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Nutritional approaches for prevention and treatment of neurodegenerative diseases

Edited by
Gorazd Drevenšek, Gang Wang, Mona E. Aboutabl and Zoltan Sarnyai

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Nutritional approaches for prevention and treatment of neurodegenerative diseases

Topic editors

Gorazd Drevenšek — University of Primorska, Slovenia

Gang Wang — Shanghai Jiao Tong University, China

Mona E. Aboutabl — National Research Center, Pharmaceutical and Drug Industries Research Division, Egypt

Zoltan Sarnyai — James Cook University, Australia

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PERSPECTIVE
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Can Ketogenic Diet Improve Alzheimer's Disease? Association With Anxiety, Depression, and Glutamate System

Jose Enrique de la Rubia Ortí[1]*, *David Fernández*[1]*, *Félix Platero*[2] and *María Pilar García-Pardo*[3]

[1] Department of Nursing, Catholic University of Valencia, Valencia, Spain, [2] Department of Medicine, University of Valencia, Valencia, Spain, [3] Department of Psychology and Sociology, University of Zaragoza, Teruel, Spain

Background: Alzheimer's disease is the most common neurodegenerative disorder in our society, mainly characterized by loss of cognitive function. However, other symptoms such as anxiety and depression have been described in patients. The process is mediated by alterations in the synaptic and extrasynaptic activity of the neurotransmitter glutamate, which are linked to a hypometabolism of glucose as the main source of brain energy. In that respect, Ketogenic diet (KD) has been proposed as a non-pharmacological treatment serving as an alternative energy source to the neurons increasing the fat percentage and reducing the carbohydrates percentage, showing promising results to improve the cognitive symptoms associated with different neurodegenerative disorders, including AD. However, the association of this type of diet with emotional symptoms and the modulation of glutamate neurotransmission systems after this dietary reduction of carbohydrates are unknown.

Objective: The aim of this short review is to provide update studies and discuss about the relationship between KD, anxiety, depression, and glutamate activity in AD patients.

Discussion: The main results suggest that the KD is an alternative energy source for neurons in AD with positive consequences for the brain at different levels such as epigenetic, metabolic and signaling, and that the substitution of carbohydrates for fats is also associated with emotional symptoms and glutamate activity in AD.

Keywords: Alzheimer's disease, ketogenic diets, anxiety, depression, glutamate

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Zoltan Sarnyai,
James Cook University, Australia

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Guoyuan Qi,
University of Arizona, United States
Isis Trujillo-Gonzalez,
University of North Carolina at Chapel Hill, United States

***Correspondence:**
Jose Enrique de la Rubia Ortí
joseenrique.delarubi@ucv.es
David Fernández
David.fernandez@ucv.es

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INTRODUCTION

At present, Alzheimer's disease (AD) is the most prevalent form of dementia, appearing mainly in the elderly and defined by a prematurely aging brain. To date there is no cure, affecting more than 50 million people worldwide (1). This disease is characterized by a progressive and irreversible memory loss. However, related to that aging brain, neuropsychiatric symptoms are also very relevant in Alzheimer's-type disorder; specially, the presence of anxiety and depression (2), which have a direct impact on the quality of life of patients (3). This is why, although they are not usually given as much attention at the therapeutic level, an adequate treatment of these neuropsychiatric symptoms

could considerably improve the quality of life, related at the same time to a better prognosis of the disease (4). Anxiety and depression in AD patients are treated pharmacologically. Nonetheless, many problems linked to the use of these drugs have been described in these patients, with even greater progression and development of the disease (5–7), neuronal damage, and, in addition, mature neurons becoming immature, which could explain why antidepressants also induce apoptosis (8).

With this in mind, it is necessary to consider other non-pharmacological options that do not pose risks to patients to improve their symptoms. In this regard, KDs, rich in medium chain fatty acids (MCFA), show promising results. This type of diet is an alternative source of energy to glucose which could improve the different symptoms of AD. By shifting metabolism from carbohydrates toward fatty acids, it has been seen that KD are able to stimulate the production of ketone bodies after hepatic metabolism, which will be used as a new energy option by the central nervous system (9). It seems that a diet based on high-fat and low-carbohydrate content induces the body to a ketosis state similar to the effect of fasting, generating a neuroprotective action on aging brain cells, reducing brain inflammation, and improving mitochondrial function (10). Specifically related to the energy activity in mitochondria, it is known that in neurodegenerative disorders there is a disruption of the brain's energy metabolism, therefore, ketone bodies can support brain energy and slow the progression of different neurodegenerative disorders such as AD (11). In fact, different current studies have evidenced the mechanism of KD for AD treatment and prevention (12–14). This activity promoted by KD intake could consequently justify not only improvements in cognitive disfunctions (15, 16), but also in mood state disorders using 3xTgAD mouse models of AD (17, 18).

Despite the evidence about the positive effect of KD and AD, further research is necessary on the etiopathogenesis of this disease that causes known neurophysiological alterations, in order to understand all the mechanisms through which this type of diet achieves improvements. In this regard, the presence and accumulation of β-amyloid (Aβ) proteins plaques seems to be particularly noteworthy (19), since there is an association between the emotional symptoms and the deposition of Aβ associated with cognitive deterioration (20), and it is even possible to see that the interaction of these variables with the amyloid state can be used to predict the speed of cognitive decline (21). As the origin of this increase in the deposition of the amyloid protein as well as the genetic causes (22, 23), alterations in the levels of neurotransmitters are also highlighted, especially the decrease in the neurotransmitter acetylcholine (24) and the increase in glutamate levels (25). Furthermore, metabolic disorders are also considered important, especially those related to insulin resistance in the brain, which would result in a misuse of glucose by certain regions involved in the development of the disease, even appearing to have a kind of insulin resistance in the brain or "type 3 diabetes" (26). Several studies have remarked the role the apolipoprotein E allele 4 (APOE4) as a common risk factor for AD and type 2 diabetes. Metabolic profiling showed that the APOE4 variant is specifically associated with one type of AD related to decreased brain glucose utilization. In fact, one and two APOE4 alleles have been used as biomarkers of AD, since carriers of this alleles showed decreased brain glucose uptake even years before the onset of clinical symptoms of AD (27–30).

Therefore, although several papers in recent years have reported the role of KD on AD, it seems that the relationship between this both variables (diet and brain disease) is complex and influenced by several factors. As it has been previously mentioned, mood disorders and alterations in different neurotransmitters have been observed in AD patients. However, very few articles have studied whether there is a relationship between the KD, mood state, neurotransmission brain systems, and AD. Thus, the aim of this short review is to provide the most current scientific evidence that shows the association between these variables in order to contribute to new therapeutical strategies for AD.

IMPACT OF GLUTAMATE ON ANXIETY AND DEPRESSION IN ALZHEIMER'S DISEASE (AD)

As for the alteration of the activity of certain neurotransmitters, excess glutamate seems to be directly related to the emotional aspects of dementia like AD, specially outlining the perception of anxiety and depression (31). This is due to the fact that in patients with AD, as a result of excessive levels of this neurotransmitter in the extrasynaptic space, there is hyperexcitability in neurons, with overstimulation especially in NMDA ionotropic receptors, leading to synaptic loss and cell death due to an increase in the cytoplasmic concentration of Ca^{++} and the generation of reactive oxygen species. This process is mainly due to a lack of activity in the glutamate transporters in charge of eliminating excess levels of the neurotransmitter, which is related to the presence of amyloid plaques (32). In this respect, when attempting to explain this relation, two mechanisms tied to different glutamate receptors have always been observed in the hippocampus.

On the one hand, a clear decrease in anxiety levels was observed when postsynaptic group II metabotropic glutamate receptors (mGluRs) were blocked in mice with AD, specifically mGlu2 and mGlu3 (mGluR2/3), using as a model Dutch mice APP (Alzheimer's amyloid precursor protein E693Q), transgenic rodents that accumulate Dutch amyloid-β (Aβ) oligomers. This is due to high levels of glutamate, which have been observed to activate these receptors in the hippocampus, increasing the production of Aβ42 amyloid peptide and not Aβ40 with less aggregation, increasing the proportion of Aβ42:Aβ40 and, in turn, the ability to form amyloid beta plaques, which decrease neurogenesis and promote the onset of anxiety and depression (33). Moreover, Kim et al. demonstrated that APP transgenic mice showed phenotype changes after treatment with BCI-838 (a drug that acts as a Group II mGluR antagonist metabolite) for 3 months. In particular, it was observed that this treatment was associated with reversal of transgene-related amnestic behavior and reduced anxiety levels (33). On the other hand, NMDA ionotropic glutamate receptors are abundantly expressed throughout the whole brain, carrying out an essential function,

not only at a cognitive level (34), but also regarding anxiety (35) and depression (36) by having an impact on synaptic plasticity. This could be explained, in part, by the amyloid-β peptide inducing the liberation of astrocytic glutamate (through its cross-interaction with nicotinic acetylcholine receptors and the entry of Ca^{2+} needed for the release of glutamate), which at the same time activates the extrasynaptic NMDA receptors (eNMDAR) in the neurons. The action of these eNMDAR causes an inward current in excess of Ca^{2+}, which sequentially stimulates the neuronal nitric oxide synthase (nNOS) generating high levels of NO, which contributes to the loss of synaptic spines (37). Blocking these receptors in animal models [adult male Wistar rats induced with sporadic Alzheimer's-like disease using microinjections of streptozotocin (3 mg/kg/5 μl)], decreased the perception of anxiety and depression. This was associated with a reduction in inflammation mediated by inflammatory cytokines, such as interleukins IL-6 and IL-1β, and tumor necrosis factor alpha (TNF-α) (38). Therefore, regulating glutamate activity in this brain area, will not only achieve cognitive improvements (39), but it is also related to levels of anxiety and depression (40, 41), and there is evidence that glutamate receptors can alter cognitive and mood state both in humans and model animals, using transgenic mouse models that have specific receptor subunits that can be targeted in specific brain regions. However, these studies have limitations since it is difficult to understand where glutamate antagonists act to induce anxiolytic or antidepressant effects and to assess the phenotypes in the animals.

Conversely, it should be noted that the glutamate role in its binding to NMDA receptors at a synaptic level (sNMDAR) is also essential for cognition and neuron survival (42, 43), and precisely in AD there is glucose hypometabolism in certain brain areas, possibly linked to an insulin resistance as aforementioned. This hypometabolism implies that the glutamate neurotransmission (GNT) at a synaptic level, which requires a glial-neuronal process with oxidation of glucose and the glutamine-glutamate cycle (44), consuming up to the 80% of ATP provided by the metabolism of glucose (45), may be diminished, and that there is no adequate synapse in its NMDA receptors. As consequence, it can be concluded that in AD the activation of sNMDAR initiates plasticity and stimulates cell survival, while the stimulation of eNMDAR promotes cell death (46). Therefore, these two different groups of glutamate receptors seem to be connected with depression and anxiety (**Figure 1A**).

DISCUSSION

As it has been showed throughout this work, the importance of non-pharmacological therapies in AD is essential to improve symptoms and to learn about different novel treatments. Among them, KD has demonstrated great results on the progression of many neurodegenerative disorders (11). Specifically, in AD the data is promising and several studies have evidenced the positive effect of this type of diet in this disorder in both animals and humans (47, 48).

The KD is actually a biochemical model of fasting. Glucose is known to be the main energy source to the neurons. However, in some conditions such as food deprivation or under fasting, brain cells use other alternative energy sources, like ketone bodies. Under these circumstances the human body starts to use fats from its own deposits with a consequent ketosis (49). This type of diet that replaces carbohydrates with fats has positive consequences on the brain at an epigenetic, metabolic and signaling level (50). On the other hand, some recent studies have showed that the neuroprotective effects of KD might be explained by indirect actions on neurons. It has been seen that there are changes in the microbiome after following this type of diet, related to an improvement in the gut-brain axis (51).

As far as the distribution of calories is concerned, in KD, 90% of the total calorie intake is from fat, while only 6% is from protein and 4% from carbohydrates (52). This can be achieved by a composition characterized by a macronutrient ratio of 4:1 (4 g of fat every 1 g of protein and carbohydrates) (50), reducing carbohydrates to ≤10% of the energy consumed (53). Nonetheless, there are some alternatives that slightly change the proportion of carbohydrates, such as de Atkins diet in which these are limited to 5% of dietary energy (54) obtaining interesting cognitive improvements (55). Besides, this ketogenesis is more effective when the fats, instead of being long chain fatty acids (LCFA) (which represents the classical version of the diet) (54) are medium chain triglycerides (MCT) made up of MCFA, as it increases the concentration of ketone bodies in blood even if carbohydrates are present in diet, making it a less restrictive diet and easier to follow (56). Precisely both preclinical (57) and clinical studies including a diet enriched with foods high in MCTs, such as coconut oil (58), has showed the positive effect of this diet. These improvements, in particular achieved with MCFA, could also be related to the metabolic activity that has been evidenced in astrocytes, where especially the administration of caprylic acid with 8 C atoms (C8:0) does not affect glycolysis, but clearly increases ketogenesis (59), so that MCFA may have benefits through the modulation of astrocyte metabolism, providing energy to neighboring neurons especially through ketone bodies (60). Moreover, concretely in AD itself, even without an extern MCFA source, it has been shown how the gliosis derived from the disease causes the astrocytes themselves to protect and repair the lesion by optimizing their metabolism through the synthesis of ketone bodies (61).

However, the influence on this type of diet on other variables of a different nature, such as neurotransmission systems or mood variables, has not been studied. In this work it has been described that the mood state and glutamate neurotransmission system can be involved on the effect of KD in AD. Regarding mood state, this work suggests that emotional improvements may be a consequence of a direct action of ketone bodies in relation to extrasynaptic glutamatergic receptors eNMDAR. It has been observed that acetone and β-hydroxybutyrate (βHB) act as glutamate inhibitors in NMDA receptor, specifically highlighting the activity exhibited by βHB, which inhibits the effects of agonists of these receptors at concentrations achieved in *vivo* (62). This process could be related to the observed decrease in glutamate availability in a neuron culture in which glucose is replaced by βHB as an energy source (63). It must be added that the improvements in anxiety and depression observed in

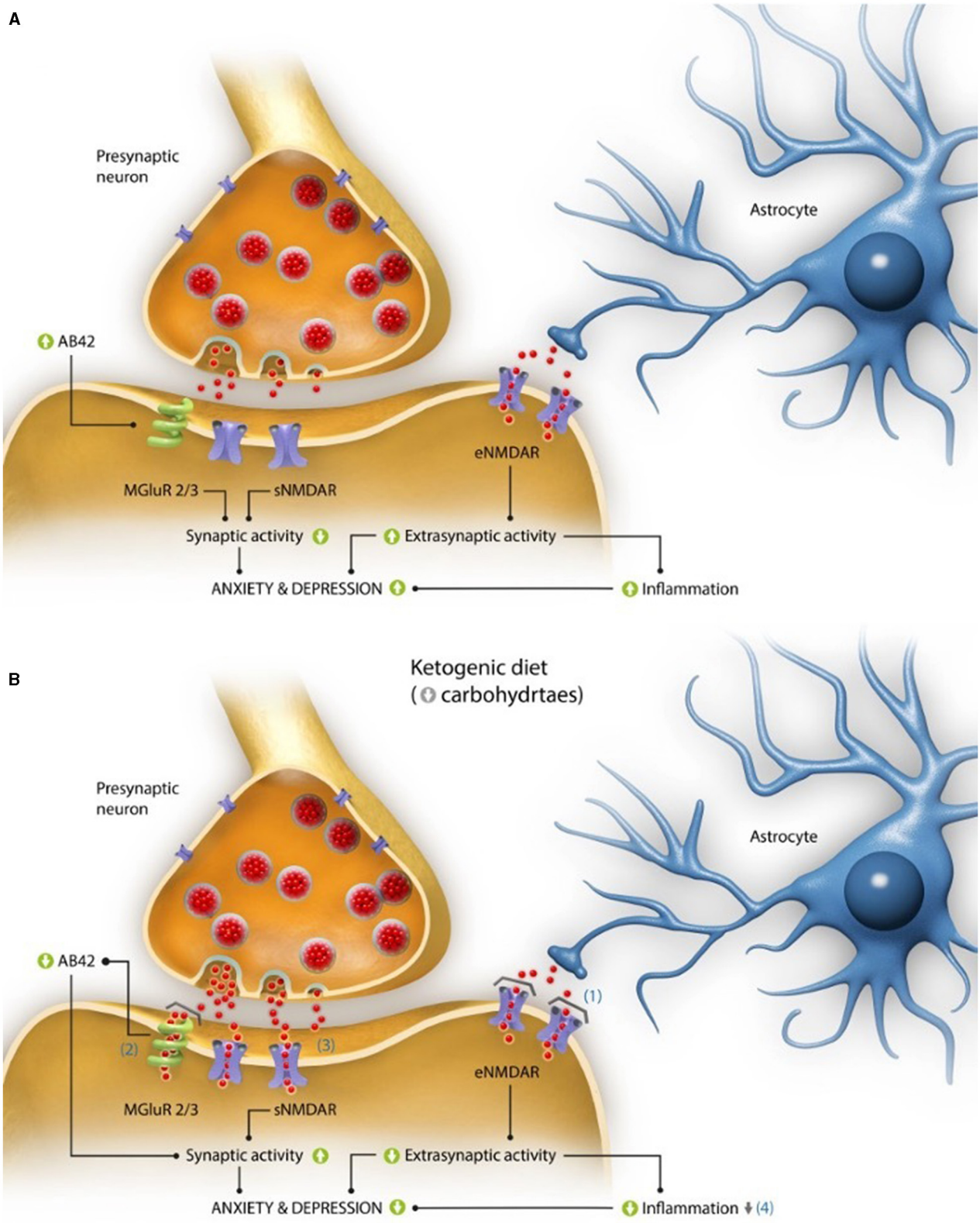

FIGURE 1 | Interaction of glutamate activity on alterations in anxiety and depression levels characteristic of the disease. **(A)** Pathogenic mechanisms based on the activity of the excitatory neurotransmitter glutamate both at synaptic and extrasynaptic levels, which could explain the elevated perception of anxiety and depression described in Alzheimer's disease (AD). At the synaptic level, in the animal model of the disease 3xTgAD mice the activation of mGluR2/3 receptors due to excess

(Continued)

FIGURE 1 | glutamate (red dots), has been linked to the formation of β-amyloid peptides with 42 residues long (Aβ42), while at the extrasynaptic level, it has been linked to high glutamate levels, which can increase the activation of its NMDA receptors (eNMDAR), producing an increase in inflammation. Both processes have been linked to the presence of anxiety and depression. **(B)** Proposed mechanisms of action of a ketogenic diet (KD) in the improvement of perception of anxiety and depression in patients with AD. (1) The production of ketone bodies derived from the intake of KDs act as glutamate inhibitors in the NMDA extrasynaptic receptor (eNMDAR), decreasing the extrasynaptic activity of glutamate (red dots) and, as a consequence, the inflammation. (2) They are also capable of blocking the toxicity derived from the formation of amyloid plaques, whose production is partly due to the activation of the mGluR2/3 glutamate receptors. (3) Moreover, they could improve the activity of glutamate at a synaptic level because of a greater ATP contribution (with regard to glucose metabolism), which would have a positive impact on the cognitive and emotional capacity. (4) Finally, the neuroprotector effect of ketone bodies (as a result of the improvement in the electron chain functioning) could lessen the levels of oxidative stress and inflammation. All these processes achieve a decrease in the perception of anxiety and depression, characteristic of this pathology.

