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Alzheimer‘s disease: exploring nature’s ‘medicinal chest’ for new therapeutic agents

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Abstract: Natural products have served humanity as a valuable source for the discovery and development of therapeutic agents. In addition, these phytochemicals can function as lead compounds for the development of synthetic analogs aimed at treating human diseases. In our aging society, Alzheimer’s disease (AD) is the most common cause of dementia, which is characterized by a significant and progressive loss of memory and other cognitive functions. As society demographics change, the predominance of AD and other age-related dementias is increasing, with concurrent financial and societal costs.

AD represents one of the most remarkable scientific challenges for drug discovery as the search for effective disease-modifying agents has been unsuccessful. Medicinal plants have been used for their “anti-aging” properties, and cognitive enhancing properties. In the past decades, natural products have been studied for their anti-AD properties, and their potential for developing therapeutic agents against several molecular targets has been evaluated. This insight evaluates the prospects of medicinal plants for providing disease-modifying, as well as disease-preventing, agents for AD.

Keywords: Alzheimer’s disease; natural products; amyloidogenesis; protein aggregation inhibitors; *Crocus sativus* L.

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Alzheimer’s Disease: Origin and Search for Disease-Modifying Agent

Alzheimer’s disease (AD) is the most common cause of senile dementia in our aging society, and it is characterized by a gradual and harmful decline in cognitive and non-cognitive function. The World Health Organization estimates that AD is the fourth leading cause of death affecting more than 47.5 million people worldwide, while there is a new case of dementia worldwide every 3 seconds with numbers projected to double every 20 years [1,2]. Therefore, it is predicted that the number of people over the age of 65 with AD in the United States will at least triple to > 15 million by 2050 from 5 million currently affected [3]. The pathological process is believed to begin 10–20 years before the first clinical symptoms arise, whereas the average lifespan of sufferers is between 7-10 years from diagnosis, and no cure is presently known. Furthermore, the majority of AD sufferers will require personal care with their needs starting early in the disease course and constantly growing over time, thus leading to high cost and contributing to widespread consequences for families, health-care systems, and society as a whole [4]. Therefore, finding ways to prevent and reverse this trend is critical and represents a worldwide priority [1].

The etiology of AD and the understanding of the disease mechanism remains elusive, and several risk factors have been proposed to account for the pathological characteristics of AD, including neuronal loss and disintegration of the neural circuits. The principal key factors are oxidative stress, inflammation, deposition of abnormal protein aggregates in the brain, and metal deposition [5]. There are several excellent reviews on the current knowledge of the underlying mechanisms, pathophysiology, epidemiology, symptoms, diagnosis, and treatment strategies of AD [6-8]. Over the last twenty years, there has been remarkable progress in our knowledge of the underlying cause of the disease, which in turn has generated more insights into where and when we can intervene. It is believed that abnormal accumulation of misfolded beta-amyloid (Aβ) peptide in

the hippocampus and entorhinal cortex is the initial and central event of the AD pathogenesis [9,10]. In addition, aggregation of hyperphosphorylated tau protein leading to intraneuronal neurofibrillary tangles (NFTs) is also considered to play a central role in AD neurodegeneration [11]. In the first formulated '*amyloid cascade hypothesis*' of AD [12], abnormal accumulation and aggregation of the Aβ peptide derived from the amyloid precursor protein (APP) by a sequential enzymatic reaction with β- and γ-secretases lead to the formation and cerebral deposition of amyloid plaques. In the second *'tau and tangle hypothesis'*, aggregation of hyperphosphorylated microtubule-associated tau protein results in NFTs, leading to disruption of axonal transport and neuronal dysfunction [13]. These senile plaques and neurofibrillary tangles have been recognized as the two key pathological hallmarks of AD [14], and they are also associated with increased levels of oxidative stress, inflammation, and nerve cell death [15]. It should be noted that these AD causative proteins have cell-to-cell spreading properties similar to those of prions [16], with the exception that the resulting disease (AD) is not communicable.

