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Review

Nutraceutical intervention in ageing brain

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Human brain ageing is associated with a number of specific neurobiological changes. As we age, the reduced neurogenesis in specific brain areas contributes to neural loss and brain shrinkage with an increased incidence of stroke, white matter lesions, and dementia. With age, myelin loss contributes to the decline of sensorimotor and cognitive processes. Moreover, β-amyloid storage, neurofibrillary tangle formation, lipofuscin accumulation, are well known physio-pathological age-related disorders. In neurons, ageing-related metabolic changes involved a switch from aerobic glycolysis to oxidative phosphorylation. Mitochondrial dysfunction and oxidative stress are two damage mechanisms that play a central role in brain ageing.
Dietary interventions have been identified as potential means to prevent biological ageing and the related cognitive decline. Increased consumption of specific nutrients such as polyphenols, fish and seafood, vitamins and oligoelements has demonstrated protective effects by targeting specific cellular markers and cellular functions. The work presented here describes patho-physiological alterations associated with brain ageing, with insights into the cellular mechanisms underlying this process. We also review recent relevant experimental and clinical data regarding the effects of supplemental substances (i.e., polyphenols, vitamins, oligoelements, ω-3 polyunsaturated fatty acids) that have demonstrated encouraging therapeutic properties on neurodegenerative processes implicated with brain ageing.

Key words: Ageing, Brain, Alzheimer's disease, Nutraceuticals, Oxidative stress, Parkinson's disease

INTRODUCTION

The human brain is constituted by a multitude of cell types each of which further divided into additional subclasses with distinct morphological and molecular differences, and different degrees of specialization. These cells include resident innate immune cells, the microglia, as well as other glial cells of support, such as astrocytes and oligodendrocytes, and highly differentiated cells, the neurons. Neurons have a significant homeostatic control of the essential physiological functions like propagation and generation of electrical and chemical signals, while glia function mainly to modulate neural functions. Insults and injuries that affect this homeostatic maintenance are observed during ageing. The connection between these modifications and the onset and progression of age-related diseases is profound as the incidence of such diseases like neurodegenerative disorders rise steeply with age. This raises the possibility to reduce or postpone the incidence of these pathologies by targeting ageing. Molecular, cellular, anatomical, and neurochemical changes are all associated with ageing and age-associated diseases [1]. Understanding the molecular mechanism underlying these processes could provide a wealth of opportunities for the development of anti-ageing therapies.

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STRUCTURAL AND METABOLIC CHANGES IN BRAIN DURING AGEING

The effects of ageing on the brain include specific structural changes, such as thinning of the cortex and reduced volume. Thickening of the arachnoid and prominence of the arachnoid granulations in the meninges as well as increased ventricular volume have been described [2]. As we age, the volume of the brain and/or its weight decline [3]. The shrinking of the grey matter can be due to different aspects such as death of neuronal cells and/or decline in neuronal volume, decrease in dendritic arbour, spines and synapses. Selective loss of neurons in specific neuroanatomical areas like hippocampus and prefrontal cortex has been described by several authors [4]. The most substantial shrinkage is observed in the prefrontal cortex, where the evidence of a relation between volume shrinkage and ageing deficits in executive and working memory function has been found [4]. Neuronal loss is closely associated with microglia-mediated neuroinflammation and *post mortem* studies have indeed suggested that activated microglia may be present in the ageing brain [5].

Histopathological and imaging studies reveal that brain ageing is associated with the degradation of white matter in specific brain regions. Myelin sheaths deteriorate during normal ageing, with a regional predominance in late myelinating regions of the frontal lobes followed by the temporal lobes [1]. White matter loss might represent the predominant neuroanatomic change in normal human ageing. Myelin loss contributes to the cognitive deficits in aged individuals, since myelin is necessary for the efficient and rapid conduction of impulses along axons [6]. These and other studies clearly correlate the cognitive impairment observed in the elderly with the decline in grey and white matter structures in the brain, indicating a complex interplay between specific brain structures and cognitive decline.

Studies in rodents demonstrated that ageing is associated with a reduction of hippocampal neurogenesis that is responsible for the decline in memory functions [7]. In fact, hippocampus plays an important role in learning and memory and in spatial navigation. However, other studies do not support this correlation and propose that defects in the dendritic structures as well as changes in the rest of the neuronal network may be responsible for the ageing-related cognitive decline [7].