AD patients could also be related to the protection ketone bodies seem to exert on cortical neurons against the β-amyloid induced toxicity (64). This mechanism of action could suggest that, even though ketone bodies have not been directly linked to changes in the activity of mGluR receptors, it has been demonstrated in animal models (using male 3xTgAD mice of the disease) that these diets significantly decrease β-amyloid peptide in the brain, which is in turn related in part to the activation of these receptors (18).

Furthermore, ketone bodies acetoacetate and βHB, after crossing the blood-brain barrier, can replace glycolysis. This change would improve glutamate activity at a synaptic level, with a better ATP efficiency; since the metabolites would act as energy substrates of complex II of the respiratory chain, bypassing complex I (which, together with complex IV, are the ones altered in the majority of diseases of mitochondrial nature) (65). Therefore, ketone bodies provide an energy source with higher ATP yield than glucose (66), which may also improve metabolic alterations due to a misuse of glucose, characteristic of the disease caused by destruction of the locus coeruleus (67).

Along these lines, improvements in the functioning of the electron chain in oxidative phosphorylation mediated by ketone bodies achieve cognitive and emotional betterments, given the link established between the mitochondrial alterations and the presence of this symptomatology (68); as a consequence of the decrease in the level of oxidative stress and inflammation, related, in turn, with the presence of anxiety and depression (69, 70).

All these processes are shown in **Figure 1B**.

In short, due to the negative effects associated to pharmacological treatments for anxiety and depression in AD, the increase of ketone bodies in blood after the administration of KDs (based on the low levels of hydrates and high levels of fat), could be an effective option for the treatment of both, not only for their neuroprotective activity (71, 72) but for their interaction in the pathogenic mechanisms of the disease mediated directly or indirectly by the glutamate activity.

It should also be considered at what point in the disease the KD could be more effective. Studies show how improvement in episodic memory, and reported vitality occurs in patients with mild cognitive impairment (MCI) on early AD after the administration of the modified Atkins diet (MAD) (55). This is in line with results in our laboratory, where the administration of coconut oil rich in MCFA improved episodic orientation and temporal and semantic memory, mainly in the mild-moderate stage of the disease (58). The explanation for these results may be due to the fact that energy hypometabolism begins to occur even decades before the onset of clinical symptoms progressing in the early stages of the disease, as we have previously highlighted (73, 74). However, in the severe phase of the disease, possibly as a consequence of the prolonged bioenergetic deficits and the high oxidative stress derived from these alterations, there is an increase in amyloid plaques (75) that activate apoptotic pathways, aberrant mitochondrial biogenesis and altered mitophagy resulting in neuronal death (76). Thus, the phase of the disease in which the diets are administered should be considered to improve their efficacy. Therefore, it could be therapeutically beneficial in the initial phases to combine diets with other nutraceutical or pharmacological treatments aimed at curbing the high oxidative stress associated with glucidic hypometabolism (77) and, in advanced stages, the combination of KDs should be given with drugs that treat the pathologic signs of the disease, fundamentally related to the formation of amyloid plaques. In this regard, the efficacy of different antioxidants that prevent and reverse AD when combined with adequate diets has been seen (78), highlighting vitamin C (79), α-lipoic acid (80) or the polyphenols epigallocatechin gallate and resveratrol (which can also prevent the neurotoxic effects of β-amyloid protein) (81), while drugs such as donepezil, galantamine and rivastigmine, which act as inhibitors of acetylcholinesterase derived from the accumulation of β-amyloid, could be more effective in advanced stages of the disease (82). Finally, and directly related to anxiety and depression variables, main focus of our study, the combination with glutamate inhibitors such as memantine or lamotrigine could improve the effectiveness of the impact of this diet on these variables, by completing the mechanisms related to the neurotransmitter already analyzed (83–86).

To conclude, and despite the benefits discussed and analyzed in this work, it is important to remark that in some studies in which KD were followed, adverse effects could be observed, mainly focused on gastrointestinal symptoms (constipation, nausea, vomiting and decreased appetite) (87–89), which even forced the interruption of the treatment (56) and transient hyperlipidaemia (90), seeing an increase in fasting serum total cholesterol, triglycerides and low-density lipoprotein (LDL) at the beginning of the treatment (91). In addition, as for the efficacy of KD, a recent review has highlighted the positive cognitive assessments obtained in the short term, and there are no published studies that have conducted follow-ups to determine whether the improvements in variables such as anxiety and depression are maintained over time, or even when the diet is discontinued (92). It should also be noted that these diets usually result in weight loss (93) and in that this loss is common, detrimental and predictive of the cognitive state of Alzheimer's patients (94), so it should be assessed and considered throughout the treatment. Therefore, more studies in this area are needed to gain further knowledge of this disease and the variables involved.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

MPG-P and JR: developed the hypothesis and wrote the manuscript. FP and DF: writing-review and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Soria Lopez JA, González HM, Léger GC. Alzheimer's disease. *Handb Clin Neurol.* (2019) 167:231–55. doi: 10.1016/B978-0-12-804766-8.00013-3
2. Chen JC, Borson S, Scanlan JM. Stage-specific prevalence of behavioral symptoms in Alzheimer's disease in a multi-ethnic community sample. *Am J Geriatr Psychiatr.* (2000) 8:123–33. doi: 10.1097/00019442-200005000-00007
3. Shin IS, Carter M, Masterman D, Fairbanks L, Cummings JL. Neuropsychiatric symptoms and quality of life in Alzheimer disease. *Am J Geriatr Psychiatr.* (2005) 13:469–74. doi: 10.1097/00019442-200506000-00005
4. Tschanz JAT, Corcoran CD, Schwartz S, Treiber K, Green RC, Norton MC, et al. Progression of cognitive, functional and neuropsychiatric symptom domains in a population cohort with Alzheimer's dementia: the cache county dementia progression study. *Am J Geriatr Psychiatry.* (2011) 19:532–42. doi: 10.1097/JGP.0b013e3181faec23
5. Biétry FA, Pfeil AM, Reich O, Schwenkglenks M, Meier CR. Benzodiazepine use and risk of developing Alzheimer's disease: a case-control study based on Swiss claims data. *CNS Drugs.* (2017) 31:245–51. doi: 10.1007/s40263-016-0404-x
6. Gray SL, Anderson M, Hubbard R. Anticholinergic use with incident dementia-reply. *JAMA Intern Med.* (2015) 175:1577. doi: 10.1001/jamainternmed.2015.2574
7. Borda MG, Jaramillo-Jimenez A, Oesterhus R, Santacruz JM, Tovar-Rios DA, Soennesyn H, et al. Benzodiazepines and antidepressants: effects on cognitive and functional decline in Alzheimer's disease and lewy body dementia. *Int J Geriatr Psychiatry.* (2021) 36:917–25. doi: 10.1002/gps.5494
8. Andrews PW, Anderson Thomson J, Amstadter A, Neale MC. Primum non nocere: an evolutionary analysis of whether antidepressants do more harm than good. *Front Psychol.* (2012) 3:117. doi: 10.3389/fpsyg.2012.00117
9. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF. Brain metabolism during fasting. *J Clin Invest.* (1967) 46:1589–95. doi: 10.1172/JCI105650
10. Rusek M, Pluta R, Ułamek-Kozioł M, Czuczwar SJ. KD in Alzheimer's disease. *Int J Mol Sci.* (2019) 20:3892. doi: 10.3390/ijms20163892
11. Jensen NJ, Wodschow HZ, Nilsson M, Rungby J. Effects of ketone bodies on brain metabolism and function in neurodegenerative diseases. *Int J Mol Sci.* (2020) 21:8767. doi: 10.3390/ijms21228767
12. Uddin MS, Kabir MT, Tewari D, Al Mamun A, Barreto GE, Bungau SG, et al. Emerging therapeutic promise of ketogenic diet to attenuate neuropathological alterations in Alzheimer's disease. *Mol Neurobiol.* (2020) 57:4961–77. doi: 10.1007/s12035-020-02065-3
13. Augustin K, Khabbush A, Williams S, Eaton S, Orford M, Cross JH, et al. Mechanisms of action for the medium-chain triglyceride KD in neurological and metabolic disorders. *Lancet Neurol.* (2018) 17:84−93. doi: 10.1016/S1474-4422(17)30408-8
14. Broom GM, Shaw IC, Rucklidge JJ. The KD as a potential treatment and prevention strategy for Alzheimer's disease. *Nutrition.* (2019) 60:118−21. doi: 10.1016/j.nut.2018.10.003
15. Ota M, Matsuo J, Ishida I, Hattori K, Teraishi T, Tonouchi H, et al. Effect of a ketogenic meal on cognitive function in elderly adults: potential for cognitive enhancement. *Psychopharmacology.* (2016) 233:3797–802. doi: 10.1007/s00213-016-4414-7
16. Ota M, Matsuo J, Ishida I, Takano H, Yokoi Y, Hori H, et al. Effects of a medium-chain triglyceride-based ketogenic formula on cognitive function in patients with mild-to-moderate Alzheimer's disease. *Neurosci Lett.* (2019) 690:232–6. doi: 10.1016/j.neulet.2018.10.048
17. Pawlosky RJ, Kashiwaya Y, King MT, Veech RL. A dietary ketone ester normalizes abnormal behavior in a mouse model of Alzheimer's disease. *Int J Mol Sci.* (2020) 21:1044. doi: 10.3390/ijms21031044
18. Kashiwaya Y, Bergman C, Lee JH, Wan R, King MT, Mughal MR, et al. A ketone ester diet exhibits anxiolytic and cognition-sparing properties, and lessens amyloid and tau pathologies in a mouse model of Alzheimer's disease. *Neurobiol Aging.* (2013) 34:1530–9. doi: 10.1016/j.neurobiolaging.2012.11.023
19. Hardy J, Allsop D. Amyloid deposition as the central event in the etiology of Alzheimer's disease. *Trends Pharmacol Sci.* (1991) 12:383–8. doi: 10.1016/0165-6147(91)90609-V
20. Jawhar S, Trawicka A, Jenneckens C, Bayer T, Wirths O. Motor deficits, neuron loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal abeta aggregation in the 5XFAD mouse model of Alzheimer's disease. *Neurobiol Aging.* (2012) 33:196.e29–40. doi: 10.1016/j.neurobiolaging.2010.05.027
21. Johansson M, Stomrud E, Lindberg O, Westman E, Johansson PM, van Westen D, et al. Apathy and anxiety are early markers of Alzheimer's disease. *Neurobiol Aging.* (2020) 85:74–82. doi: 10.1016/j.neurobiolaging.2019.10.008
22. Nistor M, Don M, Parekh M. Alpha- and beta-secretase activity as a function of age and beta-amyloid in down syndrome and normal brain. *Neurobiol Aging.* (2007) 28:1493–506. doi: 10.1016/j.neurobiolaging.2006.06.023
23. Lott IT, Head E. Alzheimer disease and down syndrome: factors in pathogenesis. *Neurobiol Aging.* (2005) 26:383–9. doi: 10.1016/j.neurobiolaging.2004.08.005
24. Shen ZX. Brain cholinesterases: II. The molecular and cellular basis of Alzheimer's disease. *Med Hypotheses.* (2004) 63:308–21. doi: 10.1016/j.mehy.2004.02.031
25. Wenk GL. Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry.* (2003) 64:7–10.
26. Nguyen TT, Ta QTH, Nguyen TK O, Nguyen TTD. van Giau V. Type 3 diabetes and its role implications in Alzheimer's disease. *Int J Mol Sci.* (2020) 21:3165. doi: 10.3390/ijms21093165
27. Bredesen DE. Metabolic profiling distinguishes three subtypes of Alzheimer's disease. *Aging.* (2015) 7:595. doi: 10.18632/aging.100801
28. Cunnane S, Nugent S, Roy M, Courchesne-Loyer A, Croteau E, Tremblay S, et al. Brain fuel metabolism, aging, and Alzheimer's disease. *Nutrition.* (2011) 27:3–20. doi: 10.1016/j.nut.2010.07.021
29. Rodriguez-Vieitez E, Saint-Aubert L, Carter SF, Almkvist O, Farid K, Schöll M, et al. Diverging longitudinal changes in astrocytosis and amyloid PET in autosomal dominant Alzheimer's disease. *Brain.* (2016) 139(Pt 3):922–36. doi: 10.1093/brain/awv404
30. Patrick RP. Role of phosphatidylcholine-DHA in preventing APOE4-associated Alzheimer's disease. *FASEB J.* (2019) 33:1554–64. doi: 10.1096/fj.201801412R
31. Javitt DC. Glutamate as a therapeutic target in psychiatric disorders. *Mol Psychiatry.* (2004) 9:984–97. doi: 10.1038/sj.mp.4001551

32. Gazulla J, Cavero-Nagore M. Glutamate and Alzheimer's disease. *Rev Neurol.* (2006) 42:427–32. doi: 10.33588/rn.4207.2005223
33. Kim SH, Steele JW, Lee SW, Clemenson GD, Carter TA, Treuner K, et al. Proneurogenic group II mGluR antagonist improves learning and reduces anxiety in Alzheimer Aβ oligomer mouse. *Mol Psychiatry.* (2014) 19:1235–42. doi: 10.1038/mp.2014.87
34. Castellano C, Cestari V, Ciamei A. NMDA receptors and learning and memory processes. *Curr Drug Targets.* (2001) 2:273–83. doi: 10.2174/1389450013348515
35. Barkus C, McHugh SB, Sprengel R, Seeburg PH, Rawlins JNP, Bannerman DM. Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion. *Eur J Pharmacol.* (2010) 626:49–56. doi: 10.1016/j.ejphar.2009.10.014
36. Dang YH, Ma XC, Zhang JC, Ren Q, Wu J, Gao CG, et al. Targeting of NMDA receptors in the treatment of major depression. *Curr Pharm.* (2014) 20:5151–9. doi: 10.2174/1381612819666140110120435
37. Talantova M, Sanz-Blasco S, Zhang X, Xia P, Akhtar MW, Okamoto S, el al. Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. *Proc Natl Acad Sci U S A.* (2015) 112:E3630. doi: 10.1073/pnas.1511280112
38. Amani M, Zolghadrnasab M, Salari AA. NMDA receptor in the hippocampus alters neurobehavioral phenotypes through inflammatory cytokines in rats with sporadic Alzheimer-like disease. *Physiol Behav.* (2019) 202:52–61. doi: 10.1016/j.physbeh.2019.01.005
39. Penner J, Rupsingh R, Smith M, Wells JL, Borrie MJ, Bartha R. Increased glutamate in the hippocampus after galantamine treatment for Alzheimer disease. *Prog Neuropsychopharmacol Biol Psychiatry.* (2010) 34:104–10. doi: 10.1016/j.pnpbp.2009.10.007
40. Sanacora G, Kendell SF, Levin Y, Simen AA, Fenton LR, Coric V, et al. Preliminary evidence of riluzole efficacy in antidepressant-treated patients with residual depressive symptoms. *Biol Psychiatry.* (2007) 61:822–5. doi: 10.1016/j.biopsych.2006.08.037
41. Mathew SJ, Amiel JM, Coplan JD, Fitterling HA, Sackeim HA, Gorman JM. Open-label trial of riluzole in generalized anxiety disorder. *Am J Psychiatry.* (2005) 162:2379–81. doi: 10.1176/appi.ajp.162.12.2379
42. Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci.* (2002) 5:405–14. doi: 10.1038/nn835
43. Hetman M, Kharebava G. Survival signaling pathways activated by NMDA receptors. *Curr Top Med Chem.* (2006) 6:787–99. doi: 10.2174/156802606777057553
44. Magistretti PJ, Pellerin L, Rothman DL, Shulman RG. Energy on demand. *Science.* (1999) 283:496–7. doi: 10.1126/science.283.5401.496
45. Attwell D, Laughlin SB. An energy budget for signaling in the grey matter of the brain. *J Cerb Blood Flow Metab.* (2001) 21:1135–45. doi: 10.1097/00004647-200110000-00001
46. Wang R, Reddy PH. Role of glutamate and NMDA receptors in Alzheimer's disease. *J Alzheimers Dis.* (2017) 57:1041–8. doi: 10.3233/JAD-160763
47. van der Auwera I, Wera S, van Leuven F, Henderson ST. A ketogenic diet reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease. *Nutr Metab.* (2005) 2:1–8. doi: 10.1186/1743-7075-2-28
48. Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, et al. Effects of β-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiol Aging.* (2004) 25:311–4. doi: 10.1016/S0197-4580(03)00087-3
49. Gano LB, Patel M, Rho JM. Ketogenic diets, mitochondria, and neurological diseases. *J Lipid Res.* (2014) 55:2211–28. doi: 10.1194/jlr.R048975
50. Fedorovich SV, Voronina PP. Waseem TV. Ketogenic diet versus ketoacidosis: what determines the influence of ketone bodies on neurons?. *Neural Regen Res.* (2018) 13:2060. doi: 10.4103/1673-5374.241442
51. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The gut microbiota mediates the anti-seizure effects of the ketogenic diet. *Cell.* (2018) 173:1728–41. doi: 10.1016/j.cell.2018.04.027
52. Pinto A, Bonucci A, Maggi E, Corsi M, Businaro R. Anti-oxidant and anti-inflammatory activity of ketogenic diet: new perspectives for neuroprotection in Alzheimer's disease. *Antioxidants.* (2018) 7:63. doi: 10.3390/antiox7050063
53. Taylor MK, Swerdlow RH, Burns JM, Sullivan DK. An experimental ketogenic diet for Alzheimer disease was nutritionally dense and rich in vegetables and avocado. *Curr Dev Nutr.* (2019) 3:nzz003. doi: 10.1093/cdn/nzz003
54. Włodarek D. Role of ketogenic diets in neurodegenerative diseases (Alzheimer's disease and Parkinson's disease). *Nutrients.* (2019) 11:169. doi: 10.3390/nu11010169
55. Brandt J, Buchholz A, Henry-Barron B, Vizthum D, Avramopoulos D, Cervenka MC. preliminary report on the feasibility and efficacy of the modified atkins diet for treatment of mild cognitive impairment and early Alzheimer's disease. *J Alzheimers Dis.* (2019) 68:969–81. doi: 10.3233/JAD-180995
56. Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutr Metab (Lond).* (2009) 6:31. doi: 10.1186/1743-7075-6-31
57. Yang X, Cheng B. Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. *J Mol Neurosci.* (2010) 42:145–53. doi: 10.1007/s12031-010-9336-y
58. de la Rubia Ortí JE, García-Pardo MP, Drehmer E, Sancho Cantus D, Julián Rochina M, Aguilar MA, et al. Improvement of main cognitive functions in patients with Alzheimer's disease after treatment with coconut oil enriched mediterranean diet: a pilot study. *J Alzheimers Dis.* (2018) 65:577–87. doi: 10.3233/JAD-180184
59. Sonnay S, Chakrabarti A, Thevenet J, Wiederkehr A, Christinat N, Masoodi M. Differential metabolism of medium-chain fatty acids in differentiated human-induced pluripotent stem cell-derived astrocytes. *Front Physiol.* (2019) 10:657. doi: 10.3389/fphys.2019.00657
60. Thevenet J, de Marchi U, Domingo JS, Christinat N, Bultot L, Lefebvre G, et al. Medium-chain fatty acids inhibit mitochondrial metabolism in astrocytes promoting astrocyte-neuron lactate and ketone body shuttle systems. *FASEB J.* (2016) 30:1913–26. doi: 10.1096/fj.201500182
61. Iglesias J, Morales L, Barreto GE. Metabolic and inflammatory adaptation of reactive astrocytes: role of PPARs. *Mol Neurobiol.* (2017) 54:2518–38. doi: 10.1007/s12035-016-9833-2
62. Pflanz NC, Daszkowski AW, James KA, Mihic SJ. Ketone body modulation of ligand-gated ion channels. *Neuropharmacology.* (2019) 148:21–30. doi: 10.1016/j.neuropharm.2018.12.013
63. Lund TM, Risa O, Sonnewald U, Schousboe A, Waagepetersen HS. Availability of neurotransmitter glutamate is diminished when beta-hydroxybutyrate replaces glucose in cultured neurons. *J Neurochem.* (2009) 110:80–91. doi: 10.1111/j.1471-4159.2009.06115.x
64. Nafar F, Clarke JP, Mearow KM. Coconut oil protects cortical neurons from amyloid beta toxicity by enhancing signaling of cell survival pathways. *Neurochem Int.* (2017) 105:64–79. doi: 10.1016/j.neuint.2017.01.008
65. Pavlakis SG, Phillips PC, DiMauro S, de Vivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes: a distinctive clinical syndrome. *Ann Neurol.* (1984) 16:481–8. doi: 10.1002/ana.410160409
66. Hertz L, Chen Y, Waagepetersen HS. Effects of ketone bodies in Alzheimer's disease in relation to neural hypometabolism, β-amyloid toxicity, and astrocyte function. *J Neurochem.* (2015) 134:7–20. doi: 10.1111/jnc.13107
67. Farah BA. Effects of caprylic triglyceride on cognitive performance and cerebral glucose metabolism in mild Alzheimer's disease: a single-case observation. *Front Aging Neurosci.* (2014) 16:1–4. doi: 10.3389/fnagi.2014.00133
68. Moretti A, Gorini A, Villa RF. Affective disorders, antidepressant drugs and brain metabolism. *Mol Psychiatry.* (2003) 8:773–85. doi: 10.1038/sj.mp.4001353
69. Vucic Lovrencic M, Pibernik-Okanovic M, Sekerija M, Prasek M, Ajdukovic D, Kos J, et al. Improvement in depressive symptoms is associated with reduced oxidative damage and inflammatory response in type 2 diabetic patients with subsyndromal depression: the results of a randomized controlled trial comparing psychoeducation, physical exercise, and enhanced treatment as usual. *Int J Endocrinol.* (2015) 2015:210406. doi: 10.1155/2015/210406
70. Labrenz F, Ferri F, Wrede K, Forsting M, Schedlowski M, Engler H, et al. Altered temporal variance and functional connectivity of BOLD signal is associated with state anxiety during acute systemic inflammation. *Neuroimage.* (2019) 184:916–24. doi: 10.1016/j.neuroimage.2018.09.056

