenol content (46,47).

5.8.3. Polyphenols in olive oil

5.8.3.1. Content of polyphenols in olive oil

The phenolic content in olive oil is implicated in defining the bitterness, pungency and astringency (28). They are secondary plant metabolites divided into groups in function of the number of phenol rings and the structural elements binding in these rings to one another. The phenolic compounds in olive oil are: flavonoids, lignans, phenolic acids, phenolic alcohols and their secoiridoids precursors (1,2,42). As olive oil contains over 30 different phenolic compounds this review will only evaluate the last two groups mentioned, concretely the polyphenols represented in the figure down below (Table 3) as they are the major phenolic compounds in olive oil (47).

Table 3. HT, Tyr, Oleocanthal and OLE present in olive oil. Adapted from (42).

PHENOLIC ALCOHOLS	SECOIRIDOIDS
 <p>Hydroxytyrosol (HT)</p>	 <p>Oleuropein (OLE)</p>
 <p>Tyrosol (Tyr)</p>	 <p>(-)- Oleocanthal</p>

Both HT and Tyr exist in the form of glycosides (bounded to glucose: HT-4- β -D-glucoside and salidroside, respectively) and secoiridoids. Secoiridoids are those molecules formed by elenolic acid linked to HT and Tyr. When the elenolic acid is in its glycosidic form - bounded to glucose- is called OLE when is bounded to HT and ligstroside when is bounded to Tyr. During the processes of crushing and malaxation β -glucosidases catalyze the deglycosylation and give the corresponding secoiridoids aglycones. OLE and ligstroside aglycones can be additionally hydrolyzed in the gut producing elenolic acid and the simple phenols HT and Tyr. HT and Tyr can also from esters with deacetoxy elenolic acid and give rise to oleacin and oleocanthal, respectively (43).

HT and Tyr are most prevalent in the olive fruit and OLE in the leaves (3). In a VOO, almost the half of the total phenolic content are conjugated forms as OLE and ligstroside aglycones, while free forms of Tyr, HT and their secoiridoid derivatives are approximately a 30% (41). OLE concentration is reduced through chemical and enzymatic reactions

during the maturation or olive oil processing, producing an increase on the principal degradation product of OLE, HT (39,48).

HT can be obtained by two possible ways: and exogenous one (when natural products that contain HT or its precursors are ingested) or an endogenous one (as a product of the oxidative metabolism of dopamine through monoaminooxidase and aldose/aldehyde reductase). HT is present at very low concentrations in fresh olive oil and may increase with olive oil aging because of the degradation of OLE. To obtaining HT in an exogenous way, OLE must suffer a double hydrolysis; firstly during olive oil maturation and storage, and then in the gastrointestinal tract (3,42,43). In the same way as HT, Tyr can also be obtained exogenously or be endogenously synthesized as byproducts of tyramine through the same enzymes as HT. However, the endogenous pathways of their synthesis are minor in normal conditions but gain importance after ethanol consumption (43).

Oleocanthal is present mainly in freshly pressed EVOO and is the responsible for the bitter taste that produces a burning sensation at the back of the throat (28,42).

5.8.3.2. Olive oil polyphenols bioavailability

The bioactivity of plant polyphenols depends on the absorption and metabolism of these products, the vehicle of administration has an important role on its bioavailability too (30,42).

Most of the dietary polyphenols are in glycosylated derivate forms in food and to be bioactive in the human body must suffer from intestinal biotransformation by digestive enzymes and microbiota metabolism. These transformations (deglycosylation by gastric acid and hydrolysis by β -glucosidase in small intestinal epithelial cells) are very important to ensure some of the beneficial effects of polyphenols before their metabolism and excretion (1).

Humans are able to absorb natural phenols as after their ingestion they appear in plasma and urine (HT and Tyr are absorbed in a dose-dependent manner), nevertheless their bioavailability is poor due to an incomplete intestinal absorption and to a rapid biotransformation that favors their urinary excretion (1).

Polyphenol aglycones diffuse passively through membranes and are better absorbed than their glycosylated parts; pass from the enterocyte to the lymph and to the blood stream (1). To exert their pharmacological effects they need to cross the BBB and achieve enough concentration in the brain (22); despite their permeability through the BBB their concentration in brain it is reported to be very low (1).

As many non-polar products they are exposed to Phase I and Phase II metabolism originating sulphated, hydroxylated and glucuronide derivatives as well as their degradation products that favor their excretion and provide them new biological activities; these metabolites can also reach the brain and contribute to the neuroprotection (1). For example, the free portion of HT in plasma is extremely low; around 98% is conjugated as sulfates (higher doses) and glucuronides (low doses) (42).