Various transmitter systems are affected by ageing, with implications on cognitive declines that are associated with normal and pathological ageing. During ageing, changes in cholinergic, serotonergic, dopaminergic, noradrenergic and glutamatergic systems render neurons vulnerable to impaired neurotransmissions [8-10].

In rats, the expression of ionotropic Gamma-Amino Butyric Acid (GABA) receptors, glutamate decarboxylase, the rate-limiting enzyme synthesizing GABA, and GABAergic neurons decrease with age [11]. GABA is the main inhibitory neurotransmitter in the mammalian central nervous system (CNS), with an important role in regulating neuronal excitability. Age-related cognitive decline could be explained, at least in part, to decrements in GABA inhibitory neurotransmission [12].

Studies also suggest age-related changes in dopamine levels and decline in dopaminergic neuromodulation [9 13]. This affects the fidelity of neural information and gives rise to less distinctive neural pattern representations that may underlie various facets of ageing cognitive and, possibly also, sensorimotor phenomena [13].

Serotonin (5-hydroxytryptamine, 5-HT) is a well known monoamine neurotransmitter that modulates/regulates different neural and behavioural activities [14-18]. Both the level of 5-HT [19] and 5-HT receptor [20] has been found to decrease during ageing [9]. Ageing also reduces the activity of different 5-HT receptor subtypes [21] such as 5-HT2A which is involved in learning [22], neuroendocrine function [23] and sleep behaviour [24]. In mammals, decline in serotonin function with ageing may account for several behavioural disturbances, such as sleep, sexual behaviour and mood [19].

Like brain serotonin, alterations of another endogenous dipeptide (β-Ala-L-His), known as carnosine, have been observed during ageing [25]. The decreased level of carnosine in olfactory bulb has been associated with the lost of smelling sense [26 27]. Aerobic glycolysis, i.e. the exclusive use of glycolysis despite the presence of oxygen, predominates in the developing brain during embryogenesis to provide biosynthetic materials necessary for the proliferation of neuronal and astrocytic stem cells [28]. Within certain regions of CNS, aerobic glycolysis seems to be associated with long-term memory formation and the maintenance of long-term potentiation of synaptic strength [29]. It is likely that astrocytes can be the main site of aerobic glycolysis into the brain and lactate produced by these cells can be then utilized by mitochondrial neurons as a fuel source for oxidative phosphorylation (OxPhos) (astrocyte-neuron lactate shuttle hypothesis) [30]. During ageing, a shift from aerobic glycolysis to OxPhos has been demonstrated in human brain [31], with a consequent loss of cell survival mechanisms that counter pathogenic processes underlying neurodegeneration [28]. Thus, aerobic glycolysis is predominately used to support axon growth and myelination during childhood whereas OxPhos is primarily used throughout human lifespan to support synaptic activity with a progressive increase in activity during ageing [28].

However, glucose may be also taken up by neurons and routed preferentially toward the pentose phosphate

pathway for antioxidant production, in addition to biosynthetic pathways required for growth and synaptic remodelling [32 33].

The delicate balance between glycolysis and cellular respiration might have a central role in ageing, inflammation and disease [34-36]. Such changes may also be induced by a reduced input of glucose or oxygen as a consequence of falls cerebrovascular efficiency in the ageing brain. Mildly impaired glucose availability in the synapse may be sufficient to impair cholinergic neurotransmission, being acetylcholine synthesis acutely sensitive to brain glucose metabolism [37 38].

Ageing is also associated to changes in sex hormone status, which in turn can affect cognitive performance [1]. This aspect is particularly relevant in women at menopause. It has been suggested that the decline in estrogens at menopause can be responsible in failing memory and that estrogen therapy may increase dopaminergic responsiveness and play a protective part in Alzheimer's disease (AD) [1].

AGE-ASSOCIATED BRAIN PATHOLOGICAL CHANGES

The ageing process can perturb molecular pathways regulating cellular homeostatic mechanisms, ultimately promoting disease states. Various changes such as senile plaques, cerebral β-amyloid (Aβ) angiopathy, neurofibrillary tangles, corpora amylacea and mineralization develop with age in the nervous system of mammals [39]. Moreover, the age-related increase in blood pressure, essentially due to brain shrinking in specific brain region including the frontal cortex, can increase the risk of stroke and ischaemia.

In humans, the incidence of several neurodegenerative diseases including AD, Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) raise sharply with age [40]. Molecular alterations that occur in the nervous system during normal ageing set the stage for the onset of such conditions. Several studies, for example, described the association between brain ageing and AD. During normal ageing, elderly individuals can develop anatomical and molecular features of AD including neuritic plaques and neurofibrillary tangles, still maintaining their cognitive abilities [41].