71. Stafstrom CE, Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol.* (2012) 3:59. doi: 10.3389/fphar.2012.00059
72. Cheng B, Yang X, An L, Gao B, Liu X, Liu S. Ketogenic diet protects dopaminergic neurons against 6-OHDA neurotoxicity *via* up-regulating glutathione in a rat model of Parkinson's disease. *Brain Res.* (2009) 1286:25–31. doi: 10.1016/j.brainres.2009.06.060
73. Crane PK, Walker R, Hubbard RA, Li G, Nathan DM, Zheng H, et al. Glucose levels and risk of dementia. *N Engl J Med.* (2013) 369:540–8. doi: 10.1056/NEJMoa1215740
74. Croteau E, Castellano CA, Fortier M, Bocti C, Fulop T, Paquet N, et al. A cross-sectional comparison of brain glucose and ketone metabolism in cognitively healthy older adults, mild cognitive impairment and early Alzheimer's disease. *Exp Gerontol.* (2018) 107:18–26. doi: 10.1016/j.exger.2017.07.004
75. Liu L, Komatsu H, Murray IV, Axelsen PH. Promotion of amyloid beta protein misfolding and fibrillogenesis by a lipid oxidation product. *J Mol Biol.* (2008) 377:1236–50. doi: 10.1016/j.jmb.2008.01.057
76. Gottlieb RA, Carreira RS. Autophagy in health and disease. 5 Mitophagy as a way of life. *Am J Physiol Cell Physiol.* (2010) 299:C203–10. doi: 10.1152/ajpcell.00097.2010
77. Smith MA, Harris PLR, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc Natl Acad Sci USA.* (1997) 94:9866–8. doi: 10.1073/pnas.94.18.9866
78. Veurink G, Perry G, Singh SK. Role of antioxidants and a nutrient rich diet in Alzheimer's disease. *Open Biol.* (2020) 10:200084. doi: 10.1098/rsob.200084
79. Zandi PP, Anthony JC, Khachaturian AS, Stone SV, Gustafson D, Tschanz JT, et al. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the cache county study. *Arch Neurol.* (2004) 61:82–8. doi: 10.1001/archneur.61.1.82
80. Maczurek A, Hager K, Kenklies M, Sharman M, Martins R, Engel J, et al. Lipoic acid as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease. *Adv Drug Deliver Rev.* (2008) 60:1463–70. doi: 10.1016/j.addr.2008.04.015
81. Menard C, Bastianetto S, Quirion R. Neuroprotective effects of resveratrol and epigallocatechin gallate polyphenols are mediated by the activation of protein kinase C gamma. *Front Cell Neurosci.* (2013) 7:281. doi: 10.3389/fncel.2013.00281
82. Sharma K. Cholinesterase inhibitors as Alzheimer's therapeutics (review). *Mol Med Report.* (2019) 20:1479–87. doi: 10.3892/mmr.2019.10374
83. Yuanyuan J, Junyan Z, Cuola D, Jingjing C, Yuhui S, Dan X, et al. Memantine attenuated alcohol withdrawal-induced anxiety-like behaviors through down-regulating NR1-CaMKII-ERK signaling pathway. *Neurosci Lett.* (2018) 686:133–9. doi: 10.1016/j.neulet.2018.09.006
84. Czarnecka K, Chuchmacz J, Wójtowicz P, Szymański P. Memantine in neurological disorders - schizophrenia and depression. *J Mol Med.* (2021) 99:327–34. doi: 10.1007/s00109-020-01982-z
85. Bowen RC, Balbuena L, Baetz M. Lamotrigine reduces affective instability in depressed patients with mixed mood and anxiety disorders. *J Clin Psychopharmacol.* (2014) 34:747–9. doi: 10.1097/JCP.0000000000000164
86. Herman E. Lamotrigine: a depression mood stabiliser. *Eur Neuropsychopharmacol.* (2004) 14 Suppl 2:S89–93. doi: 10.1016/j.euroneuro.2004.03.003
87. Fortier M, Castellano C-A, Croteau E, Langlois F, Bocti C, St-Pierre V, et al. A ketogenic drink improves brain energy and some measures of cognition in mild cognitive impairment. *Alzheimers Dement J Alzheimers Assoc.* (2019) 15:625–34. doi: 10.1016/j.jalz.2018.12.017
88. Taylor MK, Sullivan DK, Mahnken JD, Burns JM, Swerdlow RH. Feasibility and efficacy data from a ketogenic diet intervention in Alzheimer's disease. *Alzheimers Dement.* (2018) 4:28–36. doi: 10.1016/j.trci.2017.11.002
89. Krikorian R, Shidler MD, Dangelo K, Couch SC, Benoit SC, Clegg DJ. Dietary ketosis enhances memory in mild cognitive impairment. *Neurobiol Aging.* (2012) 33:425. doi: 10.1016/j.neurobiolaging.2010.10.006
90. McDonald TJW, Cervenka MC. Ketogenic diets for adult neurological disorders. *Neurotherapeutics.* (2018) 15:1018–31. doi: 10.1007/s13311-018-0666-8
91. Klein P, Janousek J, Barber A, Weissberger R. Ketogenic diet treatment in adults with refractory epilepsy. *Epilepsy Behav.* (2010) 19:575–9. doi: 10.1016/j.yebeh.2010.09.016
92. Lilamand M, Porte B, Cognat E, Hugon J, Mouton-Liger F, Paquet C. Are ketogenic diets promising for Alzheimer's disease? A translational review. *Alzheimers Res Ther.* (2020) 12:42. doi: 10.1186/s13195-020-00615-4
93. Ting R, Dugré N, Allan GM, Lindblad AJ. Ketogenic diet for weight loss. *Can Fam Physician.* (2018) 64:906.
94. Cova I, Clerici F, Rossi A, Cucumo V, Ghiretti R, Maggiore L, et al. Weight loss predicts progression of mild cognitive impairment to Alzheimer's disease. *PLoS ONE.* (2016) 11:e0151710. doi: 10.1371/journal.pone.0151710

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The Ketogenic Effect of Medium-Chain Triacylglycerides

*Ting-Yu Lin, Hung-Wen Liu and Tsung-Min Hung**

Department of Physical Education and Sport Sciences, National Taiwan Normal University, Taipei City, Taiwan

Medium-chain triacylglycerides (MCTs) are dietary supplements that can induce ketosis without the need for a traditional ketogenic diet or prolonged fasting. They have the potential to marginally delay the progression of neurodegenerative diseases, such as Alzheimer's disease. However, there have been inconsistencies in reports of the MCT dose–response relationship, which may be due to differences in MCT composition, participant characteristics, and other factors that can influence ketone generation. To resolve these discrepancies, we reviewed studies that investigated the ketogenic effect of MCTs in healthy adults. Aside from the treatment dose, other factors that can influence the ketogenic response, such as accompanying meals, fasting duration, and caffeine intake, were assessed. Based on the available literature, four practical recommendations are made to optimize the ketogenic effect of MCTs and reduce unwanted side effects (primarily gastrointestinal discomfort and diarrhea). First, the starting dose should be either 5 g of octanoic acid [caprylic acid (C8); a component of MCTs] or 5 g of a combination of C8 and decanoic or capric acid (C10; another component of MCTs), and the dose should be progressively increased to 15–20 g of C8. Second, MCTs should be consumed after an overnight fast, without an accompanying meal if tolerable, or with a low-carbohydrate meal. Third, the addition of caffeine may slightly increase the ketogenic response. Fourth, emulsifying the MCTs might increase their ketogenic effect and alleviate side effects.

Keywords: aging - old age - seniors, cognition, octanoic acid (caprylic acid) (PubChem CID: 379), decanoic acid (PubChem CID 2969), Tricaprin – Captex® 1000 (PubChem CID: 69310), Tricaprylin – Captex® 8000 (PubChem CID: 10850), ketone bodies, beta-hydroxybutyrate

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Gorazd Drevenšek,
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Robin S. B. Williams,
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Christopher Olson,
Midwestern University, United States

****Correspondence:***
Tsung-Min Hung
ernesthungkimo@yahoo.com.tw

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INTRODUCTION

Aging and dementia are two crucial issues that affect people worldwide. The World Health Organization reported that 1 billion people are now aged ≥ 60 years, with 50 million having dementia. Of those with dementia, 60–70% have Alzheimer's disease (1, 2). Therefore, the development of a cost-effective intervention to mitigate brain deterioration and prevent Alzheimer's disease is crucial.

One symptom and possible cause of brain function decline later in life is glucose hypometabolism. Older adults show a 7% reduction in glucose metabolic rate in the gray matter of the brain and a 10–14% deficit in the frontal cortex compared with younger adults [(3) (R2)]. Older adults with mild Alzheimer's disease show an additional 13% decrease in global cerebral glucose metabolic rate in the gray matter compared with their healthy counterparts [(4) (R2)]. One way to compensate for a low glucose metabolic rate is to use an alternative energy source such as ketones [β-hydroxybutyrate

(βHB) and acetoacetate], which have been reported to be metabolized normally in patients with Alzheimer's disease [(4) (T3)].

Medium-chain triacylglycerides (MCTs) are commercially available, inexpensive dietary supplements that can induce ketosis. Aside from serving as an alternative energy source, βHB has been reported to have health benefits similar to those observed upon calorie restriction, such as antiaging effects *via* epigenetic regulation [(5) (Pg306, 310–311)]. Therefore, an understanding of the relationship between the amount of MCTs consumed and the level of ketones in the blood is necessary to inform practical applications of MCTs. An earlier review reported an almost linear relationship between the amount of MCTs consumed, up to doses as high as 70 g, and their ketogenic effects [(6) (F6)]. However, another recent study showed a non-linear relationship between these two parameters at relatively low doses of MCTs (10–20 g) [(7) (F1A)]. This inconsistency may arise from differences in the compositions of MCTs and participants' health conditions, limitations of the study designs, and other cofounding factors (discussed in the following section). Thus, we aim to provide a brief review of the available literature on the ketogenic effect of oral MCT administration in healthy participants and related interacting factors, such as accompanying meals, fasting duration, and caffeine intake. We also provide a practical summary on how to maximize the ketogenic benefits of MCTs.

KETOGENIC EFFECT OF MCTS

Effect of Carbon Chain Length on the Ketogenic Effect of MCTs

The major ketogenic component of MCTs is caprylic acid (C8), followed by capric acid (C10) or lauric acid (C12) [(8) (R, F1, F3)]. Caproic acid (C6) is excluded because it is not typically consumed as a dietary supplement, partly due to its unpleasant odor. The ketogenic effect (total ketone concentration) of C8 is three and six times higher than the effects of C10 and C12, respectively [(9) (D1, F3)]. Vandenberghe et al. showed that the total plasma ketone concentration increased after the consumption of C8 for ∼4 h, but not after the consumption of C10 [(8) (R5, F3)]. Other studies have shown that consuming 18–20 mL (∼16–18 g) of C8 without an accompanying meal increases the plasma βHB concentration from <0.1 mmol/L to 0.5–0.6 mmol/L at 2 h after administration [(9) (F1)].

Before the results from recent studies, such as that conducted by Vandenberghe et al. [(8) (R)], came to light, all of the MCTs (i.e., C6, C8, C10, and C12) were considered to be ketogenic [(10) (I1)], partly because these molecules are rapidly absorbed and transported to the liver via the portal vein mainly as free fatty acids bound to serum albumin. In contrast, longer-chain triacylglycerides are dependent on acyl-CoA synthetase activity and the lymphatic system for absorption and are transported by chylomicrons [(11) (Pg951)]. However, only fatty acids with carbon chain lengths ≤8 can cross the inner membrane of the mitochondria independent of carnitine palmitoyl transferase I [(12) (T1)]. This may be why C8 has a stronger ketogenic effect than C10 and C12.

In their review, Cunnane et al. suggest the presence of a linear relationship between oral doses of MCTs up to 70 g and the maximal observed plasma βHB concentration [(6) (F6)]. However, some issues were noted after reviewing the studies cited in their figure. First, the cited studies included not only healthy adults (13, 14) but also memory-impaired adults (15) and patients with type 1 diabetes (16) and Alzheimer's disease (17). Second, the doses presented in their figure were the sum of all MCT doses (i.e., C6, C8, C10, and C12) and not just the dose of C8, which has been reported to contribute to the majority (9), if not all [(8) (R, F1, F3)], of the ketogenic response. Finally, in their analysis, Cunnane et al. did not consider whether the MCTs had been consumed with or without a meal or the number of hours the participants had fasted.

Based on the above data and other recent findings, the non-linear relationship between MCT intake and plasma ketone concentration might begin at a relatively low dose of MCTs (6 g of C8 + 4 g of C10 vs. 12 g of C8 + 8 g of C10) [(7) (F1A)]. Based on the results of several studies (7, 8, 18), it is clear that 20 g of C8 produces a significantly stronger (but perhaps not twice as high) [(7) (D2)] ketogenic response than 10 g of C8. Further studies are required to determine whether doses higher than 20 g can produce a significantly larger ketogenic response and/or a greater risk of unwanted side effects. Norgren et al. also suggested [(19) (D5)] that the dose of C8 should be limited to 15–20 g per intake to minimize potential side effects.

Repeated Use of MCTs May Influence the Acute Ketogenic Response

It is unclear whether the repeated use of MCTs can augment the acute ketogenic response. In a previous study, Freund and Weinsier showed that repeated administration of MCTs to the same participants showed reproducible responses within narrow limits [(20) (A)]. However, a 1-month interventional study showed that the daily mean plasma βHB concentration increased from ∼0.1 mmol/L to ∼0.2 mmol/L after consuming ∼6 g of C8 twice a day for 30 days [(21) (F1A-B)].

Effect of an Accompanying Meal on the Ketogenic Effect of MCTs

Consuming MCTs without an accompanying meal produces a stronger ketogenic effect than with an accompanying meal that has a substantial carbohydrate content. For example, adding 50 g of glucose to 20 g of C8 (0.27 mmol/L vs. 0.10 mmol/L from control) decreased the ketogenic effect (measured as the venous whole blood βHB concentration) by 63% [(19) (T3)]. Another study showed that the plasma ketone response was ∼2-fold higher after consuming C8 without an accompanying meal than with a meal [470 calories in the meal; 19.5 g of fat (36%), 24.2 g of protein (20%), and 55 g of carbohydrate (44%)] [(9) (A)]. As the amount of carbohydrate consumed with the MCTs increases, the ketogenic response decreases [(20) (R4, F4)]. Therefore, consuming C8 without an accompanying meal can

maximize the ketogenic effect, i.e., it can be consumed as a replacement for breakfast or as a stand-alone snack [(9) (D2)].