In conclusion, polyphenols are absorbed from the intestine and distributed through the blood flow to the whole organism. There is evidence that OLE, HT, Tyr and Oleocanthal are able to cross the BBB (1,2,40,42).

The specific microorganisms in the gut also influence the bioactivity of polyphenols. When polyphenols reach the colon they are metabolized by gut microbiota to low-molecular weight compounds; therefore the colonic microflora can metabolize and chemically modify polyphenols (2) and be in part responsible of their systemic effects (30). However there is still a little known about this impact (40). A resume of all this process is included in Figure 5.

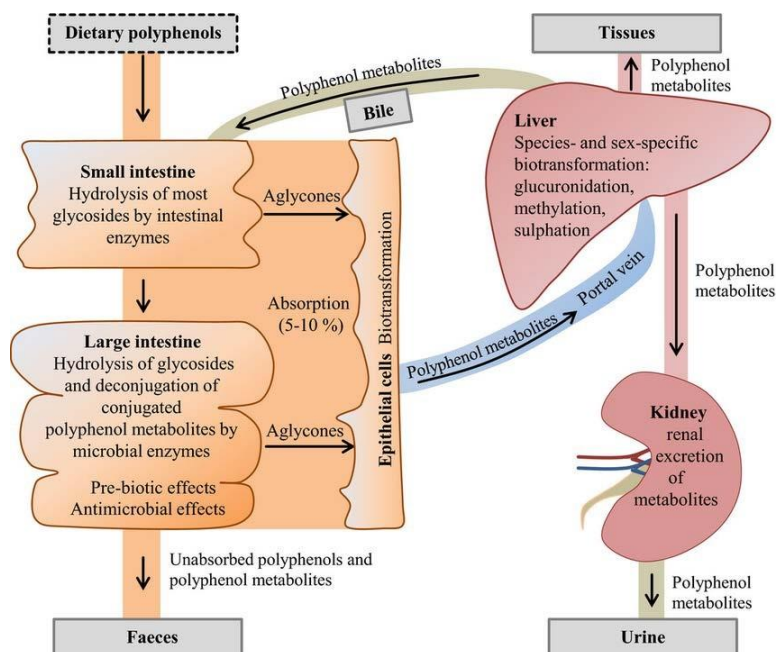


Figure 5. Absorption and metabolism of polyphenols in animals (49).

Some conditions may improve the poor bioavailability of these products; for instance, OLE stability can be increased both in the stomach and in the intestine if it is ingested with the meal (40). Therefore, recommendations of daily intakes of olive oil polyphenols to obtain neuroprotective effects need to consider functional active doses and their bioavailability (33).

Due to the reduced bioavailability of plant polyphenols in olive oil, some solutions are under study; the most investigated one is the encapsulation inside nanoparticles (1) which improves both stability and bioavailability of the molecules (40). Emulsions are considered to be one of the most popular forms of encapsulation and delivery due to the maintenance of chemical stability and the controlled release of the encapsulated molecules (50). For instance, OLE was found to enter β -cyclodextrin particles with its phenolic portion, this protected it from oxidation at the same time it increased its solubility (40). Nutraceuticals, enriched olive oils in polyphenols with concentrations much higher than those found in their natural forms, are also an emerging path (42). These olive oils with higher phenolic content are more bitter and greener than those with low phenolic content (46). But before developing these products is important to use appropriate analytical methods to estimate the polyphenol content that produces beneficial effects in order to standardize the concentration of these products (42).

5.8.4. Polyphenols activity: Where do polyphenols act in Alzheimer's disease?

Experimental evidence has demonstrated that the phenolic compounds present in olive oil are, at least partly, responsible for the beneficial effects arising from the consumption of this oil. Olive oil phenols possess different biological properties as antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antitumoral, cardioprotective and neuroprotective (3,44).

Polyphenols in olive oil do not simply act on a single step of AD, they seem to modify several different aspects of the biological and functional features of the cells and tissues where aggregation takes place (40): its mechanism involves interactions that are important in the normal functioning of cells (51).

