Chapter 53

Lipoic acid and vitamin D3 and their use in preventing brain aging

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List of abbreviations

$1,25(OH)_2D_3$ 1,25- dihydroxyvitamin D3
25(OH)D 25-hydroxyvitamin D
25(OH)D3 25-hydroxyvitamin D3
AD Alzheimer disease
APOE apolipoprotein E
Aβ neurotoxicity of β-amyloid
BBB blood-brain barrier
H_2O_2 hydrogen peroxide
HD Huntington disease
Ho homotaurine
LA lipoic acid
NFKB nuclear factor kappa-light-chain-enhancer of activated B cells
NO nitric oxide
PD Parkinson disease
PHO phosphatidylserine
ROS reactive oxygen species
SIRT1 sirtuin 1
VD vitamin D3
VDR vitamin D receptor

Mini-dictionary of terms

Aging it is a process in which tissues and organs decline causing high morbidity, increasing health risk due to accumulation of a wide variety of molecular and cellular damage over time. This leads to a gradual decrease in physical and mental capacity, growing risk of disease, and ultimately death.

Brain it is the central organ of the nervous system. It controls most of the activities of the body by processing, integrating, and coordinating the information it receives from the sense organs, and making decisions from the indications sent to the rest of the body.

Cognitive functions they refer to multiple mental abilities, including learning, thinking, reasoning, remembering, problem solving, making decision, and paying attention, which an individual applies during the life.

Inflammation the function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and damaged tissues, and initiate tissue repair.

Lipoic acid it is considered the most powerful micronutrient for its pharmacological properties. It has received more attention as a food supplement in various diseases such as neuroinflammatory disease.

Nutraceutical intervention it is considered a treatment used to reduce oxidative stress and chronic inflammation, improve vascular function, and reduce damage to the body by integrating micronutrients into the diet.

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Oxidative stress it is a process characterized by an imbalance between free radicals and antioxidants in the body. Free radicals can cause important chemical chain reactions in the body because they react very easily with other molecules.

Prevention is aiming to minimize the burden of diseases and associated risk factors.

Supplement it is a product administered orally composed of dietary ingredients, and it is added to the diet to improve physiological balance.

Vitamin D3 it is a vitamin produced by the body when exposed to UV light: it could be obtained from dietary sources as well. Vitamin D3 is also considered a hormone because of its steroid structure. It has skeletal and extraskeletal functions.

Introduction

Aging is a condition that progressively deteriorates physiological functions and leads to increasing risks of disease and death (Maharsh, Riley, Hobbs, Cortez-Cooper, & Robinson, 2014) Researchers consider aging as a multifactorial process that involves genetics, lifestyle, and environmental factors (Kennedy et al., 2014; Lopez-Otin, Blasco, Partridge, Serrao, & Kroemer, 2013). During aging, biological subsystems managed mainly by endocrine and neurovegetative systems undergo a gradual loss of homeostasis, followed by a progressive deterioration of physiological functions, increasing the susceptibility to diseases associated with aging (Singh et al., 2015; Molz & Schröder, 2017). Since life expectancy increase and the aging of a large segment of the population have made aging-related disability and morbidity increasingly important issues, more researches were done. Indeed, aging has been associated to a higher oxidized state in the redox balance (Jones & Sies, 2015); consequently, the central nervous system becomes vulnerable to oxidative stress (Arivazhagan, Shila, Kumaran, & Panneerselvam, 2002; Kidd, 2005), due to an unbalanced redox signaling, related to increased amounts of oxidants and ineffective antioxidant defenses (Go & Jones, 2017). The mitochondrial "free radical" theory defined the molecular mechanism of aging based on the endogenous production of reactive oxygen species (ROS) and their harmful effects on mitochondria. Moreover, nitric oxide (NO), when produced in high concentrations, works as a source of ROS which react with lipids, proteins, and nucleic acids, causing oxidative damage, leading to progressive decline of cellular functions (Chakravarti & Chakravarti, 2007), resulting in generalized impairment of the membrane function (Palaniappan & Dai, 2007). Nitric oxide and drugs that directly or indirectly increase NO signaling have found clinical applications in both age-related diseases and in younger individuals (Ghimire, Altmann, Straub, & Isenberg, 2017). Since, the organism possesses defense mechanisms based on antioxidant action (Jovanovic, 2014), in some cases, the endogenous defense system is not strong enough to counteract the oxidative damage caused by ROS and NO. The oxidative stress isn't the only characteristic of the aging process. While oxidative stress plays an important role in the aging process, chronic inflammation has also been implicated as a major contributing factor for cellular senescence, and it is known to be important in the etiology of many diseases since it causes molecular deterioration such as mitochondrial collapsing; DNA damage; protein, carbohydrate, and lipid oxidation (Queen & Tollefsbol, 2010). This damage can lead to early cell aging, cell death, and various chronic pathologies, like neurodegenerative disorders (Basli, 2012). Furthermore, various intervention strategies have been hypothesized, exploiting positive effects on cognitive function and other systemic functions deriving from actions on oxidative stress. The focus of these new strategies is to prepare a dietary supplementation with mitochondrial-targeted nutrients that could delay the onset or progression of cognitive dysfunction and neurodegenerative diseases. In this context we summarize the innovative effects of alpha lipoic acid (LA) and vitamin D3 (VD) in order to slow down brain aging.