AD is a progressive and irreversible brain disorder with the highest incidence in people aged over 65. AD patients experience progressive cognitive and memory deficits as well as dementia. AD neuropathological hallmarks include the creation of extra-cellular Aβ peptide plaques and the intracellular deposition of Tau protein tangles. Both alterations are frequently observed in brains over 60 years of age, particularly in the most vulnerable areas, such as hippocampus and entorhinal cortex [42]. Deposition of the microtubule-associated protein Tau occurs in AD but also during normal brain ageing [43]. Tau protein has been identified as a major constituent of paired helical filaments (PHFs) and AD is characterized by a major redistribution of the normal Tau protein pool into PHFs. In neurodegenerative conditions, hyperphosphorylation of Tau may result in microtubule destabilization, in addition to the deposition of toxic aggregates [39].

PD is the second most common neurodegenerative disorder after AD, which afflicts nearly 1% of the population above the age of 60. A relationship between PD and ageing has been proposed [43]. PD results in movement, balance, and fine motor control changes as a consequence of cell death of dopamine-containing neurons of the substantia nigra pars compacta [44]. The dopaminergic cell death is induced by reactive oxygen species (ROS) overproduction and mitochondrial dysfunction among other factors [45 46].

Pathways that lead to neural death in these conditions include local inflammatory response, accumulation of damaged molecules, mitochondrial alterations [10 40 47]. In ageing, microglia undergoes phenotypic changes compatible with its activation. Glial activation can lead to neuroinflammation, which is increasingly accepted as part of the pathogenesis of neurodegenerative diseases, including AD [48]. Furthermore, during ageing mitochondria in microglia accumulate DNA oxidative damage an increased ROS production. ROS can activate the redox-sensitive nuclear factor kappa B, which promotes more neuroinflammation and cognitive impairment [49]. Dysfunctions in mitochondrial function have been associated with an increased activity of specific enzymes including monoamine oxidase (MAO) [49]. MAO is localized in the mitochondrial outer membrane and catalyzes the oxidative deamination of biogenic amines. The reaction accounts for a quantitatively large production of H_2O_2 that contributes to an increase in the steady state concentration of ROS within the mitochondrial matrix and cytosol. Thereby a role of monoamine oxidation and MAO activity as causative factors in increased oxidative stress during physiological ageing has been underlined [50]. The amount of neuronal lipofuscin pigment in pyramidal neurons increases with age [51]. One of the most widely alterations that occur in neurons during ageing, is the accumulation of damaged molecules which form insoluble aggregates within the cell [40]. Lipofuscin consists of undigested carbohydrates, proteins, and lipids that are present in residual bodies derived from the lysosomal system. The accumulation of lipofuscin contributes to abnormal intracellular protein accumulation and elimination [51].

MITOCHONDRIAL DYSFUNCTIONS AND OXIDATIVE STRESS IN AGEING BRAIN

Neurons, as much as all mammalian cells, depend on mitochondria for their survival and ATP production [52]. Mitochondrial function generally declines during ageing due to a number of modifications that compromise the electron transport chain proteins [53-56]. In old animals, brain mitochondria show reduced rates of electron transfer in complexes I and IV, decreased membrane potential, increased content of oxidation products of phospholipids and proteins, and increased size and fragility. The complex I inactivation together with oxidative damage is usually named "complex I syndrome", a condition associated with mammalian normal brain ageing and neurodegenerative diseases [8 47 57].

The amount of cardiolipin, an acidic phospholipid associated to mitochondrial membranes, has been shown to decrease with age [58]. This phospholipid is known to have optimal electrical insulating properties, thereby contributing significantly to the transmembrane potential that drives the formation of ATP via ATP synthase. The mitochondrial decline in ATP synthesis and concomitant damage caused by ROS seems to play a key role in ageing [8 10 59].

It was in the 1972 that mitochondria were for the first time associated with the process of ageing [60]. According to this theory, free radicals generated through mitochondrial metabolism can act as causative factors of abnormal function and cell death. Mitochondria are known to be the main site of ROS production. Depending of their amount, ROS can regulate cell survival or apoptosis. Basal level of ROS can act as a second messenger to promote proliferation, whereas higher levels of ROS induce cellular damage and promote apoptosis [61].