Most of the studies that investigate where do polyphenols act in AD use cell culture experiments making it difficult to extrapolate *in vitro* data to *in vivo* conditions (33); for this reason is always necessary to verify if the discoveries can be translated into practical clinical recommendations (42). This is because the exact way polyphenolic compounds exert their neuroprotective effect is still incompletely understood. However, it is known that their mechanism on cells is not only through their antioxidant activity (for which they are most famous) but also through different pathways such as signaling cascades, anti-apoptotic processes and synthesis/degradation routes that may not occur in humans in the same way as on cells (39).

Different studies had been carried out to test the efficiency of polyphenols in AD by administrating to rodent's diet supplementation olive oils rich in polyphenols. In general those researches jump into conclusion that EVOO improves learning and behavioral deficits associated with aging and AD (26). Such as the one of Ah Young Lee et al. (34) in which it was investigated the effect of oils with different fatty acid composition on learning and memory function determined in behavioral tests (34). Or the one from Cristina Grossi et al. (26) that provides a compelling evidence that OLE aglycone intake improves the cognitive performance of young and middle-aged rats, converting OLE in a useful compound to prevent AD, or at least, to delay the appearance and to reduce the severity of its symptoms (26). In fact, there are plenty of studies that provide some evidence in this field (39). However, there are still many issues unresolved, as: dose, treatment duration and food and drink interactions, among others (22).

Next, olive oil polyphenols effects on AD pathogenesis are going to be exposed in the same way as AD mechanism in the previous sections has been presented.

5.8.4.1. Synaptic failure

Olive oil is capable of inhibiting AChE by formatting strong hydrogen bonds and multiple hydrophobic interactions with it. This interaction increases acetylcholine levels, an important neurotransmitter in maintaining cognitive functions like learning and memory, helping to maintain the normal synaptic activity (22). Many phenolic compounds in olive oil had been investigated for their AChE inhibitory activity, but the identification of these compounds has not been precisely determined yet (52). The ability of polyphenols to block NMDAr and enhance the synaptic functioning has not been given enough attention, and more research is needed to be done before jumping to any conclusions (22).

However, olive oil polyphenols are known to improve the synaptic failure present in AD; for instance, as Oleocanthal, HT, Tyr and OLE alter the oligomerization state of A β , they protect neurons from the A β oligomers induced synaptic deterioration as they reduce their binding to synapses (1,24). Moreover, polyphenols in olive oil are able to increase neurotrophins levels and the expression of their receptors as the intraperitoneal administration of HT and OLE increase levels in brain areas of neurotrophins protecting neurons from the damage caused by their deficits (3).

5.8.4.2. Abnormal accumulation of harmful proteins

Plant polyphenols act like amyloid inhibitors; they have different mechanisms to redirect the aggregation towards a path where no oligomeric or nontoxic oligomeric intermediates are formed. In some cases they can also disaggregate performed fibrils (40). Nevertheless, polyphenols and their glycosides affect amyloid aggregation in a different way: amyloid oligomers are remodeled by the aglycones by rapid conversion into large off-pathway aggregates, whereas the glycosylated forms rapidly dissociate into soluble disaggregated peptides molecules the amyloid oligomers (1,2).

Oleocanthal enhances A β clearance from the brain via up-regulation (increasing expression) of P-glycoprotein and LDL lipoprotein receptor related protein-1 (LRP1), which are major A β transport proteins at the BBB (29). It also inhibits the formation of NFTs as it interacts covalently through two aldehyde groups with the lysine residues of a tau fragment inducing conformational modifications that interfere with protein aggregation (1,42).

OLE forms a noncovalent complex with A β or its oxidized form (9,48) producing the precipitation of A β into amorphous aggregates without toxicity that finally evolve into non-harmful protofibrils. It also prevents and reduces the growth of toxic oligomers and plaque deposition (40). It is moreover capable to hinder the aggregation of tau into NFTs (1); in fact OLE, OLE aglycone and HT do inhibit tau aggregation at the same level as methylene blue (a reference compound for tau anti-aggregation) (53), but the OLE aglycone proved to be more effective inhibiting tau aggregation than OLE or HT (22). OLE induces autophagy (shown by the increase of autophagic markers and lysosomal activity) protecting neurons from A β -induced cytotoxicity (2,26,40). Because all of these, OLE improves learning and memory (3) as some studies in rodents have shown. For instance, the one of Grossi et al. (26) showed that mice dietary supplementation with OLE aglycone improved memory deficits as it reduced A β 40 and A β 42 levels as well as it reduced plaque size and *de novo* deposits and favored the already performed plaque disassembly (26).