Lipoic acid as a multifactorial nutrient

LA can be considered a powerful micronutrient that has a wide range of pharmacological properties (Rochette et al., 2013; Koufaki, 2014), and for this reason it has received great attention as a food supplement in the treatment or prevention of various pathologies (Shay, Moreau, Smith, Smith, & Hagen, 2009; Rochette et al., 2013), such as neurodegenerative diseases (Molz & Schröder, 2017). LA plays a fundamental role in mitochondrial metabolism because it is linked covalently to a lysyl residue as a lipoamide which is a substrate for the NADPH-dependent enzyme GSH reductase as well (Liu, 2008). In addition, the reduced form of LA, dihydrolipoic acid, reacts with oxidant molecules such as superoxide radicals, hydroxyl radicals, hypochlorous acid, peroxyl radicals, and singlet oxygen to protect membranes. The administration of LA is beneficial to a number of oxidative stress models such as diabetes, cataract, HIV activation, neurodegeneration, and radiation injury in animals. Finally, LA has neuroprotective effects in neuronal cells through its antioxidant effect and metal chelating activity (Liu, 2008; Molinari et al., 2019). Therefore, LA can be considered a

mitochondrial nutrient, and it is mainly studied on mitochondrial function in cellular and animal models related to brain aging and neurodegeneration. In particular, LA can reduce memory deficits in different behavioral paradigms on AD (Farr, Price, Banks, Ercal, & Morley, 2012), HD, oxidative stress (Manda, Ueno, Moritake, & Anzai, 2007), and age-associated cognitive dysfunction (Cui et al., 2006; Mahboob et al., 2016) models. In humans, two studies in AD patients have supported the positive cognitive effects of LA (Hager, Kenklies, McAfoose, Engel, & Munch, 2007). In addition, many in vivo and in vitro studies showed that LA can exert beneficial effects to counteract neurodegeneration by decreasing neuronal apoptosis to prevent mitochondrial cell death pathway and by restoring physiological level of oxidative stress, to prevent lipid peroxidation (Di Domenico, Barone, Perluigi, & Butterfield, 2015). Furthermore, in addition to inhibiting the formation of free radicals, another important effect is the chelation of transition metal ions such as iron, thus reducing its bioaccumulation in the brain (Shay et al., 2009; Rochette et al., 2013). LA shows antiinflammatory properties (Deuther-Conrad et al., 2001) and cell death control capabilities as well. These effects suggest that LA is able to reduce mitochondrial dysfunctions ameliorate the neurological damage, suggesting a potential role in preventing memory loss and in enhancing cholinergic and cognitive functions. For these reasons LA supplementation is a potential antiaging agent because it can mitigate aging-related decline in cardiovascular function, diabetic neuropathy, and decline in cognitive function (Maharsh et al., 2014). Understanding whether these health effects will be observed in "normal aging" in humans will require well-designed prospective investigations of adequate duration and adequate dosage of LA alone or combined with other substances in order to improve its absorption. Indeed, LA is found in both vegetable and animal-based foods, and it is rapidly absorbed, metabolized, and excreted (Rochette et al., 2013). Up to 93% of LA dose administered orally is absorbed in the gastrointestinal tract (Cremer, Rabeler, Roberts, & Lynch, 2006), but only 27%–34% LA orally administered is available for absorption by the tissues (Molz & Schröder, 2017). Recently, many investigations tested several combinations containing LA in order to improve its efficiency after ingestion. Dietary supplementation with antioxidants reduces the increased production of ROS in the aging brain but does not completely shut it down, which implies that the antioxidant treatment prevents direct cell damage mediated by radicals while a basal level of ROS is maintained within the cell for correct functioning of the redox signaling pathways.

Lipoic acid in supplement formulation

Numerous lifestyle interventions such as diet alone or with exercise can help strengthen the brain's endogenous defense systems and can be used as a noninvasive strategy to reduce oxidative stress and improve cognitive performance (Cotman, Head, Muggenburg, Zicker, & Milgram, 2002; Opii et al., 2008). In this context the use of supplementation is very important. For example, in rodent experiments, fruit and vegetable-based antioxidant supplements have been reported to improve learning and memory and reduce oxidative stress (Gemma et al., 2002; Thakurta et al., 2014). Various antioxidant preparations have shown the ability to reduce the brain amyloid beta load or improve behavioral and memory deficits (Sinha, Bir, Banerjee, Bhowmick, & Chakrabarti, 2016). In particular, the combination of both LA and antioxidant supplementation shows beneficial effects on cognition (Thakurta et al., 2014). Another type of combination used, N-acetylcysteine, LA, and α-tocopherol, administered with the diet to rats for a prolonged period has been shown to significantly prevent the development of mitochondrial impairment, inflammatory response, oxidative damage, and dysfunction of synaptosomes in the aged brain (Thakurta, Chattopadhya, Ghosh, & Chakrabarti, 2012; Thakurta et al., 2014). These findings support the hypothesis that a proper combination of antioxidants working at multiple levels and administered over a prolonged period from the preclinical phase may be able to prevent brain damage leading to brain aging. However, the efficacy of supplements without a nutritional deficiency remains questionable. For this reason several studies explore different combinations of LA with other molecules in order to prevent mitochondrial imbalance in addition to improving the absorption of molecules.

Vitamin D3 as an aging marker

Risk and protective factors for cognitive function in aging may affect how much individuals benefit from their environment or life experiences by preserving or improving cognitive abilities. These risk factors included apolipoprotein E (APOE), age, body mass index, blood pressure, and cholesterol. On the other hand, the protective factors include high levels of education, intelligence quotient (IQ), physical activity, fatty acids, and vitamin D (Brathen, De Lange, Fjell, & Walhovd, 2020). Two of the most important nutritional forms of VD are cholecalciferol (vitamin D3, derived from animal foods) and