Consuming carbohydrates after consuming MCTs also decreases their ketogenic effect. For instance, the subsequent consumption of sucrose suppresses the ketogenic effect of MCTs. As the amount of sucrose consumed increases, the maximal acetone concentration in alveolar air decreases [(20) (R5, D5, F5–6)].

Although it may not inhibit the ketogenic effect, consuming a low-carbohydrate meal with C8 may prolong the time required to attain the maximal plasma βHB concentration. A low-carbohydrate ketogenic breakfast with ∼110 g of fat (∼28 g of C8 and ∼43 g of C10), 25 g of protein, and 3 g of carbohydrate can elevate the plasma βHB concentration to approximately 0.7 mmol/L at 1 h and 2 h after administration, with a peak at 6 h after administration (∼1 mmol/L) [(13) (R1, F1)].

The Effect of Fasting on the Ketogenic Effect of MCTs

after an overnight fast (∼12 h), the plasma ketone concentration reaches ∼0.07–0.15 mmol/L [(8, 13, 21) (T1), 13(T1), 21(T2)]. Longer fasting periods (12, 18, and 24 h) result in greater ketogenic responses (measured as acetone concentration in alveolar air) after consuming a single dose of MCTs [30 mL, 74.7% (∼20 g) C8]; the measured acetone in alveolar air after 4 h was ∼1, 1.5, and 2.5 μg/100 mL after fasting for 12, 16, and 24 h, respectively [(20) (R7, F7)].

The Effect of Coffee/Caffeine on the Ketogenic Effect of MCTs

Caffeine, when taken with a high-carbohydrate breakfast and without MCT consumption, can increase the plasma βHB concentration [(22) (F2)]. Consuming 5 mg/kg of caffeine along with a high-carbohydrate breakfast (∼482 kcal; 71% carbohydrate, 18% fat, and 12% protein) results in a significantly higher plasma βHB concentration after 3, 3.5, and 4 h (∼150–200 vs. 50–100 μmol/L), compared with eating breakfast alone [(22) (F2B)].

Although McAllister et al. claimed in their article title that acute coffee ingestion increases the blood ketone concentration, it is not clear whether this was caused by coffee or fasting, as there was no non-coffee control condition in their study [(23) (Title, F1B)]. Combining caffeine with MCTs may indeed potentiate the ketogenic effect of MCTs, but this requires further investigation [(22) (D3)].

Emulsification Might Influence MCT Absorption

The emulsification of MCTs with beverages increases their ketogenic effect compared with the same dose of non-emulsified MCTs (increase in the 4-h area under the curve from 0.147 ± 0.094 to 0.560 ± 0.095 mmol*h/L with 12 g of C8 and from 0.311 ± 0.097 to 1.320 ± 0.336 mmol*h/L with 18 g of C8) [(18) (F1)]. However, the effect of emulsification and the optimal technique of emulsification (e.g., using a blender) require further investigation.

The Effect of Exercise on the Ketogenic Effect of MCTs

Although Vandenberghe et al. claimed that aerobic exercise increases the ketogenic effect of MCTs [(24) (Title)], all of the participants in their study underwent the same intervention sequence (control → MCTs → aerobic exercise → MCTs + aerobic exercise) [(24) (M, F1)]. As the study design did not include randomization or even a counterbalance, it remains unclear whether aerobic exercise indeed augments the ketogenic effect of MCTs. Additional studies with a more robust design are required to address this question.

The Effect of Consuming Other Fatty Acids on the Ketogenic Effect of MCTs

Adding 30 g of coconut oil (<10% C8, 5–6% C10, and 41–42% C12) (25) to 20 g of C8 does not significantly increase the venous whole blood βHB concentration over a period of 6 h compared with 20 g of C8 alone [(19) (F1, T3)]. Moreover, 30 g of coconut oil alone does not significantly increase the venous whole blood βHB concentration [(19) (T3)] after 6 h compared with the control condition (30 g of sunflower oil).

Beginning With a Low Dose and Emulsification May Reduce Side Effects

MCTs are safe at doses up to 1 g/kg [(26) (A)]. However, common side effects such as gastrointestinal discomfort [(27) (T1)] and diarrhea [(20) (R1)] do occur. If an individual is not accustomed to MCTs, then there is a significant possibility that they will experience side effects. For instance, three out of seven participants in Freund and Weinsier's study experienced abdominal discomfort and diarrhea after consuming 25 mL (∼23 g) of MCTs [(20) (R1)]. To counter this, Courchesne-Loyer et al. recommend starting with 5 g of MCTs in the morning [(18) (D4)]. Emulsification may also reduce the side effects of MCTs [(18) (T2)], but adding glucose to coconut oil or C8 does not reduce adverse effects such as nausea and upset stomach [(19) (T5)].

PRACTICAL SUMMARY

1. Start with a low dose (5 g or 6 mL) of C8 or C8 + C10. If there are no adverse effects such as diarrhea or other abdominal issues, then increase the dose up to 15–20 g of C8 (17–22 mL). For example, if consuming an MCT product with 50% C8, increase the dose from 6 to 33–44 mL (15–20 g of C8).
2. To optimize the ketogenic effect of MCTs, consume C8 after an overnight fast, without an accompanying meal if tolerable or with a low-carbohydrate meal. After consuming C8, fast for several hours to maximize the time under mild ketosis. For example, if dinner is completed at 8 p.m., then consume C8 at 8 a.m. the next day as a breakfast substitute and wait until lunch to break the carbohydrate/protein fast.
3. Consuming MCTs with caffeine may slightly increase their ketogenic effect.

4. Emulsifying MCTs (perhaps using a blender) might increase their absorption rate and decrease the risk of adverse effects.

DISCUSSION

By reviewing and citing the available literature in detail, this brief article clearly identifies the discrepancies in previous studies and provides several practical recommendations on how to consume MCTs to maximize their ketogenic benefits. However, in addition to the effects of their metabolites (i.e., ketones), C8 and C10 have been shown to directly improve neural function. For instance, C8 regulates the energy balance by affecting the activity of pro-opiomelanocortin neurons [(28) (Sec3.4)]; C10 enhances mitochondrial function in neurons by activating the SIRT1 enzyme [(29) (Sec3.3.2)] and regulates astrocyte function by inhibiting mTOR [(30) (R12, F7)]; and both C8 and C10 increase neuronal GABA synthesis by increasing the glutamine supply [(31) (R5, F5)]. Thus, the optimal dose and composition of MCTs may differ from previous recommendations and from those provided hereafter in the light of other relevant factors.

As participants with diseases such as diabetes and Alzheimer's disease may have different metabolic responses, we only included studies on healthy participants. Healthy adults and elderly individuals do not seem to show major differences in their ketogenic responses to MCTs [(13) (R1, F1)]. However, further investigations of the ketogenic effect of MCTs in different populations are necessary.

AUTHOR'S NOTE

In-text citation: A: abstract; I: introduction; M: method; R: results; D: discussion; number after abbreviation: paragraph; T: table; F: figure; Sec: section; Pg: page.

AUTHOR CONTRIBUTIONS

T-YL conceived and conducted the research and collected and analyzed the data. He is the guarantor of the study and responsible for writing the manuscript. H-WL provided advice to improve the clarity of the manuscript. T-MH is the supervisor and is responsible for reviewing the manuscript. All authors have contributed to drafting the manuscript and have approved of the final version.

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REFERENCES

1. World Health Organization. *Dementia*. (2021). Available online at: https://www.who.int/news-room/fact-sheets/detail/dementia (accessed May 7, 2021).
2. World Health Organization. *Ageing*. Available online at: https://www.who.int/health-topics/ageing#tab=tab_1 (accessed May 7, 2021).
3. Nugent S, Castellano C, Bocti C, Dionne I, Fulop T, Cunnane S. Relationship of metabolic and endocrine parameters to brain glucose metabolism in older adults: do cognitively-normal older adults have a particular metabolic phenotype? *Biogerontology*. (2016) 17:241–55. doi: 10.1007/s10522-015-9595-7
4. Castellano C-A, Nugent S, Paquet N, Tremblay S, Bocti C, Lacombe G, et al. Lower brain ^{18}F-fluorodeoxyglucose uptake but normal ^{11}C-acetoacetate metabolism in mild Alzheimer's disease dementia. *J Alzheimer Dis*. (2015) 43:1343–53. doi: 10.3233/JAD-141074
5. Veech RL, Bradshaw PC, Clarke K, Curtis W, Pawlosky R, King MT. Ketone bodies mimic the life span extending properties of caloric restriction. *IUBMB Life*. (2017) 69:305–14. doi: 10.1002/iub.1627
6. Cunnane SC, Courchesne-Loyer A, Vandenberghe C, St-Pierre V, Fortier M, Hennebelle M, et al. Can ketones help rescue brain fuel supply in later life? Implications for cognitive health during aging and the treatment of Alzheimer's disease. *Front Mol Neurosci*. (2016) 9:1–21. doi: 10.3389/fnmol.2016.00053
7. Vandenberghe C, St-Pierre V, Fortier M, Castellano C-A, Cuenoud B, Cunnane SC. Medium chain triglycerides modulate the ketogenic effect of a metabolic switch. *Front Nutr*. (2020) 7:1–9. doi: 10.3389/fnut.2020.00003
8. Vandenberghe C, St-Pierre V, Pierotti T, Fortier M, Castellano C-A, Cunnane SC. Tricaprylin alone increases plasma ketone response more than coconut oil or other medium-chain triglycerides: an acute crossover study in healthy adults. *Curr Dev Nutr*. (2017) 1:e000257. doi: 10.3945/cdn.116.000257
9. St-Pierre V, Vandenberghe C, Lowry C-M, Fortier M, Castellano C-A, Wagner R, et al. Plasma ketone and medium chain fatty acid response in humans consuming different medium chain triglycerides during a metabolic study day. *Front Nutr*. (2019) 6:1–5. doi: 10.3389/fnut.2019.00046
10. Seaton TB, Welle SL, Warenko MK, Campbell RG. Thermic effect of medium-chain and long-chain triglycerides in man. *Am J Clin Nutr*. (1986) 44:630–4. doi: 10.1093/ajcn/44.5.630
11. Bach AC, Babayan VK. Medium-chain triglycerides: an update. *Am J Clin Nutr*. (1982) 36:950–62. doi: 10.1093/ajcn/36.5.950
12. Khabbush A, Orford M, Tsai YC, Rutherford T, O'Donnell M, Eaton S, et al. Neuronal decanoic acid oxidation is markedly lower than that of octanoic acid: a mechanistic insight into the medium-chain triglyceride ketogenic diet. *Epilepsia*. (2017) 58:1423–9. doi: 10.1111/epi.13833
13. Freemantle E, Vandal M, Tremblay-Mercier J, Plourde M, Poirier J, Cunnane SC. Metabolic response to a ketogenic breakfast in the healthy elderly. *J Nutr Health Aging*. (2009) 13:293–8. doi: 10.1007/s12603-009-0026-9
14. Pi-Sunyer FX, Hashim SA, Van Itallie TB. Insulin and ketone responses to ingestion of medium and long-chain triglycerides in man. *Diabetes*. (1969) 18:96–100. doi: 10.2337/diab.18.2.96
15. Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, et al. Effects of β-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiol Aging*. (2004) 25:311–4. doi: 10.1016/S0197-4580(03)00087-3
16. Page KA, Williamson A, Yu N, McNay EC, Dzuira J, McCrimmon RJ, et al. Medium-chain fatty acids improve cognitive function in intensively treated type 1 diabetic patients and support in vitro synaptic transmission during acute hypoglycemia. *Diabetes*. (2009) 58:1237–44. doi: 10.2337/db08-1557
17. Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutr Metab*. (2009) 6:31. doi: 10.1186/1743-7075-6-31
18. Courchesne-Loyer A, Lowry C-M, St-Pierre V, Vandenberghe C, Fortier M, Castellano C-A, et al. Emulsification increases the acute ketogenic effect and bioavailability of medium-chain triglycerides in humans: protein, carbohydrate, and fat metabolism. *Curr Dev Nutr*. (2017) 1:1–8. doi: 10.3945/cdn.117.000851

19. Norgren J, Sindi S, Sandebring-Matton A, Kåreholt I, Daniilidou M, Akenine U, et al. Ketosis after intake of coconut oil and caprylic acid—with and without glucose: a cross-over study in healthy older adults. *Front Nutr.* (2020) 7:1–11. doi: 10.3389/fnut.2020.00040
20. Freund G, Weinsier RL. Standardized ketosis in man following medium chain triglyceride ingestion. *Metabolism.* (1966) 15:980–91. doi: 10.1016/0026-0495(66)90046-1
21. Courchesne-Loyer A, Fortier M, Tremblay-Mercier J, Chouinard-Watkins R, Roy M, Nugent S, et al. Stimulation of mild, sustained ketonemia by medium-chain triacylglycerols in healthy humans: estimated potential contribution to brain energy metabolism. *Nutrition.* (2013) 29:635–40. doi: 10.1016/j.nut.2012.09.009
22. Vandenberghe C, St-Pierre V, Courchesne-Loyer A, Hennebelle M, Castellano C-A, Cunnane SC. Caffeine intake increases plasma ketones: an acute metabolic study in humans. *Can J Physiol Pharmacol.* (2017) 95:455–8. doi: 10.1139/cjpp-2016-0338
23. McAllister MJ, Waldman HS, Rentería LI, Gonzalez AE, Butawan MB, Bloomer RJ. Acute coffee ingestion with and without medium-chain triglycerides decreases blood oxidative stress markers and increases ketone levels. *Can J Physiol Pharmacol.* (2020) 98:194–200. doi: 10.1139/cjpp-2019-0458
24. Vandenberghe C, Castellano C-A, Maltais M, Fortier M, St-Pierre V, Dionne IJ, et al. A short-term intervention combining aerobic exercise with medium-chain triglycerides (MCT) is more ketogenic than either MCT or aerobic exercise alone: a comparison of normoglycemic and prediabetic older women. *Appl Physiol Nutr Metab.* (2019) 44:66–73. doi: 10.1139/apnm-2018-0367
25. U.S. Department of Agriculture. *Oil, Coconut.* Available online at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/171412/nutrients (accessed March 24, 2021).
26. Traul KA, Driedger A, Ingle DL, Nakhasi D. Review of the toxicologic properties of medium-chain triglycerides. *Food Chem Toxicol.* (2000) 38:79–98. doi: 10.1016/S0278-6915(99)00106-4
27. Liu Y-mC. Medium-chain triglyceride (MCT) ketogenic therapy. *Epilepsia.* (2008) 49(Suppl. 8):33–6. doi: 10.1111/j.1528-1167.2008.01830.x
28. Haynes VR, Michael NJ, van den Top M, Zhao F-Y, Brown RD, De Souza D, et al. A neural basis for octanoic acid regulation of energy balance. *Mol Metab.* (2020) 34:54–71. doi: 10.1016/j.molmet.2020.01.002
29. Dabke P, Das AM. Mechanism of action of ketogenic diet treatment: impact of decanoic acid and beta—hydroxybutyrate on sirtuins and energy metabolism in hippocampal murine neurons. *Nutrients.* (2020) 12:2379. doi: 10.3390/nu12082379
30. Warren EC, Dooves S, Lugarà E, Damstra-Oddy J, Schaf J, Heine VM, et al. Decanoic acid inhibits mTORC1 activity independent of glucose and insulin signaling. *Proc Natl Acad Sci USA.* (2020) 117:23617–25. doi: 10.1073/pnas.2008980117
31. Andersen JV, Westi EW, Jakobsen E, Urruticoechea N, Borges K, Aldana BI. Astrocyte metabolism of the medium-chain fatty acids octanoic acid and decanoic acid promotes GABA synthesis in neurons via elevated glutamine supply. *Mol Brain.* (2021) 14:132. doi: 10.1186/s13041-021-00842-2

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REVIEW
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Neuroprotection by the Ketogenic Diet: Evidence and Controversies

*Sarah M. Gough, Alicia Casella, Kristen Jasmin Ortega and Abigail S. Hackam**

Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL, United States

The ketogenic diet (KD) is a high-fat low-carbohydrate diet that has been used for decades as a non-pharmacologic approach to treat metabolic disorders and refractory pediatric epilepsy. In recent years, enthusiasm for the KD has increased in the scientific community due to evidence that the diet reduces pathology and improves various outcome measures in animal models of neurodegenerative disorders, including multiple sclerosis, stroke, glaucoma, spinal cord injury, retinal degenerations, Parkinson's disease and Alzheimer's disease. Clinical trials also suggest that the KD improved quality of life in patients with multiple sclerosis and Alzheimer's disease. Furthermore, the major ketone bodies BHB and ACA have potential neuroprotective properties and are now known to have direct effects on specific inflammatory proteins, transcription factors, reactive oxygen species, mitochondria, epigenetic modifications and the composition of the gut microbiome. Neuroprotective benefits of the KD are likely due to a combination of these cellular processes and other potential mechanisms that are yet to be confirmed experimentally. This review provides a comprehensive summary of current evidence for the effectiveness of the KD in humans and preclinical models of various neurological disorders, describes molecular mechanisms that may contribute to its beneficial effects, and highlights key controversies and current gaps in knowledge.

Keywords: neurodegeneration, ketogenic diet, mitochondria, ketone bodies, inflammation

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****Correspondence:***
Abigail S. Hackam
ahackam@med.miami.edu

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INTRODUCTION

The ketogenic diet (KD) and related diets have been used for many decades for weight loss, managing metabolic disorders and reducing seizures in pediatric epilepsy. Recent convincing evidence for neuroprotective effects of the KD in animal models of neurologic diseases has led to a surge in interest in testing the benefits and mechanisms of action of the diet. PubMed articles using the search term "ketogenic diet" increased nearly three-fold from 2010 to 2021 compared to all years prior to 2010, indicating heightened attention to the KD within the scientific community. The KD is used in clinical practice for several non-neurological conditions, including heart disease, diabetes, obesity, autism, glioblastoma and other cancers. However, other than for epilepsy, the KD is not yet recommended for reducing symptoms and slowing degeneration in any neurological disease. The objective of this literature review is to provide a comprehensive summary of supporting evidence from clinical and experimental laboratory studies on the effectiveness of the KD in neurodegenerative disorders of the brain, spinal cord and retina. Furthermore, we describe the current understanding of molecular mechanisms that may directly contribute to the beneficial effects of the KD and highlight key knowledge gaps.