In conclusion, Oleocanthal, OLE, HT and Tyr are able to interfere in the aggregation path of A β binding to the aggregation molecules, eviting the appearance of toxic species and favoring the formation of non-toxic disordered aggregates protecting neuronal cells against A β and tau induced toxicity (2,54).

No articles related with polyphenols activity towards the aggregation path of ApoE were found during the development of the present study.

5.8.4.3. Mitochondrial dysfunction

Due to the mitochondria-related oxidative damage previously explained, a polyphenol supplementation would not only help in the process of harmful accumulation of proteins

but also, as olive oil polyphenols are excellent antioxidants, would enrich mitochondrial membranes with less oxidizable fatty acids and reduce lipid peroxidation (31).

Diets with high levels of PUFA are associated with higher levels of lipid peroxidation and DNA double-strand breaks compared to MUFA-based diets. The diet fat profile influences membranes, including the mitochondrial one, and therefore a low diet in PUFA and rich in antioxidants may help in the prevention of developing AD as it reduces reactive species and consequently mitochondrial dysfunction (31).

HT promotes mitochondrial biogenesis as it increases the activity and protein expression of mitochondrial complexes I, II, III and V, enhances oxygen consumption and reduces free fatty acid contents in the adipocytes helping in the prevention of AD reducing mitochondrial dysfunction (22).

5.8.4.4. Oxidative stress

MD polyphenols are most studied for their antioxidant activity; they are known to be excellent antioxidants both as ROS scavengers (thanks to the aromaticity of their phenolic rings) and transition metal chelators (8,9). Polyphenols in olive oil also increase antioxidant molecules, such as HDL cholesterol (41) and directly act on enzymes, proteins, receptors and several types of signaling as well as interfere in the biochemical homeostasis (1). This antioxidant activity is not only beneficial in the prevention of AD, but is also useful in the prevention of cardiovascular diseases as it helps to protect LDL from oxidative damage (42).

Oxidative stress plays an important role both in aging and in age-related diseases (22). As the brain has high metabolism, oxidative stress occurs in its neural tissue; in fact, is considered to cause the initiation and propagation of neurodegenerative diseases (23). The brain is especially sensitive to oxidative damage because its high content of PUFA (constituents of neuronal cell membranes, which are easily peroxidizable substrates), its low level of endogenous antioxidant enzymes and moreover, its high oxygen consumption rate (54). That particular evidence of oxidative stress in AD brains indicates the potential role that antioxidants, such as phenolic compounds from diet, could play in its prevention and control. Although dietary phenolic compounds are good antioxidants *in vitro*, *in vivo* such effects may be indirectly mediated through the activation of some pathways and not their intrinsic antioxidant activity (42).

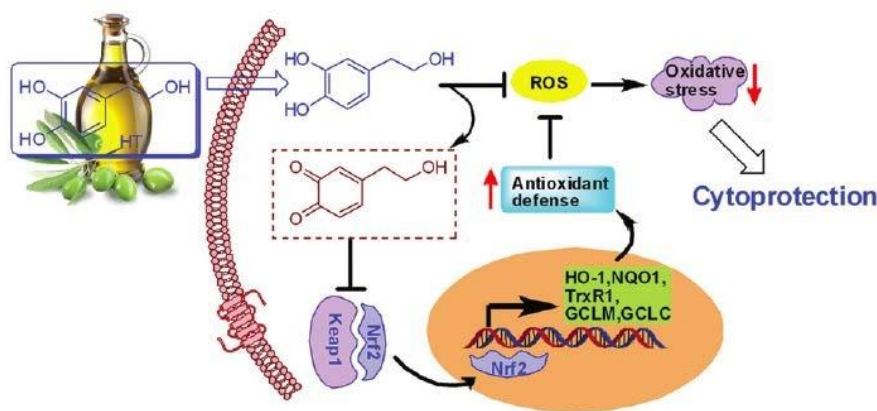


Figure 6. Dual neuroprotective model of HT: it directly neutralizes free radicals and indirectly induces the endogenous antioxidant defense systems in cells (55).

HT prevents from induced oxidative stress in cells through two different mechanisms. On one hand, as it has a small size and a lipophilic character is able to pass through the plasma membrane, get inside cells and directly stabilize ROS generated during oxidative stress. And on the other hand, is also capable of increasing endogenous defense systems by inducing phase II detoxifying enzymes (such as increasing the expression of manganese-superoxide dismutase) via nuclear factor 2 (Nrf2) activation (40,42,55) as represented in **Figure 6**.