ergocalciferol (vitamin D2, derived from plants), which can also be derived by photoirradiation from their precursors, 7-dehydrocholesterol and ergosterol, respectively (Hill, Granic, & Aspray, 2018). The discovery of the VD receptor in over 30 different body tissues together with the existence of the alpha-1-hydroxylase enzyme in these tissues provided evidence of the extraskeletal effects of VD supporting the increasing interest in VD in the context of nutritional requirements for health, including the prevention of chronic diseases of aging (Hill et al., 2018), in particular, the role of VD in brain function, including cognition, across the lifespan (Balion et al., 2012). Moreover, in vitro and in vivo studies propose neuroprotective properties of VD, since several aspects of brain aging in this animal model mirror brain aging in humans (Henderson, Kimmelman, Fergusson, Grimshaw, & Hackam, 2013). First, the initial signs of cognitive aging, characterized by subtle deficits, occur at approximately the same time in the lifespan of humans and rats, during middle age (Lynch, Rex, & Gall, 2006; Salthouse, 2009). Second, the behavioral task used here, assessing hippocampal-dependent spatial memory, also has relevance for human memory because patients with hippocampal lesions performed poorly in a virtual maze test (Foster, Defazio, & Bizon, 2012). Finally, the VD levels achieved here model clinically relevant levels found in humans, ranging from deficient to sufficient (Rosen et al., 2012), and animals with the highest 25OHD levels, considered "optimal" by some recommendations (Holick et al., 2011; Hossein-nezhad & Holick, 2013; Norman & Bouillon, 2010), outperformed animals with lower levels on a challenging cognitive task. Although recent evidence indicates that supplementation improves vitamin D status in older adults without adversely affecting health and survival (Bjelakovic et al., 2014), there is no consensus on the definition of hypovitaminosis D and upper 25(OH)D thresholds for optimum physical and mental health in old age to prevent problems with under- or over-treatment (Ross et al., 2010; Sanders, Nicholson, & Ebeling, 2013). In particular in the first prospective study, the evidence about the relationship between 25(OH)D and global cognitive function and attention in the very old people was found. Starting from this data, it could be hypothesized that the neuroprotective effects of vitamin D, mediated via expression of proteins that, for example, attenuate the toxicity of reactive oxygen species (Ibi et al., 2001) in very old neurons are attained only at moderate but not at low or high 25(OH)D concentrations. VD is generated by and has powerful effects on neurons. This molecule suppresses oxidative stress, inhibits inflammation, provides neuroprotection, and modifies a variety of neuronal and glial cell functions. It is effective in part by downregulation of inflammatory mediators and upregulation of neurotrophins. A variety of common diseases are presumably sensitive to 1,25(OH)2D3 including multiple sclerosis, Parkinson disease, Alzheimer disease, depression, bipolar disorder, and schizophrenia (Lang, Ma, & Leibrock, 2019). There is also the possibility of reverse causation (i.e., nonoptimal 25(OH)D concentrations being a consequence of prevalent cognitive impairment). Future studies that will study the efficacy of VD on health outcomes must carefully establish the dose and form of its integration, as well as the appropriate duration of observation, to ensure that the desired target concentration of 25(OH)D is achieved. There are relatively few risks associated with increased vitamin D intake, especially when taken in the inactive form (cholecalciferol) (Hathcock, Shao, Vieth, & Heaney, 2007). The most concerning potential side effect is hypercalcemia; however, hypercalcemia is rarely seen except at serum 25OHD levels far exceeding those levels recommended for optimum health (Hathcock et al., 2007). Studies should also give due consideration to VDR genotype and should control for sun exposure, season, calcium intake, baseline circulating 25(OH)D concentrations and measure potential adverse effects. Finally, in light of the widespread prevalence of dietary and biochemical VD inadequacy in many populations and its negative consequences for bone health, strategies to increase oral VD intake should be a priority. In this context many studies showed different combinations with VD in order to modulate or slow down the aging markers.

Vitamin D3 in the formulation of food supplements

Cognitive impairment, dementia, psychosis, and autism have been added to the list as well now in the interchange of decreased VD levels (Maddock et al., 2017). The importance of vitamin D3 in reducing risk of these diseases continues to rise due to the increasing number of people in developed countries having a significant VD deficiency (Welland, 2009). This is also due to the ability of VD to modulate the biosynthesis of neurotransmitters and neurotrophic factors. Western diet does not protect from VD deficiency. On the contrary, reduced VD levels found in the rising numbers of morbidly obese subjects are thought to be primarily attributed to insufficient intake of micronutrients (Kaidar-Person, Person, Szomstein, & Rosenthal, 2008). Dietary supplements may be required for the maintenance of sufficient VD levels, particularly in people living at high latitudes (37 degrees+) and in high-risk groups such as older adults (Heaney, 2006). Supplements containing 400 International Units (IU) are likely to result in a stable increase of serum 25(OH)D

levels by 7 nmol/L (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003). Animal and in vitro experiments suggest that vitamin D has therapeutic potential for the prevention and treatment of cognitive decline; in particular, low levels are a potentially modifiable risk factor for cognitive decline and dementia representing an important breakthrough commonly in older adults and supplements are inexpensive and well tolerated. Several neurologists recommend dietary supplements for brain, including B complex vitamins, fish oil supplements, and vitamin D. However many other possible combinations with other antioxidant molecules are being investigated.

New formulation that combines lipoic acid and vitamin D3 to improve cognitive function

In scientific literature, a lot of clinical data regarding the effects of supplemental substances (i.e., polyphenols, vitamins, oligo elements, ω-3 polyunsaturated fatty acids) are available, demonstrating encouraging therapeutic properties on neurodegenerative processes related to brain aging (De Domenico & Giudetti, 2017). Nutraceutical interventions can maintain physiological status or slow down pathological progression of the brain due to their antioxidant, antiinflammatory, and anti-amyloidogenic properties, regulating mitochondrial stress, apoptotic factors, free radical scavenging system, and neurotrophic factors (De Domenico & Giudetti, 2017). Aging is the major risk factor for neurodegeneration. In this context the use of LA and VD was investigated. Indeed, both LA and VD are able to cross the blood-brain barrier (BBB), as reported in Fig. 53.1 (Molinari et al., 2019), and several studies have been performed to understand their functions within the aging process of the brain (Molz & Schröder, 2017; Cui et al., 2015). Moreover, in the study by Molinari et al. (2019) a cooperative effect between LA and VD was evaluated. In this study was demonstrated the efficacy of the combination with LA and VD to slow down aging in a synergistic and cooperative manner. Indeed, this combination showed the ability to perform beneficial effects directly on viability of astrocytes, since these substances are able to cross the BBB. In addition, the increasing absorption rate of the two substances compared to control during time and to their single administration starting from 60 min supported the existence of cooperative effects of LA plus VD also in terms of brain permeability. Under oxidative conditions, this treatment improves the reduction of ROS level suppressing the effect of H_2O_2-induced mitochondrial dissipation and preventing apoptosis through the mitochondrial-mediated pathways and increasing cell survival, by the activation of ERK and Akt mediators. In addition, the expression of PKA observed in astrocytes showed a significant increase in presence of this combination, supporting the hypothesis of an antiinflammatory activity of combined LA and VD (Fig. 53.2). This study demonstrates that the combination of LA and VD is an effective treatment for astrocytes under oxidative stress conditions, indicating the possibility of developing new strategies to treat brain aging in all stages of development. Based on these findings, a successive study evaluated the opportunity to test a new formulation, which contains selected components able to act on these mechanisms in astrocytes, which are the cells that the brain mostly loses during aging. In particular, this combination can act to counteract the negative effects existing in pre-neurodegenerative conditions and can allow to develop a new formulation useful for slowing down brain aging and neurodegenerative diseases, such