KETONE BODY METABOLISM: KETOGENESIS AND KETOLYSIS

The classic ratio of fat to carbohydrate plus protein in the KD is 3:1 or 4:1, in which 80–90% of total calories comes from fats, 4% from carbohydrates and 6% from proteins. Modifications of the diet to increase palatability and improve compliance are common, although may complicate drawing comparisons among different studies. These modifications include varying the relative amounts of macronutrients, the nature and sources of the fats and duration of the diet, while maintaining the appropriate fat to carbohydrate ratio. The general goal of classic and modified KDs is to achieve ketosis, a state in which levels of ketone bodies are elevated in the blood. Ketone bodies are chemically related water-soluble molecules that are generated by normal physiological metabolism of fatty acids by β-oxidation. The most well-known ketone bodies are beta-hydroxybutyrate (BHB), acetoacetate (ACA) and acetone. Typical circulating levels of ketone bodies within the blood are 100–250 μM, whereas physiological or nutritional ketosis leads to elevated ketone body levels in the range of 0.5–5 mM. In contrast, blood levels of ketone bodies in pathological ketoacidosis can reach up to 15–25 mM. Levels of blood and urine ketones are often measured to assess adherence to the diet, although ketone concentrations do not always correlate with better outcomes (1, 2).

Ketone bodies are produced in a process known as ketogenesis, which generates ACA and BHB for use as alternative metabolic fuel sources for the body when glucose stores become depleted. Ketogenesis occurs during glycolytic inhibition from periods of fasting, intense exercise or severe carbohydrate restriction such as in the KD. Ketogenesis primarily occurs in the mitochondria of liver hepatocytes (3), in several neuronal cell types and in the retinal pigment epithelium (4), although the relative importance of extrahepatic ketogenesis is not fully known. Fatty acids derived from the diet are converted *via* β-oxidation reactions into acetyl-CoA, which enters the TCA cycle or is converted further into ketone bodies by several modulating enzymes and co-factors (**Figure 1**) (3). Acetoacetyl-CoA thiolase converts two acetyl-CoA molecules into acetoacetyl-CoA, which is combined with another acetyl-coA and is then converted into the intermediate product beta-hydroxy-methylglutaryl-CoA (HMG-CoA) by the key rate-limiting enzyme HMG-CoA synthase 2 (HMGCS2). HMG-CoA is metabolized into ACA and acetyl-CoA by the enzyme HMG-CoA lyase. Finally, ACA is reversibly converted into BHB by β-hydroxybutyrate dehydrogenase coupled with NADH oxidation (5) or converted into acetone by spontaneous decarboxylation. ACA and BHB are transported by monocarboxylate transporters (MCT) out of the liver into the blood where they are taken up and used by the brain and other organs through ketolysis.

Ketolysis is the metabolism of ketone bodies ACA and BHB for energy within the mitochondria. Ketone bodies enter extrahepatic cells down a concentration gradient through MCT transporters, are reconverted into acetyl-CoA and utilized in the citric acid cycle to yield GTP and ATP. BHB can be converted back into acetoacetate by BHB dehydrogenase coupled with the reduction of NAD+. This yields NADH to help power the electron transport chain. Additionally, although primarily exhaled, acetone may be metabolized into usable energy sources such as pyruvate, lactate and acetate.

EVIDENCE FOR BENEFICIAL EFFECTS OF KD IN NEUROLOGICAL DISEASES

Many claims have been made about the benefits of the KD for overall health. However, the only well-established use of the KD for managing a neurological disease is for seizure reduction in pediatric epilepsy (6). Intriguingly, evidence for neuroprotection in other neurologic diseases from the classic and modified KD has been reported in pilot clinical studies and in pre-clinical laboratory models, as described below and summarized in **Tables 1**, **2**. Note that studies using ketogenic-like diets that deviated from high fat/low protein/low carbohydrate content ratio, such as diets with excess fat but with normal carbohydrate levels, are not included in the summary below.

Epilepsy

Neal et al. (11) reported the first randomized, prospective and controlled clinical trial for treatment-intractable childhood epilepsy in which 3 month administration of a KD resulted in significant reduction in seizure frequency. The KD was also shown in smaller studies to reduce onset and frequency of seizures in other childhood seizure syndromes such as Dravet syndrome, myoclonic-atonic epilepsy and other conditions (1). The use of rodent models of epilepsy confirmed that the KD significantly reduces seizures and provided clues to potential mechanisms contributing to decreased neuronal excitability. For example, Masino et al. (37), using a transgenic mouse model with spontaneous seizures, found that 3-week administration of the KD reduced seizure frequency in a process that required activation of adenosine A1 receptors (A_1Rs). The anti-epileptic effect of the KD was lost in A_1R knockout mice and in mice that were treated with an A_1R inhibitor (37). In another epilepsy mouse model, the Kcna1-null mouse, seizure reduction was promoted by direct administration of BHB and depended on alterations in mitochondrial permeability transition (12). Furthermore, Olson et al. (13) used two mouse models of epilepsy to identify specific gut microbial species that are influenced by the KD and contribute to seizure reduction by modifying host production of neurotransmitters. Although the beneficial effect of the KD in adults and additional epilepsy subtypes are not yet established by clinical trials, ClinicalTrials.gov lists nearly 40 prospective randomized trials currently recruiting or completed that are investigating effectiveness and safety of KDs in treating epilepsy. Therefore, more information on the benefits of the KD for epilepsy will likely be reported in the future.

Multiple Sclerosis

Several ongoing randomized controlled studies are listed on Clinicaltrials.gov that are testing safety, tolerability and effectiveness of the KD as a therapeutic intervention for MS. Reports from several pilot studies suggest the KD may benefit patients with MS. For example, Brenton et al. (34) demonstrated that patients on a modified KD for 6 months had no worsening

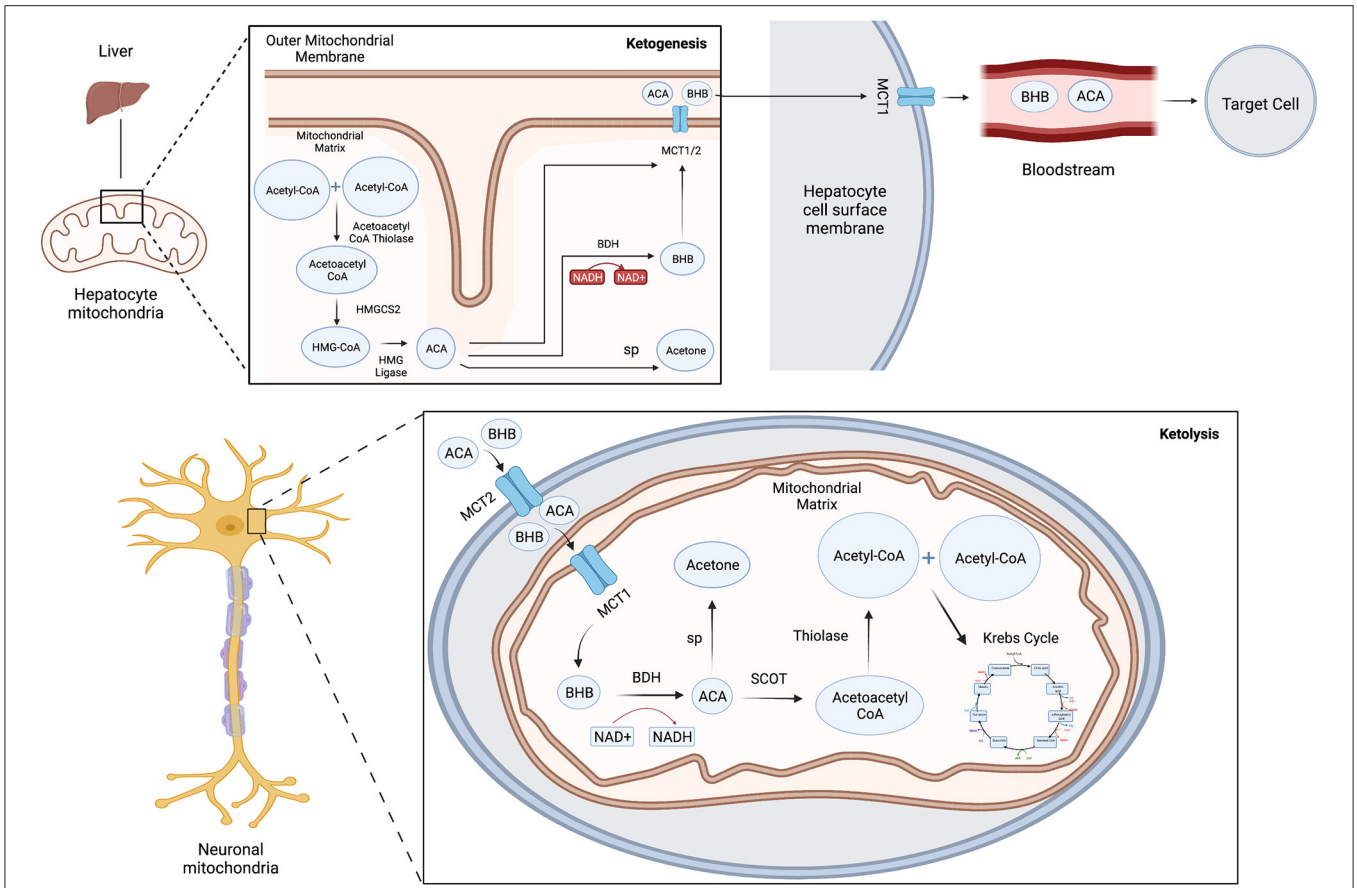

FIGURE 1 | Schematic model of ketone body metabolism. Ketogenesis: Acetoacetyl-CoA thiolase converts two acetyl-CoA molecules into acetoacetyl-CoA, which is combined with another acetyl-coA then converted into beta-hydroxy-methylglutaryl-CoA (HMG-CoA) by HMG-CoA synthase 2 (HMGCS2), which is metabolized into ACA and acetyl-CoA by HMG-CoA lyase. ACA is converted into BHB by β-hydroxybutyrate dehydrogenase (BDH) or converted into acetone by spontaneous decarboxylation (sp). ACA and BHB are transported by monocarboxylate transporters (MCT) out of the liver into the blood. Ketolysis: ACA and BHB enter cells through MCT transporters and are reconverted with the mitochondria into acetyl-CoA and utilized in the Krebs cycle to yield GTP and ATP. BHB can also be converted back into acetoacetate by BDH, which generates NADH to help power the electron transport chain.

symptoms and showed significant improvements in both fatigue and depression scores. Additionally, evidence for patients with relapsing-remitting MS from a three-armed parallel grouped, single center, controlled and randomized clinical pilot trial showed that being on the KD for 6 months led to improvements in quality of life and overall health (35).

Additional support for a neuroprotective effect of the KD comes from two mouse models of MS. Kim et al. (18) used the experimental autoimmune encephalomyelitis (EAE) mouse model fed a KD with a high fat to carbohydrate plus protein ratio of 6.3:1, which led to increased spatial learning and memory, improved motor ability and reduced lesion size compared with EAE mice fed a standard diet. These improvements were associated with reduced pro-inflammatory responses in brain tissue, including lower cytokine and chemokine levels and lower macrophage and microglia numbers, as well as decreased formation of reactive oxygen species (ROS) (18). Neuroprotection was also observed in the cuprizone-induced demyelination mouse model of MS using mice on a more tolerable fat to carbohydrate plus protein ratio of 3:1 for 5 weeks (19). Animals on the KD had significantly improved motor and behavioral deficits, increased numbers of mature oligodendrocytes and reduced hippocampal demyelination. Elevated expression of myelin basic protein and other myelin associated markers were observed, suggesting that the KD protected against demyelinating pathology (19). Although extrapolations from animal models to human MS patients must be made cautiously, these pre-clinical studies suggest that the KD may potentially reduce MS pathology and ameliorate associated symptoms.

Alzheimer's Disease

Clinicaltrials.gov lists a dozen ongoing or completed clinical trials that investigate the effectiveness of classic and modified KDs on reducing cognitive impairment. Several published reports have indicated mild improvements in cognitive function, with the ApoE4 genotype influencing the extent of improvement [see (38) for a comprehensive review]. For example, Krikorian et al. (29)

TABLE 1 | Beneficial effects of the KD or ketone bodies in animal models.

Disease	Animal model	Treatment	Outcome	References
Alzheimer's disease	3xTgAD transgenic mouse	Diet supplemented with BHB	Reduced oxidized proteins and lipids	(7)
	APP/PS1 and Tg4510 transgenic mice	KD for 3 months	Improved locomotor activity, no improvement in learning or change in amyloid or tau deposition	(8)
	3xTgAD transgenic mouse	Ketosis induced by 2-deoxy-D-glucose	Reduced accumulation of Aβ and lowered oxidative stress	(9)
	PDGFB-APPSwInd transgenic mouse	BHB and ACA subcutaneous injections for 2 months	Reduced amyloid deposition, improved learning and memory and synaptic plasticity	(10)
Epilepsy	adenosine A1 receptors (A1R)-/- and Adk-Tg mice	KD for 3 weeks	Reduced seizure frequency	(11)
	Kcna1-/- mice	KD diet, BHB administration	Reduced spontaneous recurrent seizures	(12)
	6-Hz induced seizure model and Kcna1-/-, mice	KD 2-14 days	Elevated seizure threshold, decreases seizure duration and frequency	(13)
Glaucoma	DBA/2J mutant mouse with elevated intraocular pressure	KD for 8 weeks	Increased neuronal survival	(14)
	NMDA-induced RGC death, rat	KD for 3 weeks	Increased RGC survival in juvenile not adults	(15)
	NMDA-induced RGC death, rat	Daily BHB or ACA intraperitoneal injection for 21 days	Increased RGC survival	(16)
	Blast pressure-induced ocular injury, mice	KD beginning 2 weeks prior to injury	Increased neuronal survival, decreased gliosis	(17)
MS	Experimental autoimmune encephalomyelitis (EAE) mouse	KD up to 30 days	Increased spatial learning and memory, improved motor ability and reduced lesion size	(18)
	Cuprizone-induced demyelination mouse	KD up to 35 days	Improved motor and behavioral deficits, increased mature oligodendrocytes and reduced hippocampal demyelination	(19)
Parkinson's Disease	MPTP mouse	BHB infusions	Decreased dopaminergic neurons degeneration and reduced motor deficits	(20)
	MPTP mouse	KD prior to injury	Improved motor function	(21)
	6-OHDA rat	KD for 7 weeks	None	(2)
	6-OHDA rat	KD for 2 weeks prior to and after injury	Increased neurons	(22)
Retinal degeneration	rd10 model of retinitis pigmentosa, mouse	KD+low protein 1 week prior to disease onset	Increased retina function and thickness	(23)
	Pde6a D670G model of retinitis pigmentosa, mouse	KD for 1 week	Improved retinal histology	(24)
SCI	C5 spinal hemi-contusion, rats	KD initiated 2 weeks prior to injury	Motor recovery, reduced lesion size	(25)
Stroke	Endothelin-1 model of induced ischemia, rat	KD prior to injury	Improved mobility	(26)
	Cardiac arrest-induced cerebral ischemia, rat	KD for 25 days prior to injury	Prevented neurodegeneration	(27)
	Middle cerebral artery occlusion, rat	Intravenous BHB administration after injury	Reduced cerebral infarct area and lower neurological deficits	(28)

demonstrated in a small group of patients that 6 weeks on a KD improved verbal memory performance compared with patients on a high carbohydrate diet. Similarly, a single-arm study of patients on the KD for 12 weeks showed improved cognitive scores, although comparison to a control group was lacking (30). A recent randomized crossover trial showed that 12 weeks on a modified KD led to significantly improved daily function and quality of life, although not significant improvements in memory (31). These results are promising, particularly if the benefits are sustained in longer term studies.

TABLE 2 | Summary of key published clinical studies examining the beneficial effects of the KD in neurological diseases.

Disease	Trial type	KD duration	Outcome	References
Alzheimer's disease	Randomized comparison of KD to high carbohydrate diet	6 weeks	Improved verbal memory performance	(29)
	Single-arm	12 weeks	Improved cognitive scores	(30)
	Randomized crossover	12 weeks	Improved quality of life, no improved memory	(31)
Dravet syndrome	Prospective study	3-12 months	Reduced seizures and hyperactivity	(32)
Epilepsy	Randomized, prospective and controlled	3 months	Reduced seizure frequency	(11)
Pharmacoresistant epileptic encephalopathy	Prospective study	12 weeks	Improved cognitive function, reduced seizure frequency	(33)
MS	Single-arm	6 months	Significantly improved fatigue and depression scores	(34)
	Randomized three-armed parallel grouped, single center, controlled	6 months	Improved quality of life and overall health	(35)
Parkinson's disease	Randomized, controlled, parallel-group trial	8 weeks	Small increase in cognitive abilities but no change in motor function	(36)

The effectiveness of the KD has been analyzed in several animal models of Alzheimer's disease (AD) but the beneficial effects were shown to vary widely. Administration of BHB in the 3xTgAD mouse model enhanced energy use in the hippocampus and reduced oxidized proteins and lipids, suggesting correction of metabolic defects associated with AD (7). Furthermore, APP/PS1 and Tg4510 mice on a KD for 3 months showed improved locomotor activity, although no improvement in learning or changes in amyloid or tau deposits were detected (8). In comparison, a study using 7 week oral administration of the glucose analog 2-deoxy-D-glucose, which blocks glycolysis and induces ketosis, in the 3xTgAD mouse model led to reduced accumulation of Aβ and lowered lipid peroxidation and oxidative stress response proteins, but did not decrease tau hyperphosphorylation (9). Furthermore, Yin et al. (10) used daily administration of BHB or ACA for 2 months in a mutant APP mouse model and demonstrated lower oxidative damage and reduced β-amyloid deposition, which was associated with improved learning and memory and synaptic plasticity. Therefore, the effectiveness of the KD appears to be inconsistent among different animal models of AD and may be influenced by the method of inducing ketosis. Additional studies to clarify the effect of the KD and ketone bodies on AD pathology are needed.

Parkinson's Disease

Several pilot clinical studies have reported findings on the effect of nutritional ketosis on reducing PD symptoms. For example, consumption of a KD for up to 8 weeks resulted in small increases in cognitive abilities but not motor functions compared with a low-fat diet (36). However, whether the KD improves symptoms in animal models of PD is controversial. In an early study with the commonly used MPTP neurotoxicity mouse model, Tieu et al. (20) demonstrated that BHB infusions resulted in a small but significant rescue of dopaminergic neuron degeneration and reduced motor deficits. Another study using the MPTP model indicated that mice fed the KD also showed improved motor function and lower inflammation (21). In contrast, a rat model of induced PD-like lesions from 6-OHDA injection into the medial forebrain bundle showed no protection of dopaminergic neurons or functional improvement after 7 weeks on the KD despite increased brain BHB levels (2). Although increased number of tyrosine hydroxylase-positive neurons were reported in a different study using 6-OHDA injected rats fed the KD, neuronal apoptosis or motor function were not directly measured (22). Therefore, the literature does not yet support a neuroprotective effect of the KD in PD but it may indirectly improve motor performance. Because motor improvement was not demonstrated in the human pilot studies, longer studies may be necessary, and the possibility remains that these improvements may not translate from animals to humans.