Nrf2 is regulated by its interaction with Keap1 that directs the transcription factor for proteasomal degradation of Nrf2. When one or more critical cysteine residues of Keap1 are modified (by oxidative stress for instance) Nrf2 escapes from Keap1 inhibition and is translocated to the nucleus where it interacts with the protein small Maf (sMaf) forming Nrf2-sMaf heterodimers that bind to the antioxidant responsive elements (ARE) to regulate the gene expression of several phase II detoxifying enzymes. HT is able to induce the Nrf2 translocation to the nucleus and to increase antioxidant enzymes expression (42).

Moreover, polyphenols act like caloric restriction mimickers. Caloric restriction is capable of reducing the risk of suffering age-associated diseases and can extend lifespan. Nevertheless, caloric restriction is difficult to be maintained for long periods. Plant polyphenols are able to mimic caloric restriction effects by affecting their cellular targets, mainly through the activation and increased levels of sirtuins. Sirtuins are involved in lifespan and metabolism regulation, Sirt1 protects cells against oxidative stress and DNA damage as it activates Nrf2 and reduces inflammation (2).

Tyr is also studied for its potent activity as antioxidant; however, HT and Tyr have different antioxidant profiles as they act through different mechanisms. This could be due to the fact that they have different chemical structures: HT has two OH groups and Tyr only has one (3).

OLE and its aglycone (40), similarly to HT, increase superoxide dismutase, catalase and glutathione peroxidase activities in brain aged rats (antioxidant defenses) (42).

The phenolic content of olive oil modulates the degree of lipid and LDL oxidation; being lower after an ingestion of high content phenolic olive oil, this represents a beneficial effect in the prevention of cardiovascular diseases. It is therefore important to have a high intake of antioxidants in the diet because they are key factors in the pathogenesis not only of neurodegenerative disorders but also of all kind of oxidative stress related disorders (41).

5.8.4.5. Inflammation

In olive oil, both MUFA fat and phenolic compounds have anti-inflammatory properties; in fact, EVOO rich in polyphenols has shown in some studies to be more effective in lowering LTB₄ and TXB₂ (important metabolites in inflammation) than refined olive oil with lower phenolic content (41).

Oleocanthal can display a non-steroidal anti-inflammatory activity with similar properties to ibuprofen (28): it is capable to inhibit COX-1 and COX-2 activity (9,42). During the development of the disease there is overproduction of prostaglandins that contribute to the neuropathology, thus the external support with natural phenols that are able to inhibit

the enzymes responsible of the production of prostaglandins (COX-1 and COX-2) may be helpful in the prevention and treatment of AD (40).

Diet supplementation with OLE produced migration of microglia to the amyloid plaques favoring the phagocytosis of the amyloid deposits and in addition, reduced the astrocyte reaction. In other words, it helped reducing the inflammation present in AD (2,26,40).

Moreover, EVOO polyphenols, in particular OLE, are able to regulate the mTOR pathway, whose downregulation leads to FOXO3 activation with the subsequent transcription of homeostatic genes that favor longevity and reduce inflammatory genes. mTOR is one of the most potent upstream regulators of autophagy; when autophagy is genetically inhibited degenerative modifications are produced as in AD. As olive oil polyphenols are able to participate at this level this is one more beneficial effect against the development of AD (2).

All this data from different studies supports the protection of olive oil against neurodegeneration (2). The results of clinical trials sometimes are not definitive and in some cases may also seem contradictory. This is probably a result of the many different factors (not only diet-related) involved in the pathogenesis and clinical progression of AD, as well as the difficulty of controlling that people follow precisely the planned diet for long time periods. Also, several biological interactions might occur between nutrients and lead to synergistic effects and finally, it might also be due to the extreme variability in individuals (7,40). However, there is considerable evidence from animal and first human studies that suggest the neuroprotective effects of polyphenols in preventing or even reversing changes in cognitive and motor functions (33).

5.8.5. Epidemiological studies with olive oil and its polyphenols

Epidemiological studies have an important role as they direct the attention towards observed phenomenon's opening new territories for experimental research (22). Some of the most important population-based studies with olive oil are the "Three-City Study", PREDIMED, PREDIMED-NAVARRA and EUROLIVE.

The "Three-City Study" was a multi-center cohort study, produced in three French cities and designed to estimate the risk of dementia and cognitive impairment. This study showed a lower incidence of cognitive deficit in those subjects that used olive oil moderately (just for cooking) and intensively (for dressing and cooking) in contrast to those who never used it (2,50,56).