Since 1990, there has been tremendous growth in research into the disease mechanisms (*basic biology)* of AD combined with significant pharmaceutical industry efforts to develop therapeutic or even preventing agents for AD. Nevertheless, these drugs are unable to stop or reverse the progression of the disease, and they provide only symptomatic relief in stabilizing or improving cognitive impairment of AD. Overall, poor understanding of the pathophysiology of this neurodegenerative disease accounts for inadequate definition, difficulty in diagnosis, and lack of effective drugs. The first approved and used agents for pharmacological therapy of AD-associated dementia is a group of three acetylcholinesterase (AChE) inhibitors, donepezil, rivastigmine, and galantamine [17], whereas memantine, a N-methyl-D-aspartate (NMDA) receptor antagonist, is also used in clinical practice [18] (**Table 1**). All these drugs are approved for mild to severe dementia and seem to provide some symptomatic relief, i.e., alleviate the cognitive symptoms of AD patients and improve the quality of life, but there is no clear evidence to halt or delay the progression of the disease. The disappointing clinical results of the aforementioned drugs has prompted the search for real disease-modifying agents. Of course, that reemerged the question of the best suitable drug target for curing or preventing the disease. Researchers have been divided between the two most prominent molecular pathways for AD pathogenesis, the Aβ peptide and the hyperphosphorylated tau hypotheses. It is postulated that these molecular mechanisms leading to the formation of amyloid plaques and neurofibrillary tangles, respectively, may act in parallel or in combination with each other in the development of AD [19,20]. There is also direct support of the hypothesis that altered Aβ metabolism precedes tau-related pathology and neuronal degeneration in humans [9]. Current and future research could provide more insights into the interdependence of the aforementioned Aβ and tau hypotheses and determine the distinct, common, or partly overlapping mechanisms for impairing neuronal functions.

In the past 20-30 years, several efforts have been made towards developing therapies targeted at the two protein culprits for AD pathogenesis. Several drug candidates have been developed by interfering with the two prevailing mechanisms that drive the development of the disease, Aβ- and tau-pathology (**Table 1**). Some of these targets are single drug compounds or molecular and cellular strategies to interfere with the deleterious consequences of the accumulation of toxic proteins and block neuronal cell death.

To date, most of the disease-modifying therapies for AD involve Aβ-targeted approaches focused on altering the production, deposition and clearance of Aβ according to the '*amyloid cascade hypothesis*' of AD [12] (**Fig. 1** shown by ⚡**)**. Similarly, major therapeutic approaches addressing tau pathology include anti-phosphorylation strategies and aggregation inhibitors (**Fig. 1** shown by ⚡**)**. The former involves inhibition of abnormal hyperphosphorylation through tau kinase inhibitors such as glycogen synthase kinase-3β (GSK-3β) inhibitors, whereas the latter involves the prevention of aggregation by derivatives of methylene blue, such as the derivative Rember TM. Rember TM monotherapy in mild and moderate AD patients has shown cognitive benefits although with side effects. Subsequently, a second-generation derivative of this tau protein aggregation inhibitor called TRx0237 (LMTM) is currently in clinical trials [6] (**Table 1**).

The accepted central role of the '*amyloid cascade hypothesis*' and the amyloid plaques in AD pathogenesis [12] has steered research towards the development of AD therapies by preventing or reducing the Aβ levels in the brain [21,22], including inhibition of amyloid formation by controlling APP proteolytic cleavage (β- and γ-secretase inhibitors) [23,24]. It is worth noting that β-secretase (BACE1) inhibitors are emerging as a potential target (perhaps better than those for γ-secretase) for treating AD and there are several BACE1 inhibitors in different phase trials (www.alzforum.org/therapeutics). Other therapeutic alternatives include control of Aβ degradation [25], immunization with Aβ [26], inhibition of Aβ aggregation, and/or stimulation of

Table 1: Current Disease-Modifying Therapies for Alzheimer's Disease.*

Target type	Mechanism	Drug Name	Status
Cholinergic	AChE inhibitor	Donepezil	Approved
Cholinergic	AChE inhibitor	Rivastigmine	Approved
Cholinergic	AChE inhibitor	Galantamine	Approved
Glutaminergic	NMDA receptor antagonist	Memantine	Approved
Aβ clearance	Soluble Aβ oligomers	Aducanumab (Biogen, Eisai)	Phase III
Aβ clearance	Aβ Oligomers and Fibrils	Crenezumab (Genentech)	Phase III – Discontinued
Aβ clearance	Soluble monomeric Aβ	Solanezumab (Lilly)	Phase III – Discontinued
Tau aggregation	Tau aggregation Inhibitor	TRx0237 (LMTM)	Phase III
Neuroinflammation	Microglial activation inhibitor	Azeliragon	Phase III

* This Table lists approved drugs or drug candidates discussed in the article.