Spinal Cord Injury

To date, there have been no completed clinical trials reported in the USA or European Union although promising results of the KD have been obtained in animal models of SCI. In a study on rats after C5 spinal hemi-contusion, Streijger et al. (25) found that administration of the KD promoted motor recovery, measured by ipsilateral forelimb use and range of motion, and was associated with smaller lesion sizes compared to rats fed the control diet. Blocking MCT activity prevented neuroprotection by the KD. Notably, improved forelimb function was maintained after the animals were switched back to a standard diet. This result provides compelling evidence to investigate whether the KD leads to functional improvements in patients after SCI.

Stroke

Several clinical trials investigating possible benefits of the KD have been completed according to Clinicaltrials.gov but conclusions have not yet been reported. No clinical trials are registered in the EU. Animal studies using various stroke models have demonstrated reduced pathology due to a KD or direct administration of exogenous BHB prior to injury. Shaafi et al.

(26) used the endothelin-1 model of induced ischemic stroke to demonstrate that rats fed the KD shortly before stroke induction had improved mobility, indicating a benefit of diet preconditioning. Similarly, rats fed the KD for 25 days prior to injury were protected from neurodegeneration caused by cardiac arrest-induced cerebral ischemia (27), and intravenous administration of BHB in rats after the initiation of middle cerebral artery occlusion significantly reduced cerebral infarct area and neurological deficits (28). However, it is still unknown whether providing the KD after stroke injury would be protective and whether the mechanisms of neuroprotection differ between providing the KD pre-injury or post-injury.

Glaucoma

There are no clinical trials listed at clinicaltrials.gov (USA) or clinicaltrialsregister.eu/ (European Union) on the use of a KD to treat glaucoma. Neuroprotective effects of the KD have been shown in mouse and rat models of glaucoma. In a study using the DBA/2J mouse model of elevated intraocular pressure and secondary glaucoma, mice fed the KD for 8 weeks showed significantly increased retinal ganglion cell (RGC) survival and axon number compared to mice fed the control diet (14). This neuroprotection was associated with increased mitochondria and reduced expression of pro-inflammatory molecules in the retina and optic nerves. In a second model, the NMDA-induced RGC death model, pretreatment of juvenile but not adult rats with a KD for 21 days led to increased RGC survival (15). An age-dependent neuroprotective effect of the KD was also observed in another rat CNS injury model, cortical impact injury, and may be related to variations in MCT expression and ketone uptake into the brain (39). Another study using the NMDA toxicity model showed that intraperitoneal injections of BHB and acetoacetate in rats also reduced RGC degeneration although age was not investigated as a variable (16). Additionally, mice fed the KD prior to or after pressure-induced ocular injury had decreased optic nerve gliosis, reduced optic nerve degeneration and higher visual evoked potential amplitudes (17). Together, these studies provide support for investigating the KD as a potential therapy for glaucoma.

Retinal Degenerations

There are no US or European clinical trials on the effect of the KD on common retinal degenerations, such as age-related macular degeneration or retinitis pigmentosa. However, investigation in mouse models of retinal degenerations revealed neuroprotection by the KD. Ryals et al. (23) examined the effect of a ketogenic combined with protein restriction using the rd10 mouse model of autosomal recessive retinitis pigmentosa, which causes early onset and severe photoreceptor degeneration. The combination of a protein-restricted KD led to high BHB levels, increased retinal function and increased photoreceptor layer thickness. This protective effect was observed after the mice were placed on the diet 1 week before the onset of photoreceptor degeneration, indicating rapid induction of the neuroprotective response. In contrast, rd10 mice on a KD with typical protein levels did not show neuroprotection, while wild-type mice fed the protein-restricted KD showed reduced photoreceptor function with no corresponding change in photoreceptor survival. The authors proposed that the low protein KD protected the retina by reducing phototransduction, lowering calcium influx and decreasing subsequent ROS production (23). A second study using a similar mouse model of autosomal recessive retinitis pigmentosa (Pde6a D670G mutation) also demonstrated increased photoreceptor survival after 1 week on the standard KD, measured by improved retinal histology, although improved photoreceptor function was not found (24). Therefore, these studies indicate that even a short time on the KD can lead to measurable benefits to the retina in mouse models of inherited retinal degenerations.

NEUROPROTECTIVE MECHANISMS OF THE KD

Various molecular mechanisms have been associated with neuroprotective effects of the KD and ketone body administration. Most studies report correlations between cellular pathways and neuroprotection, such as changes in oxidative stress or inflammatory proteins, but whether these pathways are altered as primary or secondary effects of the diet is not determined in many studies and remains controversial. Recently, several elegant studies using inhibitors and mouse knockout strains have provided evidence for direct contributions of specific cellular pathways in mediating neuroprotective effects of the KD. These studies are summarized below.

Energy Supply Restoration

Neuronal injury often leads to changes in glucose metabolism. The simplest mechanism for neuroprotection by the KD is that ketone bodies serve as alternative fuels for brain metabolism, which maintains mitochondrial function, ATP production and neuronal survival. For example, in the MPTP neurotoxicity mouse model discussed above, BHB induced neuroprotection by preventing the decline in mitochondrial respiration by acting on complex II (succinate-ubiquinone oxidoreductase) and restoring ATP production (20). The ketogenic diet also promoted complex II and IV activities in a glaucoma mouse model, which increased energy production and promoted neuronal survival (40). Increased mitochondrial respiration induced by BHB administration was associated with higher ROS from oxidative phosphorylation in the MPTP model, although ROS levels were not elevated in the KD fed glaucoma model, suggesting additional benefits from the diet counter elevated ROS. Therefore, the production of ketones from the KD can help cells avoid energy deficits after neuronal injury and prevent the cascade of events that lead to neuronal death.

AMP-activated protein kinase (AMPK) senses cellular energy levels and is activated when energy levels are low, which serves to reduce ATP consumption and promote ATP production. AMPK regulates inflammation by activating the major inflammatory regulator NF-κB, which leads to transcription of pro-inflammatory proteins, including TNFα, IL-1β and IL-6 (41). Analysis of retinas from a glaucoma mouse model fed the KD demonstrated reduced AMPK activation, which was

associated with lower NF-κB p65 nuclear translocation and decreased expression of pro-inflammatory molecules (40). The authors concluded that the anti-inflammatory effect of the KD in the glaucomatous retina is dependent on its ability to restore normal ATP production through the supply of ketone bodies and consequential changes in AMPK activation.

Induction of Anti-inflammatory Pathways

Neuroinflammation is an intrinsic response to neuronal injury and disease. Inflammatory cells, including microglia, macrophages, astrocytes and other non-neuronal cells, function together to repair neuronal damage by enhancing phagocytosis and secreting neuroprotective and anti-inflammatory molecules. However, unregulated neuroinflammation can cause further neuronal loss from elevated ROS levels and release of neurotoxic and pro-inflammatory cytokines. The neuroprotective effects of the KD in animal models are often correlated with reduced pro-inflammatory signaling and are characterized by lower microglia numbers, decreased expression of the inflammation inducer NF-κB p65 protein and reduced levels of pro-inflammatory molecules such as TNFα. Until recently, it was unknown whether these inflammatory changes are directly induced by ketones or secondary to other processes. In the studies described below, pharmacologic and genetic knock-outs of specific molecular pathways were used to demonstrate the importance of several mediators of inflammation that are altered directly by ketone bodies, including the receptors HCA1 and HCA2, GPR40 fatty acid receptor and adaptor protein ARRB2 (**Figure 2**). Further, BHB itself functions as a signaling molecule in addition to serving as an energy source. Therefore, evidence is accumulating that the KD, and more specifically BHB and its metabolites, directly reduce pro-inflammatory responses leading to neuroprotection.

Inflammatory signaling in the CNS is often mediated by the NLRP3 inflammasome. Upon activation by microbial PAMPs and other stress-related molecules, NLRP3 leads to the activation of caspase-1 followed by cleavage of pro-IL-1β into its active IL-β form, which promotes further inflammatory signaling. The NLRP3 inflammasome also interacts with NF-κB and induces transcription of pro-inflammatory cytokines which promotes further activation of NLRP3 (42). Investigations in cultured macrophages, human monocytes and mouse models of inflammatory diseases demonstrated that BHB inhibits NRLP3 inflammasome activation through a mechanism that involves blocking potassium efflux and preventing ATP-induced ASC oligomerization, which are both needed for inflammasome assembly (43). The other ketone bodies, acetoacetate, acetate and butyrate, did not inhibit inflammasome activity. The authors concluded that metabolites such as BHB may suppress ATP-mediated inflammatory responses, particularly involving NRLP3, during glucose-depleted states as an intrinsic mechanism to reserve ATP for the activity of ketone-dependent organs such as the brain and heart (43).

BHB binds directly to hydroxycarboxylic acid receptor 2 (HCA2) (also called GPR109A) on adipocytes (44). HCA2 is also expressed on neutrophils, macrophages and other cells in the brain, expanding the potential target cells influenced by BHB and the KD. In the MCAO mouse model of ischemic stroke, BHB was shown to activate HCA2 in macrophages that infiltrate the brain after injury and the protective effect of the KD and BHB was lost in HCA2 knock-out mice. Mechanistically, BHB activation of HCA2 resulted in activation of the enzymes HPGDS and COX1 which induces prostaglandin D2 (PDG2) synthesis. PDG2 and its conversion product 15d-PGJ2 are neuroprotective by reducing NF-κB activation, decreasing proinflammatory genes and by activating the transcription factor PPARgamma (45).

Lin et al. (46) identified mechanisms downstream of the fatty acid receptor GPR40 that inhibit proinflammatory pathways using a rat traumatic brain injury model treated with omega-3 fatty acids, which are a common component of the KD. Inhibiting GPR40 using chemical antagonists prevented the anti-inflammatory effect of the fatty acid-enriched diet. The GPR40 receptor interacts with the scaffolding protein ARRB2, which also interacts with and directly inhibits NLRP3 activity. Treatment with omega-3 fatty acids resulted in ARRB2-mediated inhibition of NLRP3, reducing IL-1β inflammatory signaling and promoting neuronal survival. Furthermore, reducing ARRB2 expression or knocking out NLRP3 inhibited ARRB2-NLRP3 binding and prevented the anti-inflammatory effect of fatty acids. Therefore, GPR40-ARRB2-inflammasome inhibition is likely an important mediator of the neuroprotective effect of dietary fats. Although the KD differs from the omega-3 enriched diet, and directly comparing the diets cannot be done, the findings of Lin et al. raise the possibility that specific fatty acid components of the KD may contribute to the observed anti-inflammatory effects. Additionally, in a mouse model of glaucoma, the KD was associated with increased expression of ARRB2 and its interacting protein and BHB target HCA1, which led to reduced levels of NLRP3 and IL-1β, consistent with inhibited NLRP3 inflammasome function (40). Therefore, the KD reduces inflammation through binding of BHB-HCA1 and fatty acids-GPR40, which leads to ARRB2-dependent suppression of NRLP3 inflammasome function (**Figure 2**).

The KD and BHB were also demonstrated to have direct effects on microglia ramifications, causing microglia to polarize toward the M2-like neuro-reparative and protective phenotype (40). BHB was shown to mediate this change in microglia morphology by stimulating Akt signaling, which involved inhibition of HDACs but not HCAR2 (47). Consequently, BHB may promote anti-inflammatory signaling by altering microglial morphology. The KD was also shown to increase anti-inflammatory responses in glaucomatous mice, evidenced by increased expression of Arginase 1, a marker of neuroprotective anti-inflammatory microglia, as well as increased expression of the anti-inflammatory cytokine IL-4 in retinas and optic nerves (40).

Cellular mechanisms induced in low energy states also regulate inflammatory responses (48). Energy metabolism influences inflammation through changes in the cytosolic NADH:NAD+ ratio (48). Shen et al. used the glycolytic inhibitor 2DG to mimic changes in glycolytic flux from the KD and demonstrated that reduced glucose usage leads to lower NADH:NAD+ ratio, which inhibits the NAD(H) sensitive transcriptional co-repressor CtBP in microglia. Reduced CtBP activation has an anti-inflammatory effect through

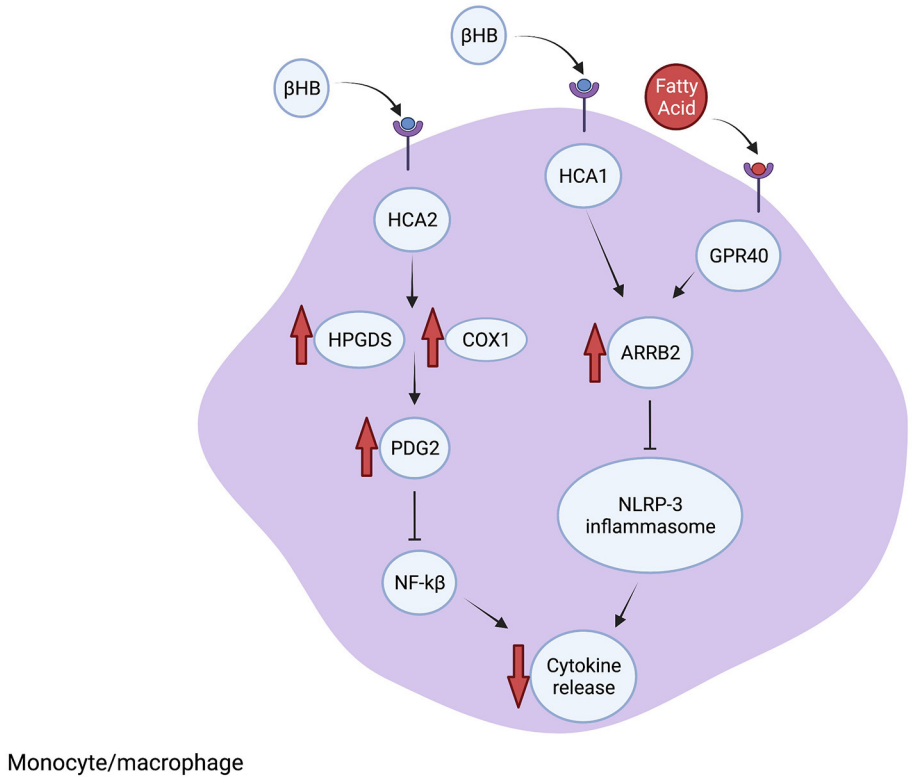

FIGURE 2 | The KD reduces inflammation. The scaffolding protein ARRB1 is elevated after BHB binds to HCA1 or fatty acids (FA) bind to GPR40. Increased ARRB1 inhibits the NRLP-3 inflammasome formation, which reduces cytokine processing and release. Furthermore, BHB interacting with HCA2 leads to increased PDG2 synthesis through increased HPGDS and COX1, which leads to inhibition of NF-kB and suppressed inflammatory signaling. Additional anti-inflammatory mechanisms are described in the text.

decreased activity of p300 and reduced NF-κB binding to the promoters of proinflammatory genes (49). Therefore, the KD may alter inflammation through bioenergetic changes in the NADH:NAD+ ratio.

Complicating the effect of the KD on inflammation is the finding that acetoacetate activates inflammatory pathways through TNFα, which is in contrast to the anti-inflammatory properties of BHB (50). Additionally, high concentrations of BHB induce inflammatory signaling molecules NF-κB, TNFα, IL-6 and IL-1β (51). Therefore, the overall effect of the KD on inflammation is influenced by local concentrations of ketone bodies in the affected tissues and the ratio of BHB to acetoacetate.

Reducing Oxidative Stress

Oxidative stress is a secondary effect of neuronal injury and is caused by mitochondrial dysfunction and subsequent production of reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive electrophile species (RES), all of which have been associated with neurotoxicity and neuronal death in neurodegenerative diseases (52). Blocking oxidative stress using chemical or genetic modulators reduces excitotoxicity and mitochondrial dysfunction, leading to cellular protection in animal models of many neurodegenerative diseases (52).

The KD is associated with decreased markers of oxidative stress in animal models, including reduced nitric oxide synthase-2 (NOS2) in the retina of glaucomatous mice (40), reduced retinal superoxide and elevated anti-oxidant proteins such as SOD2 in the retina after blast injury (17) and increased antioxidant responses and regulation of ROS in rat cortex after impact injury and kidney tissue from mice treated with BHB (53, 54). Physiological concentrations of BHB are able to scavenge ROS and hydroxyl radicals and directly reduce cellular ROS levels, which preserves mitochondrial functioning and increases cell survival (55). For example, in the cuprizone-induced demyelination mouse model of MS, the KD led to increased activity of glutathione peroxidase, an enzyme that breaks down H_2O_2, and reduced expression of the oxidative stress marker malondialdehyde; these molecular changes in oxidative stress markers were correlated with improved myelination in the hippocampus (19). Interestingly, brain region-specific changes in antioxidant enzyme activities were noted in rats fed a KD, with the hippocampus showing increased levels of antioxidant enzymes that were not observed in the cerebral cortex or cerebellum (56). However, the implication for neuroprotection in specific brain regions is not known.