It has been reported that old cell mitochondria do not properly control the generation of ROS and this can in turn generate damage to mitochondrial mtDNA and membranes resulting in alterations of mitochondrial morphology and progressive impairment of mitochondrial functions [62-64]. Moreover, oxidation renders mtDNA more susceptible to mutation because oxidized bases are misread during replication and this leads to nucleotide substitution [65]. The increased in ROS production is also associated to a decrease age-related in the free radical scavenging system so that increased escape of ROS occurs. Indeed, a decrease in the activity of several ROS-scavenging enzymes including superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase was also measured in the brain of AD patients [66].

As a consequence of ROS production, different mutations can accumulate with advancing age because of the number of replications of mtDNA that occur independently of the cell cycle. Several studies have found increased levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG), a biomarker of the oxidative DNA damage, in mtDNA in the aged brain [67 68]. High levels of 8-OHdG have been found in both nuclear DNA and mtDNA in post-mortem brains of aged subjects [69].

Recent data have also described the importance for naturally occurring replication errors, such as large deletions, in the formation of age-associated mtDNA mutations, suggesting that a model solely based on ROS does not properly explain the natural history of mtDNA mutations [70]. Consistent with this hypothesis, other cellular and metabolic mitochondrial alterations may contribute to the ageing process [70]. This enlarges the nutritional targets to a large spectrum of cellular pathways.

Furthermore, it is clear the association between mitochondrial dysfunctions, mainly as decreases in respiratory chain complex activities, with the onset and progression of ageing-related neurodegenerative disorders including PD and AD [10 47 71]. Thus, mitochondria-targeted protective compounds, that prevent or minimize mitochondrial dysfunctions, constitute potential therapeutic strategies in the prevention and treatment of these CNS diseases.

NUTRACEUTICAL INTERVENTIONS

Several researches and results of both laboratory and clinical studies suggest that traditional herbs and derivate phytochemicals may mitigate the decline in cognitive functions associate with ageing and disorders like dementia, AD and PD. Nutraceutical interventions slow physiological or/and pathological progression due to their anti-oxidative, anti-inflammatory and anti-amyloidogenic properties. Nutraceuticals also regulate mitochondrial stress, apoptotic factors, free radical scavenging system, and neurotrophic factors by targeting specific cellular targets [72 73]. Here, we summarized a series of nutraceutical compounds and the main studies reporting the beneficial function of nutraceuticals on brain functions. Main results are listed in Table I.

(Poly)phenols

Phenolic compounds have at least one aromatic ring with several hydroxyl groups attached. These molecules are classified in flavonoids and non-flavonoids, but it is common referred to as (poly)phenols. Based on variations in the saturation of the ring system, their alkylation and/or glycosylation and the hydroxylation

Table I. Nutraceutical compounds with potential effects on age associated brain alterations.

Nutraceutical	Experimetal model	Effect	Ref.
Polyphenols			
Flavanones	Cell cultures	Neuroprotective and neuromodulator.	80
Procyanidin (pine-bark estracts)	Clinical study	Beneficial effects on oxidative stress and cognition.	77, 88
Flavonoids	Cell cultures Clinical study	Apoptosis inhibition-neuronal differentiation. Vascular blood flow promotion. ROS scavengers.	83 83 84
EGb 761 (Ginkgo biloba)	Cell cultures and clinical study	Enhances neurocognitive functions in AD and in older adults.	96, 97
Grape, pomegranate, strawberry and blueberry flavonoids	Rat and clinical study	Enhances the efficiency of memory.	93-95
Soy isoflavones	Clinical study	Improve neurocognitive function and mood in post- menopause.	102, 103
Resveratrol	Cell cultures	Anti-inflammatory effect. Therapeutic effect in PD.	107 109
Epigallochatechin-3-gallate	Epidemiological study	Decreases the incidence of neurodegenerative disorders.	117-119
Curcumin	Rat, mouse and clinical study	Anti-inflammatory and antioxidant in neurodegeneration. Inhibits formation of Aβ oligomers, fibrils, binds plaques, and reduces amyloid.	112-115 113-116
Vitamins and oligoelements			
Ascorbic acid	Cell cultures, mouse and clinical study	Antioxidant effects.	123
Vitamin E	Cell culture and animal study	Improves cognitive behaviors in rodents. Neuroprotective in apoE-deficient mice. Modifies Aβ toxicity in cultured hippocampal neurons. Improves brain mitochondrial function.	124 125 126 57
Vitamins C and E	Clinical study	Reduce AD incidence.	127
1,25(OH)2D3	Cell cultures	Inhibits TNF-α, IL-6, and nitric oxide production by the stimulated microglia.	130
Lipoic acid	Rat	Prevents mitochondria damage.	131
Zinc	Mouse Clinical study	Improves myelination. Improves brain compensatory capacity.	135 136
PUFA			
ω-3 PUFA (DHA)	Animal and clinical study	Reduce impaired cognitive functions. Protective against Aβ production and dendritic pathology in AD. Attenuate oxidative stress. Reduce Aβ and Tau accumulation. Diminish Parkinsonism symptoms and dyskinesia. Enhancing the expression of neurotrophic factors.	140-143 152-154 156,159 175-159 160, 161 162
Other compounds			
Carnosine	Cell cultures, monkeys, rat, rabbit	Antioxidant effects. Modulates MAO activity. Controls toxic effect of Aβ.	167-170 172 173-175
Creatine	Clinical study	Efficacious as a treatment in PD.	179
Acetyl-L-carnitine	Animal study	Reverses decline in mitochondrial functions. Improves clinical features of AD. Improves energy to nerve terminals.	180, 181, 183-185 181, 183 182
Taurine	Cell cultures Mouse	Ameliorates neuroinflammation. Ameliorates age-dependent decline in spatial memory.	186 187