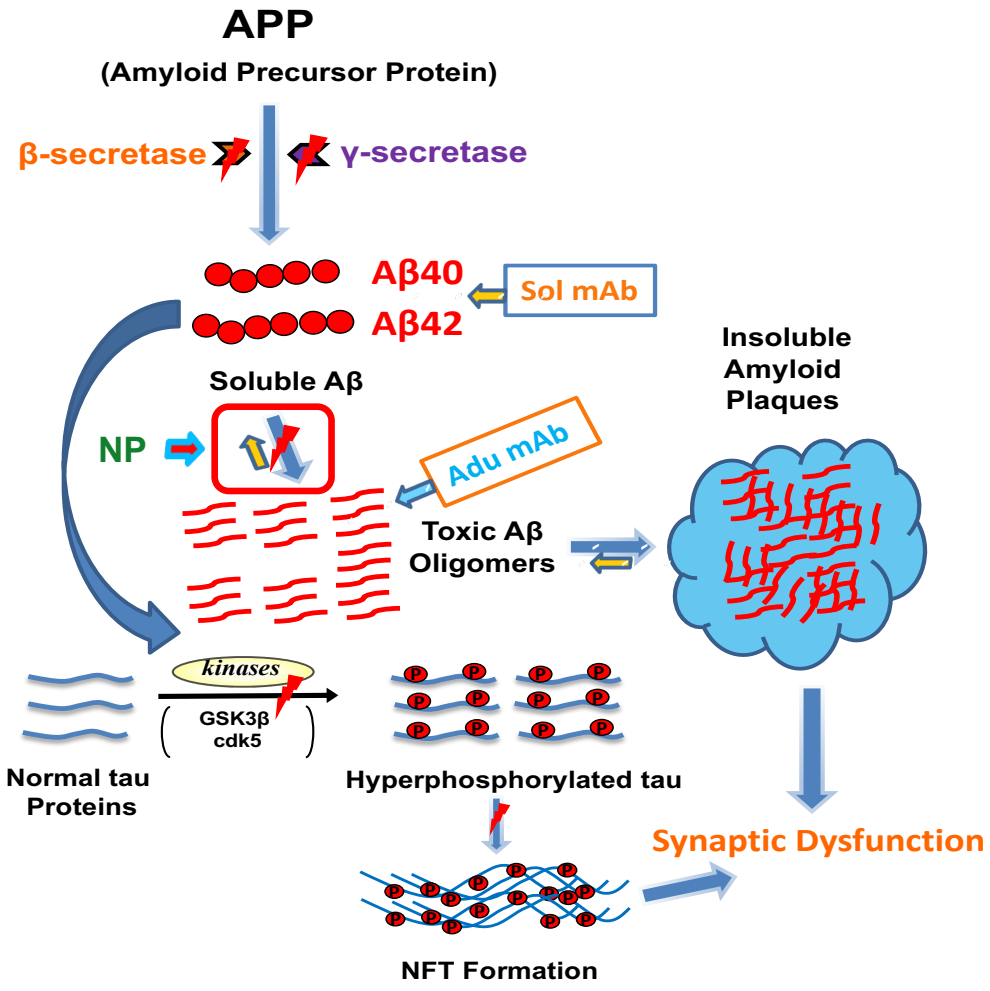


Figure 1: Alzheimer's prominent mechanisms and interventions (⚡) of most disease-modifying therapies for AD. NP: Natural Product; Solanezumab (Sol mAb) and Aducanumab (Adu mAb) are immunotherapies targeting soluble monomeric Aβ and soluble Aβ aggregates and insoluble fibrils, respectively.