Several molecular mechanisms that contribute to KD-mediated reduction of oxidative stress have been described

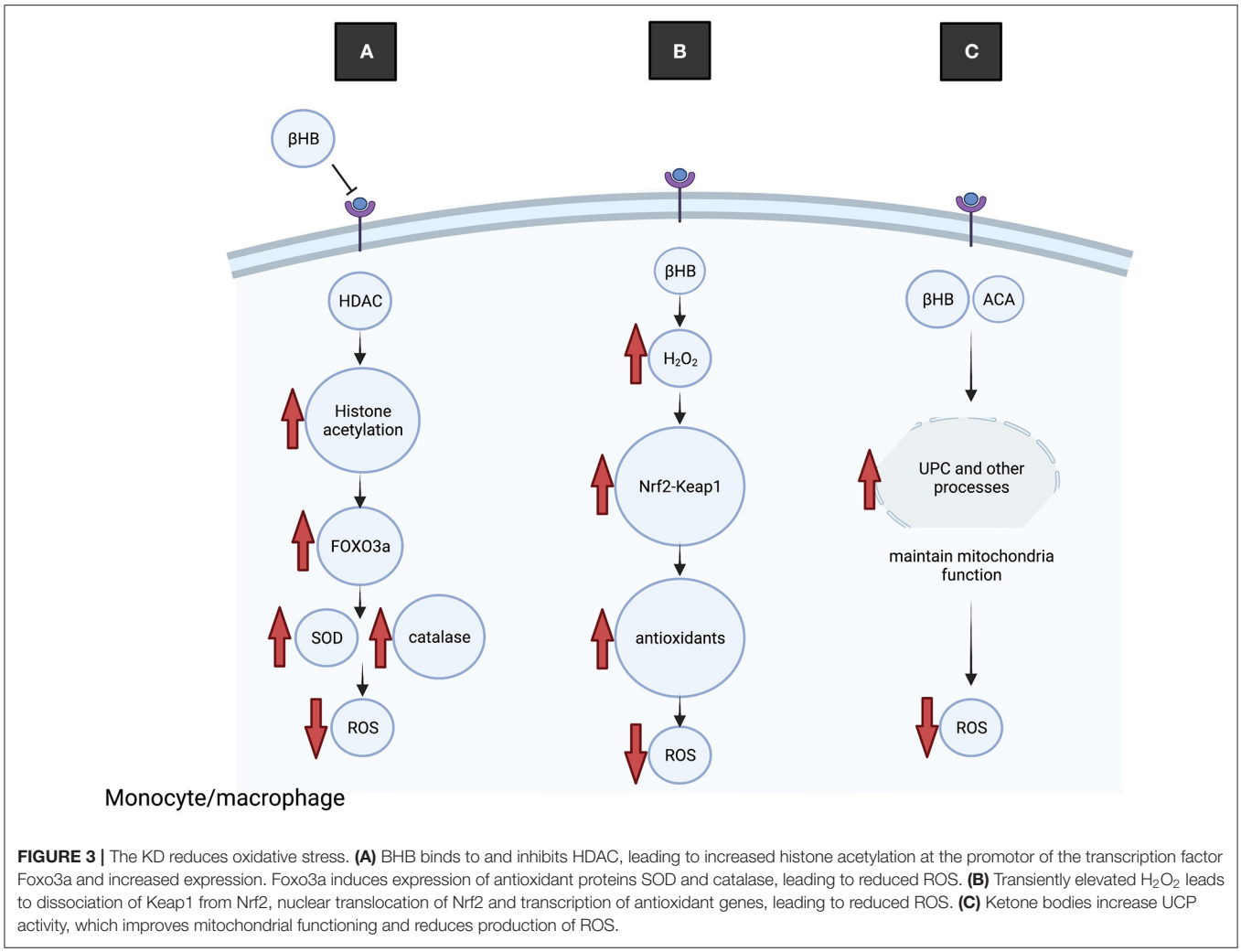

FIGURE 3 | The KD reduces oxidative stress. **(A)** BHB binds to and inhibits HDAC, leading to increased histone acetylation at the promotor of the transcription factor Foxo3a and increased expression. Foxo3a induces expression of antioxidant proteins SOD and catalase, leading to reduced ROS. **(B)** Transiently elevated H_2O_2 leads to dissociation of Keap1 from Nrf2, nuclear translocation of Nrf2 and transcription of antioxidant genes, leading to reduced ROS. **(C)** Ketone bodies increase UCP activity, which improves mitochondrial functioning and reduces production of ROS.

(**Figure 3**). Mice fed the KD had higher mitochondrial respiration rates and increased expression of mitochondrial uncoupling proteins (UCP) than mice on a control diet (57). Increased UCP activity reduces mitochondrial membrane potential and lowers production of ROS and reactive oxygen nitrogen species. Ketone bodies may increase UCP expression through regulating Sirtuin 1 activity (58), potentially via altered NAD+ levels (59), although the precise mechanisms are not yet known.

Nrf2 is a key endogenous protective transcription factor that leads to increased transcription of antioxidative enzymes including glutathione reductase, heme oxygenase-1, thioredoxin and peroxiredoxin. An interesting study on rats fed the KD demonstrated acute and transient increases in oxidative (H_2O_2) and electrophilic (lipid peroxidation end product 4-HNE) stress in the brain, which appears to conflict with the general antioxidant properties of ketone bodies (60). However, the elevated H_2O_2 levels were shown to stimulate the Nrf2 antioxidant pathway through Nrf2-Keap 1 redox sensing, and elevated Nrf2 was detected in brain and liver tissue after 1 week on the diet. Because H_2O_2 increases Nrf2 binding to DNA, a potential neuroprotective mechanism is that transient elevation of H_2O_2 and altered redox states from the KD serve to activate Nrf2 leading to induction of antioxidation pathways (60).

Gut Microbiome Alterations

The gut microbiome has been associated with several neurological disorders, such as Alzheimer's disease, epilepsy, Parkinson's disease and multiple sclerosis. Changes in the gut microbiome are observed in children on the KD to prevent seizures as early as 1 week into the diet (61), although the significance to improved epilepsy symptoms is not understood. Additionally, the colonic microbiome of MS patients on the KD for 6 months showed increased bacterial concentrations and bacterial species diversity (62). Microbial species in the gut secrete various metabolites, such as tryptophan, short-chain fatty acids, neurotransmitters and immunomodulatory molecules. Therefore, changes in the microbiome caused by the KD may also affect microbial-derived molecules that play a role in CNS homeostasis and possible neuroprotection (63).

Olson et al. (13) used two epilepsy mouse models fed a KD to determine the role of specific gut microbial species influenced by the diet. Mice fed the KD had significantly fewer seizures than mice fed a control diet, as well as increased levels of the gut bacteria species *Akkermansia* and *Parabacteroides*. These bacteria caused reduced gamma-glutamyltranspeptidase in the stomach and lowered gamma-glutamylated amino acids in the colon and vascular system, which led to higher GABA production in the brain and an elevated seizure threshold (13). The authors treated the mice with antibiotics to deplete the gut microbiota or reared them in germ-free conditions which confirmed that changes in the microbiome were responsible for the ketogenic anti-epileptic effect. Additionally, transplanting samples of microbiota from mice fed the KD into control mice, as well as transplanting *Akkermansia* and *Parabacteroides* bacteria into mice fed the control diet, resulted in similar levels of seizure protection as mice directly fed the KD (13). Therefore, the anti-convulsant properties of the KD in these mice were mediated by changes in specific bacterial species in the gut. In humans, the gut microbiome is affected by many different factors, making the connection between KD, gut microbes and neuroprotection currently only correlative and requiring further study.

Epigenetic Mechanisms

BHB regulates the epigenome by modifying histone acetylation by inhibiting HDAC or activating Sirtuin 1. BHB increases histone acetylation by inhibiting class I histone deacetylases (HDACs), which are a family of proteins that regulate transcription of numerous genes through histone modifications. Removal of acetyl groups by HDACs alters DNA conformation and leads to transcriptional repression. Mice treated directly with BHB or placed on caloric restriction to elevate endogenous BHB showed evidence of HDAC inhibition and increased histone acetylation of promoters of oxidative stress resistance genes, including the transcription factor Foxo3a and metallothionein 2 (54). Targets of Foxo3a include the antioxidant genes mitochondrial superoxide dismutase and catalase, which were also elevated in BHB-injected mice. However, these studies were performed in kidney tissue and it remains to be determined whether BHB also inhibits HDAC in the CNS, and whether induction of Foxo3a-related antioxidants after HDAC inhibition contributes to neuroprotection by the KD (54). In another study, analysis of liver cells in mice fed the KD showed induction of Sirtuin 1 activity (64), an NAD+-dependent protein deacetylase. Therefore, the role of acetylation-dependent transcription regulation in mediating neuroprotection by the KD may be influenced by opposite effects of ketones on HDAC and Sirtuin 1.

BHB itself directly modifies lysine residues on histones in regions of active gene promoters (65) in a process called β-hydroxybutyrylation. A recent study demonstrated that the acyltransferase p300 adds β-hydroxybutyrate to lysine, while HDAC1 and HDAC2 remove it (66). Over 1,300 other proteins in addition to histones were identified as targets of ketone-related modifications, suggesting that this is a major regulatory pathway that is potentially altered by the KD (66).

DISCUSSION

Controversies and Adverse Effects of the KD

The mechanisms of neuroprotection described above have often been studied in a single animal model and the generalizability to other sources of neuronal injury and different neuronal tissues are unknown. Notably, the effect of ketone bodies on antioxidants has been shown to differ across brain regions (56) and exhibit age-specific effects (15), which may underly variations in outcomes of the KD among different studies. As noted above, high concentrations of BHB were found to induce pro-inflammatory signaling which is the opposite effect of lower concentrations (51); because of this dose-dependent BHB effect on inflammation, it is important to measure serum and/or tissue BHB levels to properly assess the effect of the KD. Additionally, beneficial effects of the KD in animals have been shown to not always translate to a benefit in humans, for example, motor improvements were shown in rat models of PD but not patients with PD. Therefore, extrapolating the results of studies from animals to humans must always been done cautiously. Furthermore, it is important to consider that molecular mechanisms that lead to reduced inflammation and lower oxidative stress may change with longer durations of the diet, and the reported short-term mechanisms of neuroprotection may not necessarily contribute to its long-term effects.

Adopting the KD would appear to contradict current dietary guidelines that recommend reduced intake of total fat. However, potential negative effects of elevated dietary fat intake appear to be balanced by the beneficial effects of reduced carbohydrates and elevated ketones. The KD is popular with the general public for weight loss and side effects generally are minor. However, long-term strict adherence to the diet is associated with adverse effects, and gastrointestinal issues (such as diarrhea, abdominal distention, reflux), cardiac problems (arrhythmia) and poor growth have been noted in pediatric epilepsy patients on the KD (67–69), although a confounding variable is that these patients often have underlying metabolic and other health issues. Less common but more severe side effects include specific nutrient deficiencies, kidney stones, bone fractures and increased infections, which can be treated with standard therapies and appropriate vitamin and nutrient supplementation while maintaining the diet (70). For example, several case reports indicate that complications of the KD included protein–losing enteropathy, edema and hypoalbuminemia; in a recent case report these issues were resolved by reducing the fat to carbohydrate ratio in the diet (71). Another report demonstrated that most gastrointestinal and nutrient deficiencies occurring early (< 4 weeks on the diet) and complications such as anemia and cardiomyopathy occurring late (> 4 weeks) were transient and successfully managed (69).

The effect of the KD on growth and skeletal health was reviewed by Merlotti et al. (72). Multiple studies in rats on the KD and modified KD combined with other treatments indicated reduced biomechanical function, reduced bone mineral density and increased osteoporosis. In contrast, most studies with

patients on the KD showed no or minimal negative effects on growth, bone mass or molecular markers of bone health over 24 months (72). In a long-term retrospective study of pediatric patients on the diet for 8 years, a low but significant frequency of individuals with reduced bone mineral density was observed (73). This observation may be explained by a potential relationship between the genetic cause of epilepsy and skeletal damage, for example GLUT1 gene mutations (72). Additionally, in a study on children who had followed the KD for a median of 6 years and then discontinued the diet at least 6 months prior to the study, no long-term effects were observed on physical growth, lipid levels and other measurements (74). Therefore, current evidence does not indicate significant effects on bone metabolism, although most studies may not be long enough duration to determine increased risk of fractures or altered bone health.

FUTURE RESEARCH DIRECTIONS

There are several key gaps in knowledge about the KD that require further investigation. First, determining whether the KD reduces pathology and alleviates neurological symptoms of the diseases discussed above should be clarified with randomized clinical trials using blinded clinical assessments whenever possible and appropriate control diets. Second, due to potential side effects and potential non-compliance with the diet, it is important to consider whether exogenous ketones can be used to achieve therapeutic levels of circulating ketone bodies, as suggested from studies in animal models of disease. Third, multiple cell types react to and metabolize ketone bodies, and further investigation into whether inflammatory cells, glia and neurons respond differently to ketone bodies should be explored. Fourth, understanding mechanisms of brain region-specific and age-dependent neuroprotection of the KD may reveal new insights into the regulation of neuronal survival pathways and the influence of bioenergetic pathways. Fifth, identification of potential mechanisms of neuroprotection may be used to develop biomarkers or indicators of the effectiveness of the diet, such as changes in specific plasma cytokines. Sixth, further detailed characterization of the mechanisms that modify the effect of KD-induced neuroprotection is needed, including the role of expression levels of relevant transcription factors, BHB receptors and MCT transporters, local ketone body concentration, gut microbiome species and mitochondrial density and function, all of which could contribute to variation in the response to KD. Finally, additional studies should determine whether modified KDs, which are less restrictive and may be easier to adopt, would also have beneficial effects for the conditions described above including investigation of improving quality of life in the aging population.

CONCLUSIONS

As described above, the KD has been used for many years to reduce seizures in treatment-intractable pediatric epilepsy and was confirmed to be effective in a randomized control clinical trial (11). Furthermore, recent studies have demonstrated neuroprotection from the KD or direct administration of ketone bodies in numerous animal models of neurological disease, including stroke, glaucoma and spinal cord injury, which raises the possibility that the KD may reduce symptoms in patients with these conditions. However, the existing literature on the effectiveness of the KD in several neurological disorders shows that it reduces symptoms and improves quality of life in several diseases when tested in small pilot studies, such as MS and Alzheimer's disease, but results from larger randomized clinical trials have not yet been reported. Furthermore, the duration of the beneficial effects is unknown because all studies have been short-term, except for analysis of side effects in epilepsy patients. Finally, variations in the composition of the diet, duration of treatments, and frequently small sample sizes in the study groups, make it difficult to compare and draw conclusions across studies. Therefore, although adverse effects of the KD are minimal, evidence does not yet indicate adopting the KD for reducing neurodegeneration in common neurological diseases.

AUTHOR CONTRIBUTIONS

The manuscript was conceived and developed by AH. SG, AC, KO, and AH performed the literature review, manuscript writing and editing. Figures preparation were performed by AH and KO. All authors read and approved the final manuscript.

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REFERENCES

1. Dhamija R, Eckert S, Wirrell E. Ketogenic diet. *Can J Neurol Sci.* (2013) 40:158–67. doi: 10.1017/S0317167100013676
2. Kuter KZ, Olech L, Glowacka U, and Paleczna M. Increased beta-hydroxybutyrate level is not sufficient for the neuroprotective effect of long-term ketogenic diet in an animal model of early Parkinson's disease. Exploration of brain and liver energy metabolism markers. *Int J Mol Sci.* (2021) 22:7556. doi: 10.3390/ijms22147556
3. Puchalska P, Crawford PA. Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. *Cell Metab.* (2017) 25:262–84. doi: 10.1016/j.cmet.2016.12.022
4. Adijanto J, Du J, Moffat C, Seifert EL, Hurle JB, Philp NJ. The retinal pigment epithelium utilizes fatty acids for ketogenesis. *J Biol Chem.* (2014) 289:20570–82. doi: 10.1074/jbc.M114.565457
5. Lehninger AL, Sudduth HC, Wise JB. D-beta-Hydroxybutyric dehydrogenase of muitochondria. *J Biol Chem.* (1960) 235:2450–5. doi: 10.1016/S0021-9258(18)64641-1
6. McNally MA, Hartman AL. Ketone bodies in epilepsy. *J Neurochem.* (2012) 121:28–35. doi: 10.1111/j.1471-4159.2012.07670.x
7. Pawlosky RJ, Kemper MF, Kashiwaya Y, King MT, Mattson MP, Veech RL. Effects of a dietary ketone ester on hippocampal glycolytic and tricarboxylic acid cycle intermediates and amino acids in a 3xTgAD mouse model of Alzheimer's disease. *J Neurochem.* (2017) 141:195–207. doi: 10.1111/jnc.13958