pattern, flavonoids may be divided into seven subclasses: flavonols, flavones, flavanones, flavanonols, flavanols, anthocyanidins, and isoflavones.

Regular and moderate consumption flavonoid-rich plant foods such as wine, tea, berries and cocoa may result in cognitive benefits [74-77]. Preclinical and epidemiological studies suggest that (poly)phenols can reverse neurodegenerative pathology and age-related loss in memory, learning and neurocognitive performance. They can act in several ways regulating the peripheral and cerebrovascular blood flow, interacting with intracellular signalling and reducing neuronal damage induced by neurotoxins and neuroinflammation [78-82]. Indeed, three main processes may account for flavonoids effects. First, they present a selective interaction with a number of protein kinase and lipid kinase (such as the phosphatidylinositol 3-kinase, PI3K/Akt, and mitogen-activated protein kinase, MAPK) involved in signalling cascades that regulate pro-survival transcription factors and gene expression. This action at the brain site leads to the inhibition of apoptosis caused by neurotoxic species and promotes neuronal survival and differentiation [74]. Second, flavonoids induce angiogenesis, and new nerve cell growth in the hippocampus promoting in this way the peripheral and cerebral vascular blood flow [83]. Third, these phenolic molecules react directly with and scavenge neurotoxic species and pro-inflammatory agents produced in the brain as a result of both normal and abnormal brain ageing [84].

A discussed topic regards the possibility of flavonoids of exerting their functions in specific brain regions. Some experimental works describe their localization in the brain and their direct neuroprotective and neuromodulatory actions [82]. Flavanones such as hesperetin, naringenin and their *in vivo* metabolites, some dietary anthocyanins, cyanidin-3-rutinoside and pelargonidin-3-glucoside, have been shown to traverse the blood-brain barrier (BBB) *in vitro* and *in vivo* models [85]. Their ability to penetrate the BBB is dependent on compound lipophilicity [86] and their interactions with specific efflux transporters expressed in BBB such as the P-glycoprotein [87].

As regards to their action on cognition and memory, studies show a beneficial effect of procyanidin-rich pine bark extracts on perception and oxidative stress in older individuals [77 88] and flavan-3-oil-rich cocoa on cognitive function in young healthy female adults [89]. Consuming of flavonoid-rich foods such as grape juice, blueberry or cocoa results in positive effects on cognitive outcome measures [90-92]. Moreover, grape, pomegranate, strawberry and blueberry (1%-2% [w/w freeze-dried] juice) affect several aspects of memory and learning [93]. Blueberry-derived flavonoids may enhance the efficiency of spatial memory acting on a hippocampal sub-region (the dentate gyrus) most sensitive to ageing; in rats, blueberry-supplementation increase the proliferation of precursor cells in this region [94 95].

Ginkgo biloba extract EGb 761, rich in flavonoids and terpens, improves cognitive performance in AD patients with mild to moderate cognitive impairment [96]. Moreover, the same extract given for short-term period (i.e., 6 weeks) demonstrated its efficacy in enhancing certain neurocognitive functions/processes of cognitively intact old adults [97]. In rats, the oral administration of EGb 761 is effective in preventing the appearance of enlarged mitochondria, decreased membrane potential and increased levels of mtDNA damage [98].