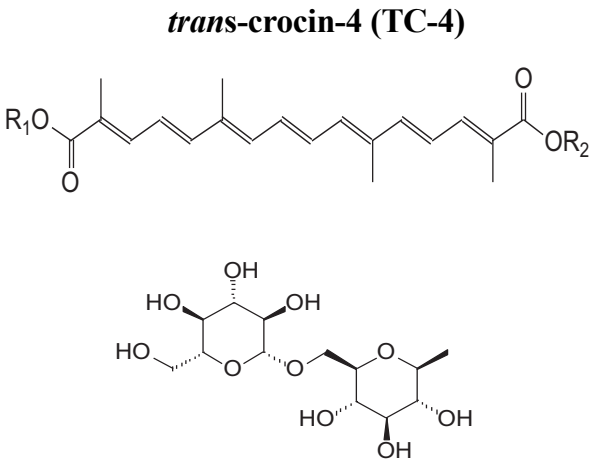
its disaggregation by molecules that potentially bind on the peptide and stabilize its structure; hence, becoming potential inhibitors of amyloidosis [27,28]. Moreover, anti-inflammatory agents have been recently investigated as a potential therapeutic approach, such as the microglial activation inhibitor Azeliragon. Azeliragon has been shown to reduce brain Aβ levels and improve cognitive performances in animal models [29] (**Table 1**). An update on AD therapeutic agents, including those targeting aggregated Aβ, aggregated tau, and neuroinflammation, is summarized in recent reviews [6, 29].

In another approach, inhibiting the formation of oligomeric fibrils and aggregates of Aβ has been examined as a therapeutic option, as recent studies suggest that the soluble, oligomeric form of Aβ is more toxic to neurons than the mature fibrils. The Aβ oligomers induce synaptic deterioration and neurodegeneration [30,31], and the cerebral concentration of soluble oligomeric Aβ correlates with the severity of AD [32]. Therefore, in addition to the prevention of Aβ production by modulating the α-, β-, or γ-secretase activities, the inhibition of Aβ aggregation and destabilization of pre-formed Aβ aggregates are also attractive therapeutic targets as anti-AD agents (**Fig. 1)**. Several drugs, known as monoclonal antibodies (mAb), may prevent beta-amyloid from clumping into plaques and help the body clear the beta-amyloid from the brain. The status of ongoing clinical studies of anti-Aβ therapies for AD and related disorders was the focus of a recent review [33]. These trials include active and passive anti-Aβ immunotherapies. The former involves the administration of an Aβ antigen that can stimulate an immunological response against Aβ. The latter consist of monoclonal anti-Aβ antibodies such as solanezumab and crenezumab that recognize soluble monomeric Aβ and oligomeric Aβ, respectively [33]. Early research on the solanezumab (Sol mAb), which binds to the soluble monomeric Aβ and Aβ plaques (**Fig. 1**) found a possible benefit for people with mild-to-moderate AD. Nevertheless, recent results of the EXPEDITION3 phase III trial in patients with mild AD showed no significant slowing in cognitive decline compared to placebo, thus leading to the drug's abandonment [34]. On the other hand, aducanumab (Adu mAb) that binds to soluble Aβ aggregates and insoluble fibrils (**Fig. 1**), has shown promise in the preliminary phase III ENGAGE and EMERGE studies for the treatment of prodromal AD. Patients who received aducanumab experienced significant improvements related to cognition and function, including memory, orientation, and language, thus leading to filing for market approval for an investigational treatment for early AD [34,35].

Natural Products as Disease - Modifying Agents for AD

Natural products (NP) have played a dominant role in the discovery of lead compounds and the synthesis of structural mimics thereof, which may serve as potential therapeutic agents against several diseases, such as cancer and neurodegenerative diseases. In particular, 63% of the small molecule drugs developed from 1981 to 2006 are natural products or natural product-derived compounds [36]. These reports suggest that natural products have a strong potential to be the source of bioactive compounds with anti-amyloidogenic activity. It should be noted that some of the AD drugs available are natural products or mimics thereof, e.g., the AChE inhibitor galantamine, which is an alkaloid obtained from the bulbs of *Galanthus nivalis*. In addition, several clinical studies using the standardized formulation of *Ginkgo biloba* L. extract EGb 761® have shown a statistically significant advantage of the *Ginkgo biloba* extract compared to placebo in improving cognition of AD patients [37]. Therefore, it was envisioned that natural products might produce biologically active compounds against AD. In particular, plants from the Mediterranean basin, a global biodiversity "hot-spot" [38], in which only the southern part of Greece offers 6,000 plant species, and 1,200 endemics [39] are worth investigation.