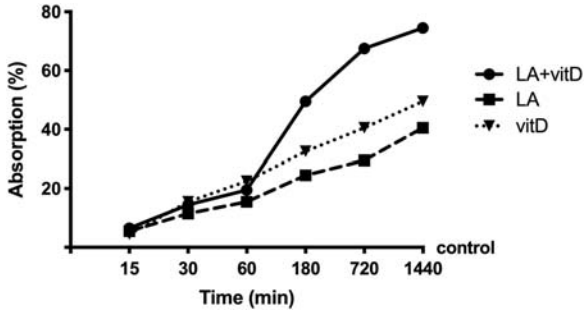

FIGURE 53.1 Absorption of LA, VD, and their combination. BBB permeability, represented as the absorption capacity through the BBB of vitamin D and LA alone and combined during time. Data are expressed as means ± SD (%) of five independent experiments normalized to control values (0% line). *Data are from Molinari, C., Morsanuto, V., Ghirlanda, S., Ruga, S., Notte, F., Gaetano, L., & Uberti, F. (2019). Role of combined lipoic acid and vitamin D3 on astrocytes as a way to prevent brain ageing by induced oxidative stress and iron accumulation.* Oxidative Medicine and Cellular Longevity, *2843121.*

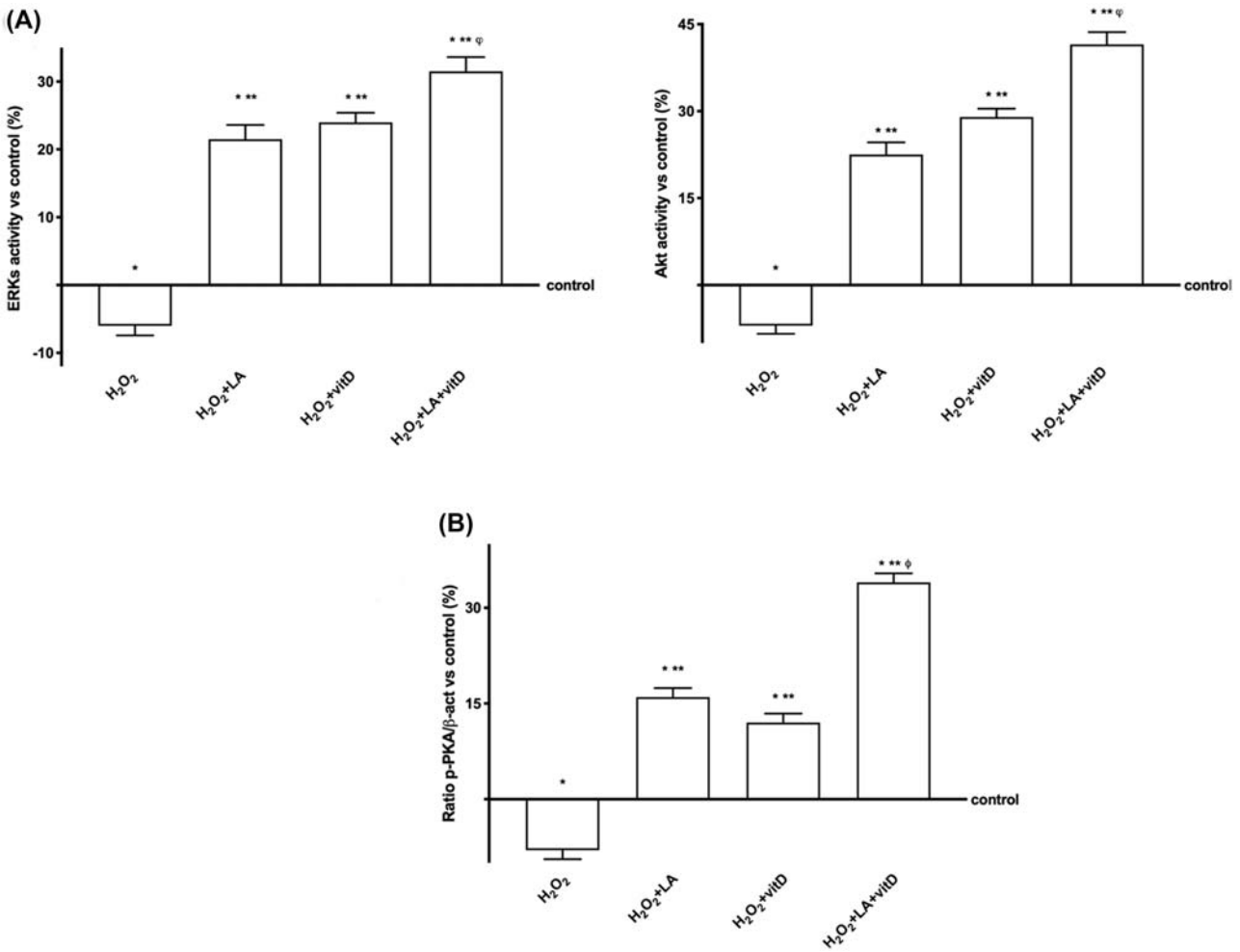

FIGURE 53.2 **Beneficial effects of LA and VD and their combination.** In (A) survival kinases ERKs/MAPK and Akt/PI3K and in (B) a densitometric analysis of key inflammatory marker PKA. All these markers were analyzed under oxidative condition. Data are expressed as means ± SD (%) of five independent experiments normalized to control values (0% line) in (A) and normalized and verified on β-actin detection in densitometric analysis (B). *$p < 0.05$ versus control; **$p < 0.05$ versus control; ϕ $p < 0.05$ versus LA and VD alone. *Data are from Molinari, C., Morsanuto, V., Ghirlanda, S., Ruga, S., Notte, F., Gaetano, L., & Uberti, F. (2019). Role of combined lipoic acid and vitamin D3 on astrocytes as a way to prevent brain ageing by induced oxidative stress and iron accumulation.* Oxidative Medicine and Cellular Longevity, *2843121.*