Soy extracts contain isoflavones such as genistein, diadzein and glycetin, which are structurally similar to estrogen. The well reported efficacy of isoflavones on cognition and memory is related to their potential to mimic the action of estrogens in the brain and to influence the synthesis of acetylcholine and neurotrophic factors [99 100]. Moreover, supplementation with soy isoflovones in post-menopausal women improves ratings of quality of life and has positive effects on neurocognitive function and mood [101-104].

Resveratrol (3,5,4'-trihydroxytrans-stilbene), is a naturally occurring phenolic compound that is found in a variety of food sources, including wine, soy, peanuts, and peanut products [105 106]. Resveratrol can modulate neuroinflammation and neurodegeneration within the brain. The neuroprotective effect of resveratrol has been investigated in several *in vitro* and *in vivo* models of AD [107]. An *in vitro* study performed on microglia cells, demonstrated that resveratrol suppressed the mRNA expression of the pro-inflammatory mediator TNF-α, and inhibited the activation of the transcription factor NF-κB. Resveratrol exerts their beneficial effects also promoting the mRNA expression of the anti-inflammatory molecule interleukin-10 (IL-10) [108]. Resveratrol has been proposed as promising therapy for PD, because of its neurotrophic effects on dopaminergic neurons and the induction of neurotrophic factors release [109].

Curcumin is a phenolic compound and main constituent of the spice turmeric (*Curcuma longa*). Curcumin intake is positively related to cognitive function in healthy elderly individuals [110] with evidence that concentrations of Aβ and Tau are lower in populations that consume large amounts of curcumin [111 112]. There is also evidence supporting the use of curcumin as a potent antioxidant and anti-inflammatory agent in neurodegenerative conditions and its ability to reduce amyloid plaque burden and disaggregating preformed Aβ fibrils [113-116].

(-)-Epigallochatechin-3-gallate (EGCG) is the main and active flavonol in green tea, together with (-)-epigallochatechin, (-)-epicatechin and (-)-epichatechin-3-gallate. Despite the relatively small number of investigations

into the neuroprotective properties of EGCG in humans, epidemiological evidences report that higher consumption of tea/green tea, rich in EGCG, is associated with a reduced risk of neurodegenerative disorders and a lower prevalence of cognitive impairment decreasing the incidence of dementia, AD and PD [117-119]. The mechanism by which tea chatechins exert their effects are very broad, due to their ability to chelate metal ion, above all iron [118], to promote an anti-inflammatory response [120], to facilitate cholinergic transmission [121] and to enhance neurite outgrowth [122].

Vitamins

Ascorbic acid is the reduced form of vitamin C, with important antioxidant properties. A clear link between ascorbic acid deficiency and oxidative-induced neuronal death during neurodegeneration has been demonstrated [123]. Ascorbic acid inhibits ROS production that is generated in neurons during synaptic activity and CNS metabolism. Glial cells function as ascorbic acid reservoir; under brain activity the vitamin is released in the synaptic cleft and taken up by neurons. Moreover, ascorbic acid can switch neuronal metabolism from glucose to lactate consumption to sustain synaptic activity [123].

Vitamin E is a dietary antioxidant that includes a group of structural-related antioxidants: four tocopherols and tocotrienols. The molecule, when administered to rodents has multiple effects including an improvement of cognitive behaviours [124], a neuroprotective action in apoE-deficient mice [125], a reduction of Aβ toxicity in cultured hippocampal neurons [126], and improved neurological and brain mitochondrial function in ageing rodents [57].

Although clinical trials have produced controversial results, a study conducted in 2004 has demonstrated that a supplement of vitamin E and vitamin C in combination is associated with reduced prevalence and incidence of AD in an elderly population [127].

Vitamin D levels are lower in people with AD than normal subjects [128], and patients with severe deficit in vitamin D have a significant increased risk of cognitive decline over 6 years [129]. Moreover, the hormone 1,25-dihydroxyvitamine D(3) seems to be involved in the maintenance of brain homeostasis, inhibiting in a concentration-dependent manner the production of TNF-α, IL-6, and nitric oxide by stimulated microglia [130].

In animal models, α-lipoic acid is able to prevent age-related decline in neurological functions and oxidative damage in brain mitochondria [131].

Folates are vitamins (B_9 family) essential to the development of the CNS. A compromised folic acid status is common in older people due to a reduced dietary intake, and intestinal malabsorption. Cognitive decline, dementia and depression in healthy and neuropsychiatric older individuals have been associated to folate deficiency. The mechanisms underlying include hyperhomocysteinemia, lower occurrence of methylation reactions and tetrahydrobiopterin levels in neurons, increased incorporation of uracil into DNA, and shorter telomere length [132]. No consistent evidences support the effect of folic acid supplementation, alone or in combination with other B vitamins, in the prevention of cognitive decline or neuropsychiatric diseases in old patients [132].