In view of the suggested mechanistic link between oxidative stress, inflammation, and neurodegeneration [40], neuroprotection by plant-derived and dietary antioxidants may offer a motivating therapeutic route for protection against the risk of AD [36,41,42]. Some natural products, such as *Ginkgo biloba* L. extracts and epigallocatechin-3-gallate (EGCG), the main phenolic constituent of green tea, have been reported to enhance the non-amyloidogenic pathway by promoting α-secretase proteolytic process, thus increasing the production of α-secretase cleaved sAPPα fragments [43,44]. On the other hand, resveratrol, a polyphenol that occurs in abundance in grapes and red wine, and derivatives thereof have been reported to exert its neuroprotection by inhibiting the amyloidogenic pathway and enhancing the Aβ clearance pathway in AD [45]. Recent studies have shown that resveratrol's neuroprotective effect is largely due to its antioxidant and anti-inflammatory properties and the attenuation of the Aβ-induced oxidative stress [46,47]. Another "natural arsenal"- derived Aβ oligomer inhibitor is 1,2,3,4,6- penta-O-galloyl-β-D-glucopyranose isolated from *Paeonia suffruticosa* Andrews (Paeoniaceae) [48]. In addition, curcuminoids such as curcumin and



a. b.

Figure 2. Chemical structure of the bioactive component trans-crocin-4 (TC-4) isolated from the stigmas of *Crocus sativus* L. (a). TC-4 (b) is one of the major crocins of saffron and is chemically a crocetin molecule esterified with two 1β-D-gentiobiosyl units GB (R1 and R2 = GB).

demethoxycurcumin inhibit the aggregation of Aβ into oligomers (Aβ aggregation inhibitor). The potential of some plant-derived natural compounds as β-secretase and γ-secretase inhibitors, as well as Aβ aggregation inhibitors, has been previously reported [49].

In recent studies, several bioactive phytochemicals derived from plants endemic in Greece were screened for forming noncovalent complexes with the Aβ peptide, which is one of the alleged protein culprits for AD pathogenesis. These bioactive compounds have been derived from plants which are constituents of the Mediterranean diet that has been associated with various health benefits and decreased risk of many diseases, such as cardiovascular and neurodegenerative diseases [50]. The screening of these natural compounds in terms of their binding ability to Aβ was performed to identify potential inhibitors for the Aβ peptide aggregation, especially the Aβ oligomer formation. The latter is more significant due to the neurotoxicity of the soluble Aβ oligomers, which seems to play a pivotal role in AD pathogenesis. Our research efforts to identify potential aggregation inhibitors for the Aβ peptide incorporated screening of the noncovalent interaction between Aβ and several natural products by electrospray ionization (ESI) mass spectrometry (MS) and spectroscopic techniques, such as NMR. This *in vitro* screening was complemented with cell viability assays using differentiated neuronal SH-SY5Y cells to assess any potential toxic effects of the selected substances. The formation of 1:1 noncovalent complex of Aβ with certain antioxidants such as the endogenous antioxidant melatonin (M) [51] and oleuropein (OE) [52] has been previously demonstrated by ESI MS, while their interaction with the hydrophobic region of the peptide has been reported by ESI MS proteolytic mapping studies [53] and NMR [54]. More promising candidate bioactive compounds were isolated from *Crocus sativus* L., which is cultivated in Greece for its red styles (saffron) **(Fig. 2a)**. The major *C. sativus* L.-derived bioactive constituents are crocins, which are mono- and bis-ester glycosides of crocetin [trans-crocin-2 (TC-2), trans-crocin-3 (TC-3) and trans-crocin-4 (TC-4)], with TC-4 representing the most abundant crocin component **(Fig. 2b)**. Similar noncovalent interactions between the Aβ peptide and the main crocin components TC-2, TC-3 and TC-4, were also observed in this study [55]. The specific nature of these noncovalent interactions was also evaluated at low concentration levels, i.e., 5-100 μM, where the occurrence of nonspecific aggregation in the gas-phase can be prevented. For example, the ESI signals arising from the noncovalent interactions of Aβ with OE and crocins were present for all concentration levels, thus indicating a very specific interaction. Furthermore, the noncovalent complexes of Aβ with OE and TC-2/TC-4 showed considerable stability even under experimental conditions, which usually do not favor noncovalent interactions [52].