as AD and PD, and represent a new food supplement. In this study, the usefulness of including in the formulation phosphatidylserine (PHO) and homotaurine (Ho) in addition to the already tested LA and VD was demonstrated. In fact, a decline in brain phosphatidylserine has been associated with impaired memory and mental cognitive impairment (Moré, Freitas, & Rutenberg, 2014). It is well known that Ho (3-aminopropanesulfonate) may interfere with several cellular pathways, both in vitro and in vivo experimental models, and it can exert neuroprotective and neurotrophic effects through different mechanisms including action against the oxidative damage to DNA, antifibrillogenic activity, and antinociceptive or analgesic activities, and prevents the neurotoxicity of β-amyloid (Aβ) peptide by reducing amyloid aggregation (Davinelli, Chiosi, Di Marco, Costagliola, & Scapagnini, 2017). This combination is able to improve cell viability by preventing ROS production and decreasing NFKB activity in primary astrocytes. Since in neurodegenerative conditions, SIRT1, amyloid-beta precursor, and tau protein are very important (Fig. 53.3), this combination is able to modulate them confirming the hypothesis that this combination acts in very early stages of brain imbalance (Molinari et al., 2019; Uberti et al., 2019).

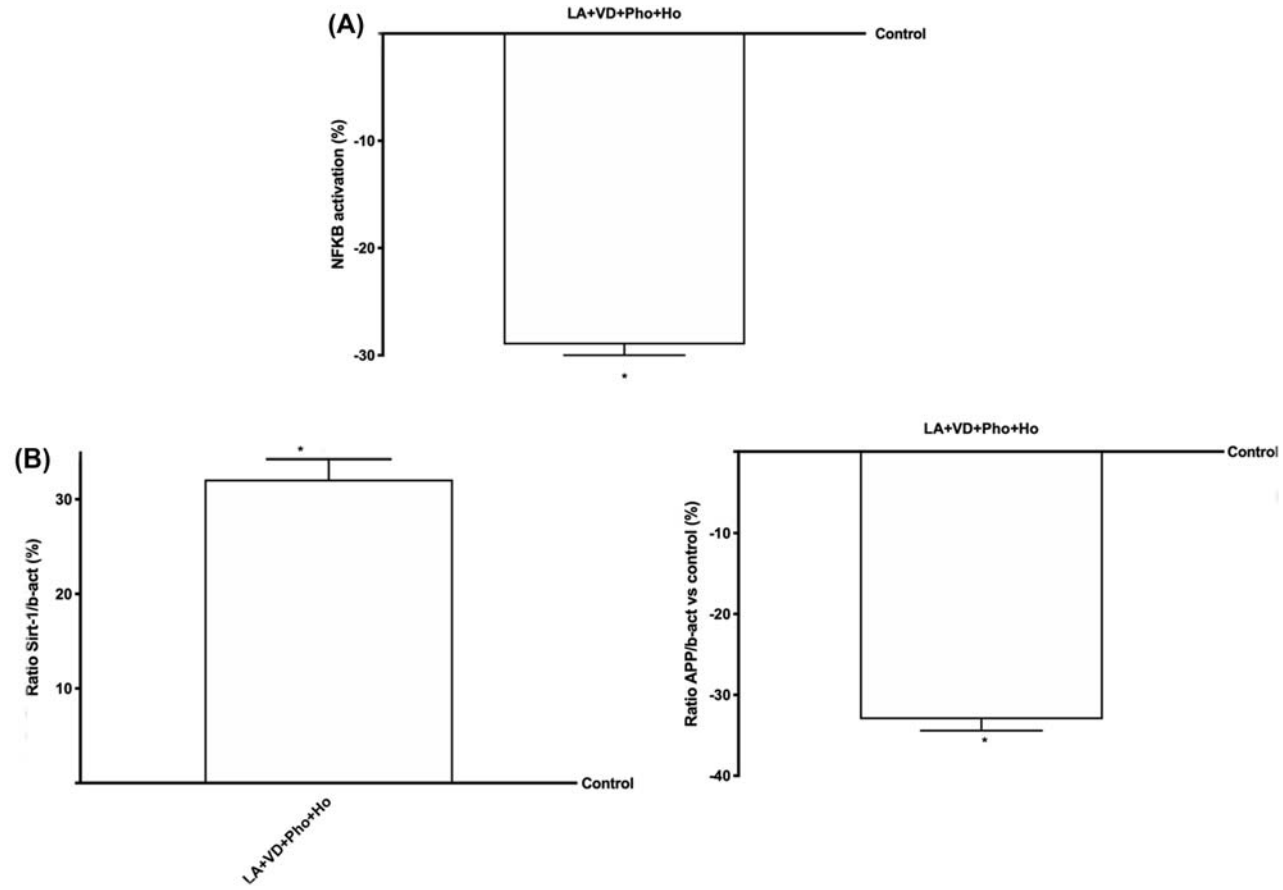

FIGURE 53.3 **Beneficial effects of a new food supplement on aging markers.** In (a) NFKB activity to demonstrate antiinflammatory activity and in (b) a densitometric analysis of SIRT1, linked to chronic degeneration, and APP linked to cognitive impairment. Data are expressed as means ± SD (%) of four independent experiments normalized to control values and normalized and verified on β-actin detection in densitometric analysis. *$p < 0.05$ versus control. *Data are from Uberti, F., Morsanuto, V., Ruga, S., Stoppa, I., Galla, R., Notte, F., & Molinari, C. (2019). Effect of mixed lipoic acid, vitamin D, phosphatidylserine and homotaurine to obtain a new formulation for brain ageing prevention.* EC Neurology, *302–312, (11.5).*

Conclusion

In this article a new strategy has been described that addresses the problem of brain aging through the use of a formulation containing selected components capable of acting on the mechanisms underlying aging in the astrocytes, which are the cells that the brain especially loses during aging.