The results of this study were confirmed by two multicenter, randomized, controlled trials: PREDIMED (PREvención con Dieta MEDiterránea) and PREDIMED-NAVARRA, they were carried out in Spain with people at high cardiovascular risk. PREDIMED was a prevention trial originally designed to test the long-term effects of the MD on the incidence of cardiovascular diseases, but some readjustments were made and it finally also tested the cognitive performance. It showed that an intervention with a MD enriched in EVOO improved cognition, and that this result was mainly due to the EVOO phenolic content. It also suggested that the consumption of EVOO is associated with a reduced risk of cardiovascular disease (2,57).

PREDIMED-NAVARRA investigated after 6, 5 years of nutritional intervention diet–gene interaction across different genetic profiles and the effect on cognition; it was a randomized trial in which subjects were divided in three groups depending on the diet. It

revealed that cognitive performance was superior for non-ApoE4 and for ApoE4 carriers of the MD groups compared to controls. This lead to the conclusion that an intervention with MD modulates the effect of genetic factors on cognition and this effect might be greater in subject's carriers of a more favorable genetic profile (1,2).

EUROLIVE (effect of olive oil consumption on oxidative damage in European populations) was a large, crossover, multicenter, clinical trial carried out in 200 individuals from five European countries. Subjects where divided in three groups depending on the phenolic content of the olive oil they daily took. It was seen a linear decrease of the total cholesterol/HDL-cholesterol ration and of oxidative stress markers with the increase of the phenolic content of olive oil (2,41).

These epidemiological studies corroborate the hypothesis that polyphenols in the MD are capable of reducing the risk of suffering from age related neurodegeneration.

6. CONCLUSIONS

AD is characterized by extracellular senile plaques with amyloid-beta peptide deposits and intracellular neurofibrillary tangles with hyperphosphorylated tau-protein aggregates, which ultimately cause neuronal death (58). These changes begin years before clinical symptoms emerge and when they do, there are multiple stages.

As the actual therapeutic targets only provide symptomatic benefits and modifiable lifestyle-related factors are associated with cognitive decline, it seems reasonable directing research towards dietary prevention. MD is associated with lower rates of cognitive decline, more concretely the polyphenols present in olive oil seem to have an important role at this point. The actual evidence of the effective activity of natural phenols in dementia prevention is still limited, however epidemiological and clinical evidence is increasingly available. Yet, what is clear is that the best option rather than single nutrients is a balanced diet, caloric restriction, physical activity and adequate rest (2).

Polyphenols in olive oil are known to act in several steps of AD pathogenesis. They are able to inhibit AChE rising levels of the neurotransmitter acetylcholine and improving synapses, they are also capable of redirecting the aggregation towards a path where no oligomeric or nontoxic oligomeric intermediates are formed; even they can also disaggregate performed fibrils. Moreover, they enrich mitochondrial membranes with less oxidizable fatty acids avoiding their dysfunction and reduce the oxidative damage as they supplement the endogenous antioxidant defense system. Finally, they also have an anti-inflammatory activity, favor the phagocytosis of amyloid deposits and reduce the astrocyte reaction. The major polyphenols present in olive oil are Tyr, HT, OLE and Oleocanthal; yet not every polyphenol is able to act in every step of the pathogenesis.

Even though research in polyphenols has been rapidly growing, there is still necessary further research to be done; as AD is a complex disease and there are some pieces of the puzzle still missing. To help developing diagnoses and treatment is necessary to establish the precise mechanisms involved in the onset and progression of the disease, as well to develop early and accurate biomarkers. Moreover, some of the polyphenols molecules display different *in vitro* and *in vivo* activities so it is always necessary to verify the discoveries in living organisms.

The effective daily dose of olive oil polyphenols to be administered to humans to obtain health benefits is still lacking, however the amount of polyphenols present in foods is not adequate to ensure daily doses to get short term effects: it is necessary the continuous assumption of these in long term or to increase the intake of these substances by assuming specific nutraceutical products (like an EVOO enriched in polyphenols). In addition, some solutions are rising up to increase the limited bioavailability of plant polyphenols and help to obtain the neuroprotective effect such as polyphenol encapsulation inside nanoparticles.

The protection provided by olive oil polyphenols goes beyond their known antioxidant activity, therefore to understand their exact mechanism it is important to consider all of their properties. In fact, it is thanks to their several effects why they are interesting as potential compounds to combat AD; because of their multi-functionality. Olive oil consumption, particularly EVOO because of its higher phenolic content, with a controlled intake, may help in the prevention of AD.

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