In an integrated approach, the *C. sativus* L. natural compounds TC-4 and *trans*-crocetin (CRC) were selected for in-depth molecular characterization of their potentially protective effects against AD, utilizing two AD neuronal cell culture models (SHSY5Y overexpressing APP and PC12 expressing hyperphosphorylated tau). TC-4 significantly decreased β-secretase, a key enzyme of the amyloidogenic pathway, and APP-C99, while it decreased γ-secretases that generate toxic beta-amyloid peptides. On the other hand, it proved effective in suppressing the active forms of GSK3β and ERK1/2 kinases and significantly reducing total tau and tau phosphorylation. Therefore, these studies demonstrated a potent effect of TC-4 and CRC in suppressing key molecular pathways of AD pathogenesis, rendering them a promising tool in preventing and potentially treating AD [56]. In subsequent *in vivo* studies of these bioactive antioxidants, the bioavailability of TC-4 in plasma has been demonstrated after *i.p.* administration, along with the detection of TC-4 in mouse brains for the first time; thus, providing preliminary evidence on TC-4's ability to penetrate the BBB and localize inside the brain, albeit its extremely hydrophilic character [57]. Furthermore, recent studies incorporating ESI - ion mobility spectrometry MS, electron microscopy, and Thioflavin-T kinetics revealed that interaction of these plant-derived compounds, such as TC4, prompted a substantial alteration in the monomer/oligomer distribution of Aβ1-40 accompanied by a re-direction of the Aβ aggregation pathway [58].

Future Perspectives

In the last two decades, there have been major efforts to understand the basic biology and mechanisms underlying the pathology of AD. Current and future research could provide more insight into the two prevailing Aβ and tau hypotheses and determine the distinct, common or partly overlapping mechanisms leading to neuronal impairment. A better understanding of the Aβ amyloid and the tau pathways has been the driving force behind drug discovery research. The significant obstacles for drug development in AD arise from the lack of prognostic and diagnostic biomarkers of the disease, along with the extremely long, symptom-free prodromal phase of AD. Consequently, there is a high failure rate of drugs which pass into clinical trials, and no available curative treatments for AD. That has increased the health care and the direct societal cost, especially if we consider the drastic change of the demographics in many societies. Therefore, there is an urgent need for disease-modifying therapies for AD, as well as for developing and testing drugs for disease prevention. Moreover, the early diagnosis of dementia renders the identification of novel and validated biomarkers as the key to AD prevention, and timely and appropriate care, thus improving the lives of patients and their families. The development of novel and robust biomarkers and diagnostic tools for AD will also assist the proper design of clinical trials for disease-modifying agents, mainly due to the long prodromal phase of AD. Any new disease-modifying therapies will be more effective when initiated during the early stages of the disease, thus making the early detection of prodromal AD very important.

Medicinal plants can provide a valuable source of bioactive natural products that can serve as therapeutic or disease preventive agents for AD. They can also serve as *scaffolds* for providing structural mimics of natural compounds through various synthetic routes. These NPs can target the Aβ and tau therapeutic targets, as well as other AD-correlated targets such as neuroinflammation. They can regulate specific molecular pathways in AD pathogenesis, i.e., modulate the amyloidogenic pathway as β-secretase and γ-secretase inhibitors, as well as inhibit the formation of Aβ oligomers which are thought to be the most neurotoxic form of Aβ. In addition, the NP interaction may be useful in promoting a substantial alteration in the monomer/oligomer distribution of the Aβ peptide and re-directing the pathway of Aβ aggregation [58]. Finally, these dietary antioxidants could not only be a promising way to prevent amyloid toxicity, but also provide a combinatorial treatment strategy for AD as they target different pathogenic pathways (e.g., amyloid/tau, inflammation, oxidative stress); thus, slowing disease progression or even successfully preventing the disease from advancing to dementia.

In that respect, nutraceutical intervention through specific diets could be an effective means for preventing and reducing the risk of age-associated neurodegenerative diseases [59,60]. People adopting specific diets, such as the Mediterranean diet and Asian diet, usually receive moderate amounts of natural antioxidants, e.g., polyphenolic and carotenoid substances, continuously during their lifespan. In particular, higher adherence to the Mediterranean diet has been associated with a lower risk of age-associated diseases, particularly cardiovascular diseases and neurodegeneration [61]. Recent results from the HELIAD study [62] suggest that adherence to the Mediterranean diet is associated with better cognitive performance and lower dementia rates in Greek elders; thus, forming a worldwide prototype that may provide cognitive benefits. Finally, we should

emphasize that more research is required to enhance the bioavailability and BBB permeability of the Mediterranean diet constituents, which are potent antioxidant and anti-inflammatory agents.

Conflict of interest: The author states no conflict of interest.

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