Applications to other areas of aging

In this chapter we reviewed the effects of LA and VD on brain aging. We clearly show that this combination is an efficient scavenger of free radicals and is able to prevent the early phase of nervous tissue degeneration. Since the food supplements are substances widely used to add lacking nutrients or elements to the diet or to lower risk of diseases, the exploration of a new strategy based on supplements which, after being adequately tested on humans, can complement the new drug therapies for neurodegeneration is very important. Indeed, these specific dietary components have also been investigated in order to find evidence supporting protective roles, for example, of n-3 PUFA, polyphenols, VD, and B-vitamins (Uberti et al., 2017). In this context, the new formulation described in the article is important to prevent or slow down aging due to the action of oxidative stress and inflammatory network. Since aging involves all organs, LA and VD can usefully be adopted to treat systemic aging, for example, in sarcopenia or in chronic pain or also to reduce heart disease risk factors (Skibska & Goraca, 2015; Tessier & Chevalier, 2018; Kim et al., 2012).

Key facts of aging process

- Aging process is a physiological condition which happens with age. However, worsening of body conditions fastens the process of aging.
- The main theory involves oxidative stress and neuronal inflammation. Moreover, additional elements can negatively affect this process, such as VD deficiency.
- Many molecules are studied to detect a marker of brain aging. However, the use of nutrients which are lacking in the body to improve the recovery mechanism of the body is a new strategy which has no negative effects.
- Food supplements used for aging are often very effective in vitro and in vivo, but sometimes efficacy in humans is poor. In this case the setting of the dosage and the duration of the treatments are the most important points.
- A combination of lipoic acid and vitamin D3 is able to slow down the physiological aging process. It is important to known that this effect is observed not only on the brain but also in all organs in which these components are able to act.

Summary points

- Main mechanism following brain aging theory.
- Features include neuroinflammation and oxidative stress.
- Summarized the use of food supplements to slow down aging process.
- Findings reported the in vitro and in vivo results useful to develop new human strategy.
- The elements proposed have been well known to have beneficial properties to humans.

References

Arivazhagan, P., Shila, S., Kumaran, S., & Panneerselvam, C. (2002). Effect of DL-alpha-lipoic acid on the status of lipid peroxidation and antioxidant enzymes in various brain regions of aged rats. *Experimental Gerontology, 37*, 803−811.

Balion, C., Griffith, L. E., Strifler, L., Henderson, M., Patterson, C., Heckman, G., … Raina, P. (2012). Vitamin D, cognition, and dementia: A systematic review and meta-analysis. *Neurology, 79*(13), 1397−1405.

Basli, A. (2012). Wine polyphenols: Potential agents in neuroprotection. *Oxidative Medicine and Cellular Longevity, 80*, 57−62.

Bjelakovic, G., Gluud, L. L., Nikolova, D., Whitfield, K., Wetterslev, J., Simonetti, R. G., … Gluud, C. (2014). Vitamin D supplementation for prevention of mortality in adults. *Cochrane Database of Systematic Reviews*, (1). CD007470.

Bråthen, A. C. S., De Lange, A. G., Fjell, A. M., & Walhovd, K. B. (2020). Risk- and protective factors for memory plasticity in aging. *Aging, Neuropsychology, and Cognition*, 1−17.

Chakravarti, B., & Chakravarti, D. N. (2007). Oxidative modification of proteins: Age-related changes. *Gerontology, 53*, 128−139.

Cotman, C. W., Head, E., Muggenburg, B. A., Zicker, S., & Milgram, N. W. (2002). Brain aging in the canine: A diet enriched in antioxidants reduces cognitive dysfunction. *Neurobiology of Aging, 23*, 809−818.

Cremer, D. R., Rabeler, R., Roberts, A., & Lynch, B. (2006). Safety evaluation of alpha-lipoic acid (ALA). *Regulatory Toxicology and Pharmacology, 46*, 29−41.

Cui, X., Gooch, H., Groves, N. J., Sah, P., Burne, T. H., Eyles, D. W., & McGrath, J. J. (2015). Vitamin D and the brain: key questions for future research. *Journal of Steroid Biochemistry and Molecular Biology*. https://doi.org/10.1016/j.jsbmb.2014.11.004.

Cui, X., Zuo, P., Zhang, Q., Li, X., Hu, Y., & Long, J. (2006). Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-alpha-lipoic acid. *Journal of Neuroscience Research, 84*, 647−654.

Davinelli, S., Chiosi, F., Di Marco, R., Costagliola, C., & Scapagnini, G. (2017). Cytoprotective effects of citicoline and homotaurine against glutamate and high glucose neurotoxicity in primary cultured retinal cells. *Oxidative Medicine and Cellular Longevity*, 2825703.

De Domenico, S., & Giudetti, A. M. (2017). Nutraceutical intervention in ageing brain. *Japanese Journal of Genetics, 65*, 79−92.

Deuther-Conrad, W., Loske, C., Schinzel, R., Dringen, R., Riederer, P., & Munch, G. (2001). Advanced glycation endproducts change glutathione redox status in SH-SY5Y human neuroblastoma cells by a hydrogen peroxide dependent mechanism. *Neuroscience Letters, 312*, 29−32.

Di Domenico, F., Barone, E., Perluigi, M., & Butterfield, D. A. (2015). Strategy to reduce free radical species in Alzheimer's disease: An update of selected antioxidants. *Expert Review of Neurotherapeutics, 15*(1), 19−40.