Zinc

Zinc (Zn) is the most abundant trace metal in the brain. Zn deficiency is quite common in elderly due to an inadequate food chewing, intestinal malabsorption, and other psychosocial factors. Despite this, the beneficial effect of Zn supplementation for brain function is controversial. Current investigations suggest that zinc may rapidly induce Aβ aggregation in senile plaque leading to AD development [133]. Moreover, Zn can accumulate in post-synaptic neurons causing neuronal toxicity, cell-death and necrosis [134]. On the contrary, other results support the neuroprotective role of Zn supplementation through the improvement of myelination [135], and an increased function of Zn-related proteins that contribute to maintain brain compensatory capacity [136]. In addition, Zn metabolism and homeostasis have been suggested to play a major role in many processes related to brain ageing and in the onset and development of age-related neurodegenerative diseases [137].

ω-3 long-chain polyunsaturated fatty acids

ω-3 PUFAs (polyunsaturated fatty acids), including EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), are dietary fats found in oily fish and seafood, with small amounts of DHA found in algae. DHA is highly incorporated into the brain with beneficial neuroprotective effects [138, 139]. Several epidemiological studies have established that moderate fish consumption is associated with a reduced risk of impaired cognitive functions in both healthy aged individuals [140, 141], and patients with AD [142, 143]. Lower DHA and ω-3 PUFA levels were detected in plasma [144] as well as in brain [145] of elderly and AD demented patients where a widespread loss of synaptic contacts takes place in neurons.

In longitudinal observation studies, an inverse relation between fish intake/DHA serum concentrations and cognitive impairment was reported. DHA (900 mg/d) administration for 6 month was able to improve learning memory function in age-related cognitive decline in healthy adults [146]. However, it is likely that DHA may delay the onset of age-related cognitive decline, but not in individuals with already diagnosed AD progression.

Encouraging results come from a study in which the combination therapy (i.e., in combination with other dietary compounds or supplements, such as lipoic acid) stabilized or improved memory scores in patients with mild AD [147-149].

Epidemiological and clinical studies have shown that elevated intake of DHA or higher intakes of food rich in ω-3 PUFA is associated with reduced AD risk [150 151]. Dietary DHA could be protective against Aβ production and increases cerebral blood volume in AD mouse model [152 153]. In an AD mouse model, DHA also protects against dendritic pathology [154]. DHA supplementation was shown to attenuate oxidative stress, specifically lipid peroxidation, and protect against memory loss in various rat models of AD and ageing [155-159] as well as reduce interneuronal Aβ and Tau accumulation [157-159].

Animal models of PD supplemented with DHA showed diminished Parkinsonism symptoms and decreased dopaminergic neuronal death [160], moreover DHA supplementation protects dopaminergic neurons in experimental rat model of PD by targeting inflammatory signalling pathways and by enhancing the expression of two neurotrophic factors glial-derived neurotrophic factor (GDNF) and neurturin (NT-3) [161]. A meta-analysis of 21 cohort studies demonstrated that DHA and fishery products are associated with lower risk of cognitive impairment, a lower risk of dementia and AD but without a linear dose-response relation [162].

Other compounds

Carnosine is a naturally occurring dipeptide (β-Ala-L-His) present in muscle, brain and circulation [163 164]. Carnosine has antioxidant and antiglycating properties [164-166], and neuroprotective effect towards brain diseases caused by oxidative stress [167-170]. When used in combination with EGCG, L-carnosine prevents neurodegenerative diseases by reducing the neuronal age-associated damage caused by oxidative stress [171]. In addition, carnosine attenuates the ageing-induced increase in the activity of MAO, thus indicating that carnosine may bring about oxidative stress and changes in brain regional serotonin level and hence behaviour [172].

It has been demonstrated that carnosine might be used to control AD for its ability to suppress the toxic effect of Aβ in cultured cells [173 174] and in transgenic mice [175]. Additionally, carnosine has been shown to suppress mitochondrial dysfunction in animal models of AD [175]. A similar efficacy was observed in PD disease, through the modulation of some biochemical events associated with this pathology [176 177].