Farr, S. A., Price, T. O., Banks, W. A., Ercal, N., & Morley, J. E. (2012). Effect of alpha-lipoic acid on memory, oxidation, and lifespan in SAMP8 mice. *Joyrnal of Alzheimer's Disease, 32*, 447−455.

Foster, T. C., Defazio, R. A., & Bizon, J. L. (2012). Characterizing cognitive aging of spatial and contextual memory in animal models. *Frontiers in Aging Neuroscience, 4*, 12.

Gemma, C., Mesches, M. H., Sepesi, B., Choo, K., Holmes, D. B., & Bickford, P. C. (2002). Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. *Journal of Neuroscience, 22*, 6114−6120.

Ghimire, K., Altmann, H. M., Straub, A. C., & Isenberg, J. S. (2017). Nitric oxide: what's new to NO? *American Journal of Physiology − Cell Physiology, 312*(3), C254−C262.

Go, Y. M., & Jones, D. P. (2017). Redox theory of aging: Implications for health and disease. *Clinical Science, 131*, 1669−1688.

Hager, K., Kenklies, M., McAfoose, J., Engel, J., & Munch, G. (2007). Alpha-lipoic acid as a new treatment option for Alzheimer's disease—a 48 months follow-up analysis. *Journal of Neural Transmission Supplementum*, (72), 189—193.

Hathcock, J. N., Shao, A., Vieth, R., & Heaney, R. (2007). Risk assessment for vitamin D. *American Journal of Clinical Nutrition, 85*(1), 6—18.

Heaney, R. P. (2006). Barriers to optimizing vitamin D3 intake for the elderly. *Journal of Nutrition, 136*(4), 1123—1125.

Heaney, R. P., Davies, K. M., Chen, T. C., Holick, M. F., & Barger-Lux, M. J. (2003). Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *American Journal of Clinical Nutrition, 77*(1), 204—210.

Henderson, V. C., Kimmelman, J., Fergusson, D., Grimshaw, J. M., & Hackam, D. G. (2013). Threats to validity in the design and conduct of preclinical efficacy studies: A systematic review of guidelines for in vivo animal experiments. *PLoS Medicine, 10*(7), e1001489.

Hill, T. R., Granic, A., & Aspray, T. J. (2018). Chapter 8: Vitamin D and ageing. In J. R. Harris, & V. I. Korolchuk (Eds.), *Biochemistry and cell biology of ageing: Part I biomedical science* (pp. 191—220).

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., … Weaver, C. M. (2011). Evaluation, treatment, and prevention of vitamin D deficiency: An endocrine society clinical practice guideline. *Journal of Clinical Endocrinology and Metabolism, 96*(7), 1911—1930.

Hossein-nezhad, A., & Holick, M. F. (2013). Vitamin D for health: A global perspective. *Mayo Clinic Proceedings, 88*(7), 720—755.

Ibi, M., Sawada, H., Nakanishi, M., Kume, T., Katsuki, H., Kaneko, S., … Akaike, A. (2001). Protective effects of 1 alpha, 25-(OH)(2)D(3) against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology, 40*(6), 761—771.

Jones, D. P., & Sies, H. (2015). The redox code. *Antioxidants and Redox Signaling, 23*, 734—746.

Jovanovic, Z. (2014). Antioxidative defense mechanisms in the aging brain. *Archives of Biological Sciences, 66*(1), 245—252.

Kaidar-Person, O., Person, B., Szomstein, S., & Rosenthal, R. J. (2008). Nutritional deficiencies in morbidly obese patients: A new form of malnutrition? Part A: Vitamins. *Obesity Surgery, 18*(7), 870—876.

Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., … Sierra, F. (2014). Geroscience: Linking aging to chronic disease. *Cell, 159*(4), 709—713.

Kidd, P. M. (2005). Neurodegeneration from mitochondrial insufficiency: Nutrients, stem cells, growth factors, and prospects for brain rebuilding using integrative management. *Alternative Medicine Review, 10*, 268—293.

Kim, H., Kim, H. J., Lee, K., Kim, J. M., Kim, H. S., Kim, J. R., … Lee, S. J., et al. (2012). α-Lipoic acid attenuates vascular calcification *via* reversal of mitochondrial function and restoration of Gas6/Axl/Akt survival pathway. *Journal of Cellular and Molecular Medicine*. https://doi.org/10.1111/j.1582-4934.2011.01294.x.

Koufaki, M. (2014). Therapeutic applications of lipoic acid: A patent review (2011—2014). *Expert Opinion on Therapeutic Patents, 24*, 993—1005.

Lang, F., Ma, K., & Leibrock, C. B. (2019). 1, 25(OH)2D3 in brain function and neuropsychiatric disease. *Neurosignals, 27*(1), 40—49.

Liu, J. (2008). The effects and mechanisms of mitochondrial nutrient alpha-lipoic acid on improving age-associated mitochondrial and cognitive dysfunction: An overview. *Neurochemical Research, 33*(1), 194—203.

Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrao, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell, 153*, 1194—1217.

Lynch, G., Rex, C. S., & Gall, C. M. (2006). Synaptic plasticity in early aging. *Ageing Research Reviews, 5*(3), 255—280.

Maddock, J., Zhou, A., Cavadino, A., Kuźma, E., Bao, Y., Smart, M. C., … Hyppönen, E. (2017). Vitamin D and cognitive function: A Mendelian randomisation study. *Scientific Reports, 7*(1), 13230.

Maharsh, K. P., Riley, M. A., Hobbs, S., Cortez-Cooper, M., & Robinson, V. G. B. (2014). Can α-lipoic acid mitigate progression of aging-related decline caused by oxidative stress? *Southern Medical Journal, 107*(12), 780—787.

Mahboob, A., Farhat, S. M., Iqbal, G., Babar, M. M., Zaidi, N. U., & Nabavi, S. M. (2016). Alpha-lipoic acid-mediated activation of muscarinic receptors improves hippocampus- and amygdala-dependent memory. *Brain Research Bulletin, 122*, 19—28.

Manda, K., Ueno, M., Moritake, T., & Anzai, K. (2007). Radiation-induced cognitive dysfunction and cerebellar oxidative stress in mice: Protective effect of alpha-lipoic acid. *Behavioural Brain Research, 177*, 7—14.

Molinari, C., Morsanuto, V., Ghirlanda, S., Ruga, S., Notte, F., Gaetano, L., & Uberti, F. (2019). Role of combined lipoic acid and vitamin D3 on astrocytes as a way to prevent brain ageing by induced oxidative stress and iron accumulation. *Oxidative Medicine and Cellular Longevity*, 2843121.

Molz, P., & Schröder, N. (2017). Potential therapeutic effects of lipoic acid on memory deficits related to aging and neurodegeneration. *Frontiers in Pharmacology, 8*, 849.

Moré, M. I., Freitas, U., & Rutenberg, D. (2014). Positive Effects of Soy Lecithin-Derived Phosphatidylserine plus Phosphatidic Acid on Memory, Cognition, Daily Functioning, and Mood in Elderly Patients with Alzheimer's Disease and Dementia. *Advances in Therapy*. https://doi.org/10.1007/s12325-014-0165-1.

Norman, A. W., & Bouillon, R. (2010). Vitamin D nutritional policy needs a vision for the future. *Experimental Biology and Medicine (Maywood, N.J.), 235*(9), 1034—1045.

Opii, W. O., Joshi, G., Head, E., Milgram, N. W., Muggenburg, B. A., Klein, J. B., … Butterfield, D. A. (2008). Proteomic identification of brain proteins in the canine model of human aging following a long-term treatment with antioxidants and a program of behavioral enrichment: Relevance to Alzheimer's disease. *Neurobiology of Aging, 29*, 51—70.

Palaniappan, A. R., & Dai, A. (2007). Mitochondrial ageing and the beneficial role of α-lipoic acid. *Neurochemical Research, 32*(9), 1552—1558.

Queen, B. L., & Tollefsbol, T. O. (2010). Polyphenol and aging. *Current Aging Science, 3*(1), 34—42.

Rochette, L., Ghibu, S., Richard, C., Zeller, M., Cottin, Y., & Vergely, C. (2013). Direct and indirect antioxidant properties of alpha-lipoic acid and therapeutic potential. *Molecular Nutrition and Food Research, 57*, 114—125.

Rosen, C. J., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., Durazo-Arvizu, R. A., … Taylor, C. L. (2012). IOM committee members respond to Endocrine Society vitamin D guideline. *Journal of Clinical Endocrinology and Metabolism, 97*(4), 1146−1152.

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., … Shapses, S. A. (2010). The 2011 report on dietary reference intakes for calcium and vitamin D from the institute of medicine. *Journal of Clinical Endocrinology and Metabolism, 96*(1), 53−58.

Salthouse, T. A. (2009). When does age-related cognitive decline begin? *Neurobiology of Aging, 30*(4), 507−514.

Sanders, K. M., Nicholson, G. C., & Ebeling, P. R. (2013). Is high dose vitamin D harmful? *Calcified Tissue International, 92*(2), 191−206.

Shay, K. P., Moreau, R. F., Smith, E. J., Smith, A. R., & Hagen, T. M. (2009). Alpha-lipoic acid as a dietary supplement: Molecular mechanisms and therapeutic potential. *Biochimica et Biophysica Acta, 1790*(10), 1149−1160.

Singh, G., Pachouri, U. C., Khaidem, D. C., Kundu, A., Chopra, C., & Singh, P. (2015). Mitochondrial DNA damage and diseases. *F1000Research, 4*, 176.

Sinha, M., Bir, A., Banerjee, A., Bhowmick, P., & Chakrabarti, S. (2016). Multiple mechanisms of age-dependent accumulation of amyloid beta protein in rat brain: Prevention by dietary supplementation with N-acetylcysteine, α-lipoic acid and α-tocopherol. *Neurochemistry International*. https://doi.org/10.1016/j.neuint.2015.10.003.

Skibska, B., & Goraca, A. (2015). The protective effect of lipoic acid on selected cardiovascular diseases caused by age-related oxidative stress. *Oxidative Medicine and Cellular Longevity*, 313021.

Tessier, A. J., & Chevalier, S. (2018). An update on protein, leucine, omega-3 fatty acids, and vitamin D in the prevention and treatment of sarcopenia and functional decline. *Nutrients, 10*(8), 1099.

Thakurta, I. G., Banerjee, P., Bagh, M. B., Ghosh, A., Sahoo, A., Chattopadhyay, S., & Chakrabarti, S. (2014). Combination of N-acetylcysteine, alpha-lipoic acid and alpha-tocopherol substantially prevents the brain synaptosomal alterations and memory and learning deficits of aged rats. *Experimental Gerontology, 50*, 19−25.

Thakurta, I. G., Chattopadhya, M., Ghosh, A., & Chakrabarti, S. (2012). Dietary supplementation with N-acetylcysteine, α-tocopherol and α-lipoic acid reduces the extent of oxidative stress and proinflammatory state in aged rat brain. *Biogerontology, 13*, 479−488.

Uberti, F., Morsanuto, V., Aprile, S., Ghirlanda, S., Stoppa, I., Cochis, A., … Molinari, C. (2017). Biological effects of combined resveratrol and vitamin D3 on ovarian tissue. *Journal of Ovarian Research, 10*, 61.

Uberti, F., Morsanuto, V., Ruga, S., Stoppa, I., Galla, R., Notte, F., & Molinari, C. (2019). Effect of mixed lipoic acid, vitamin D, phosphatidylserine and homotaurine to obtain a new formulation for brain ageing prevention. *EC Neurology*, 302−312, 11.5.

Welland, D. (2009). New studies show low vitamin D levels may impair cognitive function. *Scientific American*. https://doi.org/10.1038/scientificamericanmind1109-14.