Creatine is a molecule that is produced both endogenously in the liver, and acquired exogenously from foods. It plays a crucial bioenergetic role in several tissues acting as a spatial energy buffer. In older adults, creatine ingestion can improve quality of life, and ultimately may reduce the disease burden associated with sarcopenia and cognitive dysfunction [178]. Current literature suggests that exogenous creatine supplementation is most efficacious as a treatment paradigm in PD improving patient mood and leading to a smaller dose increase of dopaminergic therapy [179].

Acetyl-L-carnitine (ALC) is a metabolic intermediate that functions as an important trans-mitochondrial membrane transporter of long-chain fatty acids for β-oxidation. ALC is produced through endogenous biosynthesis of lysine and methionine, primarily in the brain, liver, and kidneys, and can also be consumed through foods and supplementation. It has been reported that ALC significantly reverses the age-associated decline of mitochondrial membrane potential and of cardiolipin level [180]. Moreover, ALC increases cellular oxygen consumption, which declines with age, to the level of young rats [181]. Additional studies and reviews showed that ALC can slow pathologic decline in young patients with AD, improve clinical features of AD [181] and, when administered as a component of a vitamin formula, can delay cognitive decline in both early- and late-stage of AD [180]. Chronic administration of ACL to animals induced a positive modulation of the synaptic structural dynamics through improvements in energy provision at nerve terminals [182].

ALC chronically administered to rats induced a lower age-dependent decline in the mitochondrial oxidation rate of NAD-dependent substrates [183] and in the mitochondrial gene expression of complexes I, IV, and V and of adenine nucleotide translocase [184 185].

Taurine or 2-aminoethanesulfonic acid, is an organic compound that is widely distributed in animal tissues. It has been reported that taurine may ameliorate neuroinflammation sustained by activated microglia by switching the microglia from a M1 to a M2 activation status [186]. Moreover, chronic supplementation of taurine to aged mice significantly ameliorated the age-dependent decline in spatial memory [187].

CONCLUDING REMARKS

Normal ageing is associated with deficits in cognitive performance, even in healthy individuals. Neurological and cellular modifications observed during ageing represent risk factors for certain pathological conditions. For this reason, a major understanding of the mechanisms underlying age-related changes in neuronal functions can help to reduce risk for neurological disease. Two principal processes are under investigation for possible and promising therapeutic interventions: neuroinflammation and neurodegeneration. Basic research into the

molecular mechanisms of these two processes allowed to identify specific cellular targets and more general cellular processes and biological functions altered, among these the role that oxidative stress has in ageing brain.
In consideration of this, many studies suggest that a balance between pro-oxidants and antioxidants to delay oxidative damage to the brain results in decrements in neuronal and behavioural decays. Approaches such as nutritional interventions have demonstrated to reverse or delay the onset of patho-physiological modifications associated with normal ageing. It is quite likely that this approach would impact on neurodegenerative diseases.
Most of the molecules discussed above emerged as potential nutrients capable of improving health during ageing and neurodegenerative processes. Research has demonstrated that supplementing diets with fruits or vegetables has slowed and, in some cases, even reversed deficits in brain function, motor performance, and memory in old animals and humans. Increasing dietary intake of fruits and vegetables high in antioxidant activity may be an important component of a healthy living strategy designed to maximize neuronal and cognitive functioning into old age. How phytochemicals exert their beneficial effect is not only related to their anti-oxidant activity, but also to their capacity to bind in a selective way with specific intracellular targets. Examples of molecules and pathways targeted by neuroprotective phytochemicals include (i) protein and lipid kinase that regulate pro-survival transcription factors (such as PI3K/Akt and MAPK), (ii) the inactivation of pro-inflammatory mediator TNF-α and NF-κB transcription factor, (iii) acetylcholine and neurotrophic factors induced by hormone-like molecules and finally (iv) mtDNA, Tau and Aβ. These markers regulate a complex set of cellular processes that are important in neuronal plasticity. Despite these results, specific cellular targets remain largely unknown for a large class of nutrients. For this reason, there is yet an unexplored potential for investigating in detail these molecular targets and the molecular concentrations required to obtain these beneficial effects.
Moreover, some of promising molecules exhibited efficacy only in cellular or preclinical models and further *in vivo* studies are necessary to demonstrate their clinical potential. Nutraceutics that have demonstrated to protect cells against ageing and age-disorders in human studies include a large number of phytochemical compounds, such as phenols (anthocyanidins, catechins, isoflavones and curcumin) and vitamins (C and E), and some animal derived molecules (PUFAs and creatine). At the moment, they represent an important pool of molecules for the development of therapeutic strategies.

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