8. Brownlow ML, Benner L, D'Agostino D, Gordon MN, Morgan D. Ketogenic diet improves motor performance but not cognition in two mouse models of Alzheimer's pathology. *PLoS ONE.* (2013) 8:e75713. doi: 10.1371/journal.pone.0075713
9. Yao J, Chen S, Mao Z, Cadenas E, Brinton RD. 2-Deoxy-D-glucose treatment induces ketogenesis, sustains mitochondrial function, and reduces pathology in female mouse model of Alzheimer's disease. *PLoS ONE.* (2011) 6:e21788. doi: 10.1371/journal.pone.0021788
10. Yin JX, Maalouf M, Han P, Zhao M, Gao M, Dharshaun T, et al. Ketones block amyloid entry and improve cognition in an Alzheimer's model. *Neurobiol Aging.* (2016) 39:25–37. doi: 10.1016/j.neurobiolaging.2015.11.018
11. Neal EG, Chaffe H, Schwartz RH, Lawson MS, Edwards N, Fitzsimmons G, et al. The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *Lancet Neurol.* (2008) 7:500–6. doi: 10.1016/S1474-4422(08)70092-9
12. Kim DY, Simeone KA, Simeone TA, Pandya JD, Wilke JC, Ahn Y, et al. Ketone bodies mediate antiseizure effects through mitochondrial permeability transition. *Ann Neurol.* (2015) 78:77–87. doi: 10.1002/ana.24424
13. Olson CA, Vuong HE, Yano JM, Liang QY, Nusbaum DJ, Hsiao EY. The gut microbiota mediates the anti-seizure effects of the ketogenic diet. *Cell.* (2018) 174:497. doi: 10.1016/j.cell.2018.06.051
14. Harun-Or-Rashid M, Pappenhagen N, Palmer PG, Smith MA, Gevorgyan V, Wilson GN, et al. Structural and functional rescue of chronic metabolically stressed optic nerves through respiration. *J Neurosci.* (2018) 38:5122–39. doi: 10.1523/JNEUROSCI.3652-17.2018
15. Zarnowski T, Choragiewicz TJ, Schuettauf F, Zrenner E, Rejdak R, Gasior M, et al. Ketogenic diet attenuates NMDA-induced damage to rat's retinal ganglion cells in an age-dependent manner. *Ophthalmic Res.* (2015) 53:162–7. doi: 10.1159/000379753
16. Thaler S, Choragiewicz TJ, Rejdak R, Fiedorowicz M, Turski WA, Tulidowicz-Bielak M, et al. Neuroprotection by acetoacetate and beta-hydroxybutyrate against NMDA-induced RGC damage in rat—possible involvement of kynurenic acid. *Graefes Arch Clin Exp Ophthalmol.* (2010) 248:1729–35. doi: 10.1007/s00417-010-1425-7
17. Bernardo-Colon A, Vest V, Clark A, Cooper ML, Calkins DJ, Harrison FE, et al. Antioxidants prevent inflammation and preserve the optic projection and visual function in experimental neurotrauma. *Cell Death Dis.* (2018) 9:1097. doi: 10.1038/s41419-018-1061-4
18. Kim DY, Hao J, Liu R, Turner G, Shi FD, Rho JM. Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. *PLoS ONE.* (2012) 7:e35476. doi: 10.1371/journal.pone.0035476
19. Liu C, Zhang N, Zhang R, Jin L, Petridis AK, Loers G, et al. Cuprizone-induced demyelination in mouse hippocampus is alleviated by ketogenic diet. *J Agric Food Chem.* (2020) 68:11215–28. doi: 10.1021/acs.jafc.0c04604
20. Tieu K, Perier C, Caspersen C, Teismann P, Wu DC, Yan SD, et al. D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *J Clin Invest.* (2003) 112:892–901. doi: 10.1172/JCI200318797
21. Yang X. and Cheng B, Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. *J Mol Neurosci.* (2010) 42:145–53. doi: 10.1007/s12031-010-9336-y
22. Cheng B, Yang X, An L, Gao B, Liu X, Liu S. Ketogenic diet protects dopaminergic neurons against 6-OHDA neurotoxicity via up-regulating glutathione in a rat model of Parkinson's disease. *Brain Res.* (2009) 1286:25–31. doi: 10.1016/j.brainres.2009.06.060
23. Ryals RC, Huang SJ, Wafai D, Bernert C, Steele W, Six M, et al. A ketogenic and low-protein diet slows retinal degeneration in rd10 mice. *Transl Vis Sci Technol.* (2020) 9:18. doi: 10.1167/tvst.9.11.18
24. Wert KJ, Velez G, Kanchustambham VL, Shankar V, Evans LP, Sengillo JD, et al. Metabolite therapy guided by liquid biopsy proteomics delays retinal neurodegeneration. *EBioMedicine.* (2020) 52:102636. doi: 10.1016/j.ebiom.2020.102636
25. Streijger F, Plunet WT, Lee JH, Liu J, Lam CK, Park S, et al. Ketogenic diet improves forelimb motor function after spinal cord injury in rodents. *PLoS ONE.* (2013) 8:e78765. doi: 10.1371/journal.pone.0078765
26. Shaafi S, Sharifi-Bonab M, Ghaemian N, Mokhtarkhani M, Akbari H. Early motor-behavioral outcome of ischemic stroke with ketogenic diet preconditioning: interventional animal study. *J Stroke Cerebrovasc Dis.* (2019) 28:1032–9. doi: 10.1016/j.jstrokecerebrovasdis.2018.12.024
27. Tai KK, Nguyen N, Pham L, Truong DD. Ketogenic diet prevents cardiac arrest-induced cerebral ischemic neurodegeneration. *J Neural Transm (Vienna).* (2008) 115:1011–7. doi: 10.1007/s00702-008-0050-7
28. Suzuki M, Suzuki M, Kitamura Y, Mori S, Sato K, Dohi S, et al. Beta-hydroxybutyrate, a cerebral function improving agent, protects rat brain against ischemic damage caused by permanent and transient focal cerebral ischemia. *Jpn J Pharmacol.* (2002) 89:36–43. doi: 10.1254/jjp.89.36
29. Krikorian R, Shidler MD, Dangelo K, Couch SC, Benoit SC, Clegg DJ. Dietary ketosis enhances memory in mild cognitive impairment. *Neurobiol Aging.* (2012) 33:425 e19–27. doi: 10.1016/j.neurobiolaging.2010.10.006
30. Taylor MK, Sullivan DK, Mahnken JD, Burns JM, Swerdlow RH. Feasibility and efficacy data from a ketogenic diet intervention in Alzheimer's disease. *Alzheimers Dement.* (2018) 4:28–36. doi: 10.1016/j.trci.2017.11.002
31. Phillips MCL, Deprez LM, Mortimer GMN, Murtagh DKJ, McCoy S, Mylchreest R, et al. Randomized crossover trial of a modified ketogenic diet in Alzheimer's disease. *Alzheimers Res Ther.* (2021) 13:51. doi: 10.1186/s13195-021-00783-x
32. Nabbout R, Copioli C, Chipaux M, Chemaly N, Desguerre I, Dulac O, et al. Ketogenic diet also benefits Dravet syndrome patients receiving stiripentol: a prospective pilot study. *Epilepsia.* (2011) 52:e54–7. doi: 10.1111/j.1528-1167.2011.03107.x
33. Wu Q, Wang H, Fan YY, Zhang JM, Liu XY, Fang XY, et al. Ketogenic diet effects on 52 children with pharmacoresistant epileptic encephalopathy: a clinical prospective study. *Brain Behav.* (2018) 8:e00973. doi: 10.1002/brb3.973
34. Brenton JN, Banwell B, Bergqvist AGC, Lehner-Gulotta D, Gampper L, Leytham E, et al. Pilot study of a ketogenic diet in relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflamm.* (2019) 6:e565. doi: 10.1212/NXI.0000000000000565
35. Choi IY, Piccio L, Childress P, Bollman B, Ghosh A, Brandhorst S, et al. A diet mimicking fasting promotes regeneration and reduces autoimmunity and multiple sclerosis symptoms. *Cell Rep.* (2016) 15:2136–46. doi: 10.1016/j.celrep.2016.05.009
36. Krikorian R, Shidler MD, Summer SS, Sullivan PG, Duker AP, Isaacson RS, et al. Nutritional ketosis for mild cognitive impairment in Parkinson's disease: a controlled pilot trial. *Clin Park Relat Disord.* (2019) 1:41–7. doi: 10.1016/j.prdoa.2019.07.006
37. Masino SA Li T, Theofilas P, Sandau US, Ruskin DN, Fredholm BB, et al. A ketogenic diet suppresses seizures in mice through adenosine A(1) receptors. *J Clin Invest.* (2011) 121:2679–83. doi: 10.1172/JCI57813
38. Pinto A, Bonucci A, Maggi E, Corsi M, Businaro R. Anti-oxidant and anti-inflammatory activity of ketogenic diet: new perspectives for neuroprotection in Alzheimer's disease. *Antioxidants.* (2018) 7:63. doi: 10.3390/antiox7050063
39. Appelberg KS, Hovda DA, Prins ML. The effects of a ketogenic diet on behavioral outcome after controlled cortical impact injury in the juvenile and adult rat. *J Neurotrauma.* (2009) 26:497–506. doi: 10.1089/neu.2008.0664
40. Harun-Or-Rashid M. and Inman DM, Reduced AMPK activation and increased HCAR activation drive anti-inflammatory response and neuroprotection in glaucoma. *J Neuroinflammation.* (2018) 15:313. doi: 10.1186/s12974-018-1346-7
41. Nunes AK, Raposo C, Rocha SW, Barbosa KP, Luna RL, da Cruz-Hofling MA, et al. Involvement of AMPK, IKbetaalpha-NFkappaB and eNOS in the sildenafil anti-inflammatory mechanism in a demyelination model. *Brain Res.* (2015) 1627:119–33. doi: 10.1016/j.brainres.2015.09.008
42. Kelley N, Jeltema D, Duan Y, and He Y, The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. *Int J Mol Sci.* (2019) 20:3328. doi: 10.3390/ijms20133328
43. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, et al. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. *Nat Med.* (2015) 21:263–9. doi: 10.1038/nm.3804
44. Taggart AK, Kero J, Gan X, Cai TQ, Cheng K, Ippolito M, et al. (D)-beta-Hydroxybutyrate inhibits adipocyte lipolysis via the nicotinic acid receptor PUMA-G. *J Biol Chem.* (2005) 280:26649–52. doi: 10.1074/jbc.C500213200
45. Rahman M, Muhammad S, Khan MA, Chen H, Ridder DA, Muller-Fielitz H, et al. The beta-hydroxybutyrate receptor HCA2 activates a

neuroprotective subset of macrophages. *Nat Commun.* (2014) 5:3944. doi: 10.1038/ncomms4944
46. Lin C, Chao H, Li Z, Xu X, Liu Y, Bao Z, et al. Omega-3 fatty acids regulate NLRP3 inflammasome activation and prevent behavior deficits after traumatic brain injury. *Exp Neurol.* (2017) 290:115–22. doi: 10.1016/j.expneurol.2017.01.005
47. Huang C, Wang P, Xu X, Zhang Y, Gong Y, Hu W, et al. The ketone body metabolite beta-hydroxybutyrate induces an antidepression-associated ramification of microglia via HDACs inhibition-triggered Akt-small RhoGTPase activation. *Glia.* (2018) 66:256–78. doi: 10.1002/glia.23241
48. Liu TF, Brown CM, El Gazzar M, McPhail L, Millet P, Rao A, et al. Fueling the flame: bioenergy couples metabolism and inflammation. *J Leukoc Biol.* (2012) 92:499–507. doi: 10.1189/jlb.0212078
49. Shen Y, Kapfhamer D, Minnella AM, Kim JE, Won SJ, Chen Y, et al. Bioenergetic state regulates innate inflammatory responses through the transcriptional co-repressor CtBP. *Nat Commun.* (2017) 8:624. doi: 10.1038/s41467-017-00707-0
50. Jain SK, Kannan K, Lim G, McVie R, Bocchini Jr JA. Hyperketonemia increases tumor necrosis factor-alpha secretion in cultured U937 monocytes and Type 1 diabetic patients and is apparently mediated by oxidative stress and cAMP deficiency. *Diabetes.* (2002) 51:2287–93. doi: 10.2337/diabetes.51.7.2287
51. Shi X, Li X, Li D, Li Y, Song Y, Deng Q, et al. beta-Hydroxybutyrate activates the NF-kappaB signaling pathway to promote the expression of pro-inflammatory factors in calf hepatocytes. *Cell Physiol Biochem.* (2014) 33:920–32. doi: 10.1159/000358664
52. Espinos C, Galindo MI, Garcia-Gimeno MA, Ibanez-Cabellos JS, Martinez-Rubio D, Millan JM, et al. Oxidative stress, a crossroad between rare diseases and neurodegeneration. *Antioxidants.* (2020) 9:313. doi: 10.3390/antiox9040313
53. Greco T, Glenn TC, Hovda DA, Prins ML. Ketogenic diet decreases oxidative stress and improves mitochondrial respiratory complex activity. *J Cereb Blood Flow Metab.* (2016) 36:1603–13. doi: 10.1177/0271678X15610584
54. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science.* (2013) 339:211–4. doi: 10.1126/science.1227166
55. Haces ML, Hernandez-Fonseca K, Medina-Campos ON, Montiel T, Pedraza-Chaverri J, Massieu L. Antioxidant capacity contributes to protection of ketone bodies against oxidative damage induced during hypoglycemic conditions. *Exp Neurol.* (2008) 211:85–96. doi: 10.1016/j.expneurol.2007.12.029
56. Ziegler DR, Ribeiro LC, Hagenn M, Siqueira IR, Araujo E, Torres IL, et al. Ketogenic diet increases glutathione peroxidase activity in rat hippocampus. *Neurochem Res.* (2003) 28:1793–7. doi: 10.1023/A:1026107405399
57. Sullivan PG, Rippy NA, Dorenbos K, Concepcion RC, Agarwal AK, Rho JM. The ketogenic diet increases mitochondrial uncoupling protein levels and activity. *Ann Neurol.* (2004) 55:576–80. doi: 10.1002/ana.20062
58. Deng M, Wang D, He S, Xu R, Xie Y. SIRT1 confers protection against ischemia/reperfusion injury in cardiomyocytes via regulation of uncoupling protein 2 expression. *Mol Med Rep.* (2017) 16:7098–104. doi: 10.3892/mmr.2017.7452
59. Scheibye-Knudsen M, Mitchell SJ, Fang EF, Iyama T, Ward T, Wang J, et al. A high-fat diet and NAD(+) activate Sirt1 to rescue premature aging in cockayne syndrome. *Cell Metab.* (2014) 20:840–55. doi: 10.1016/j.cmet.2014.10.005
60. Milder JB, Liang LP, Patel M. Acute oxidative stress and systemic Nrf2 activation by the ketogenic diet. *Neurobiol Dis.* (2010) 40:238–44. doi: 10.1016/j.nbd.2010.05.030
61. Xie G, Zhou Q, Qiu CZ Dai WK, Wang HP Li YH, et al. Ketogenic diet poses a significant effect on imbalanced gut microbiota in infants with refractory epilepsy. *World J Gastroenterol.* (2017) 23:6164–71. doi: 10.3748/wjg.v23.i33.6164
62. Swidsinski A, Dorffel Y, Loening-Baucke V, Gille C, Goktas O, Reisshauer A, et al. Reduced Mass and Diversity of the Colonic Microbiome in Patients with Multiple Sclerosis and Their Improvement with Ketogenic Diet. *Front Microbiol.* (2017) 8:1141. doi: 10.3389/fmicb.2017.01141
63. Zhu S, Jiang Y, Xu K, Cui M, Ye W, Zhao G, et al. The progress of gut microbiome research related to brain disorders. *J Neuroinflammation.* (2020) 17:25. doi: 10.1186/s12974-020-1705-z
64. Vila-Brau A, De Sousa-Coelho AL, Mayordomo C, Haro D, Marrero PF. Human HMGCS2 regulates mitochondrial fatty acid oxidation and FGF21 expression in HepG2 cell line. *J Biol Chem.* (2011) 286:20423–30. doi: 10.1074/jbc.M111.235044
65. Xie Z, Zhang D, Chung D, Tang Z, Huang H, Dai L, et al. Metabolic regulation of gene expression by histone lysine beta-hydroxybutyrylation. *Mol Cell.* (2016) 62:194–206. doi: 10.1016/j.molcel.2016.03.036
66. Huang H, Zhang D, Weng Y, Delaney K, Tang Z, Yan C, et al., The regulatory enzymes and protein substrates for the lysine beta-hydroxybutyrylation pathway. *Sci Adv.* (2021) 7:abe2771. doi: 10.1126/sciadv.abe2771
67. Schoeler NE, Simpson Z, Zhou R, Pujar S, Eltze C, Cross JH. Dietary management of children with super-refractory status epilepticus: a systematic review and experience in a single UK tertiary centre. *Front Neurol.* (2021) 12:643105. doi: 10.3389/fneur.2021.643105
68. Vining EP. Long-term health consequences of epilepsy diet treatments. *Epilepsia.* (2008) 49 Suppl 8:27–9. doi: 10.1111/j.1528-1167.2008.01828.x
69. Kang HC, Chung DE, Kim DW, Kim HD. Early- and late-onset complications of the ketogenic diet for intractable epilepsy. *Epilepsia.* (2004) 45:1116–23. doi: 10.1111/j.0013-9580.2004.10004.x
70. Kossoff EH, Zupec-Kania BA, Auvin S, Ballaban-Gil KR, Christina Bergqvist AG, Blackford R, et al. Optimal clinical management of children receiving dietary therapies for epilepsy: updated recommendations of the International Ketogenic Diet Study Group. *Epilepsia Open.* (2018) 3:175–92. doi: 10.1002/epi4.12225
71. Wang J, Jiang L, Cheng M. A girl with protein-losing enteropathy during a ketogenic diet: a case report. *BMC Pediatr.* (2020) 20:102. doi: 10.1186/s12887-020-1991-8
72. Merlotti D, Cosso R, Eller-Vainicher C, Vescini F, Chiodini I, Gennari L, et al., Energy metabolism and ketogenic diets: what about the skeletal health? a narrative review and a prospective vision for planning clinical trials on this issue. *Int J Mol Sci.* (2021) 22:435. doi: 10.3390/ijms22010435
73. Draaisma JMT, Hampsink BM, Janssen M, van Houdt NBM, Linders E, Willemsen MA. The ketogenic diet and its effect on bone mineral density: a retrospective observational cohort study. *Neuropediatrics.* (2019) 50:353–8. doi: 10.1055/s-0039-1693059
74. Patel A, Pyzik PL, Turner Z, Rubenstein JE, Kossoff EH. Long-term outcomes of children treated with the ketogenic diet in the past. *Epilepsia.* (2010) 51:1277–82. doi: 10.1111/j.1528-1167.2009.02488.x

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Efficacy and Safety of Ketone Supplementation or Ketogenic Diets for Alzheimer's Disease: A Mini Review

Matthieu Lilamand[1,2,3]*, *François Mouton-Liger*[1,4], *Emmanuelle Di Valentin*[2], *Marta Sànchez Ortiz*[1] and *Claire Paquet*[1,3,4]

[1] Groupe Hospitalier Lariboisiere FW Saint-Louis, Cognitive Neurology Center Paris Nord Ile de France, AP-HP.Nord, Paris, France, [2] Department of Geriatrics, Bichat and Bretonneau University Hospitals, AP-HP.Nord, Paris, France, [3] INSERM UMR-S1144, Université de Paris, Paris, France, [4] Department of Histology and Biology of Aging, Groupe Hospitalier Lariboisiere FW Saint Louis, AP-HP.Nord, Paris, France

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Gorazd Drevenšek,
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Dariusz Włodarek,
Warsaw University of Life Sciences, Poland

***Correspondence:**
Matthieu Lilamand
mlilamand@hotmail.fr

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Alzheimer's disease (AD) is the most frequent age-related neurodegenerative disorder, with no curative treatment available so far. Alongside the brain deposition of β-amyloid peptide and hyperphosphorylated tau, neuroinflammation triggered by the innate immune response in the central nervous system, plays a central role in the pathogenesis of AD. Glucose usually represents the main fuel for the brain. Glucose metabolism has been related to neuroinflammation, but also with AD lesions. Hyperglycemia promotes oxidative stress and neurodegeneration. Insulinoresistance (e.g., in type 2 diabetes) or low IGF-1 levels are associated with increased β-amyloid production. However, in the absence of glucose, the brain may use another fuel: ketone bodies (KB) produced by oxidation of fatty acids. Over the last decade, ketogenic interventions i.e., ketogenic diets (KD) with very low carbohydrate intake or ketogenic supplementation (KS) based on medium-chain triglycerides (MCT) consumption, have been studied in AD animal models, as well as in AD patients. These interventional studies reported interesting clinical improvements in animals and decrease in neuroinflammation, β-amyloid and tau accumulation. In clinical studies, KS and KD were associated with better cognition, but also improved brain metabolism and AD biomarkers. This review summarizes the available evidence regarding KS/KD as therapeutic options for individuals with AD. We also discuss the current issues and potential adverse effects associated with these nutritional interventions. Finally, we propose an overview of ongoing and future registered trials in this promising field.

Keywords: Alzheimer's disease, nutrition, ketone bodies, ketogenic diet, medium chain triglycerides

INTRODUCTION

Alzheimer's disease (AD) is the most frequent age-related neurodegenerative disorder, characterized by brain abnormal deposition of β-amyloid peptide (Aβ) and phosphorylated tau (pTau) accumulation. These protein deposits cause neuronal death, cognitive decline and behavior disorders (1). Industrial research has focused on disease-modifying therapeutics (mostly anti-amyloid agents), which have failed so far to significantly change the course of the disease (2). Nutrition has been suggested as a key modifiable factor to fight against protein-driven cognitive symptoms or to postpone the onset of cognitive impairment (3). Aβ deposition and tau lesions are accompanied by activated microglia and astrocytes (4). These cells release proinflammatory cytokines and chemokines causing chronic neuroinflammation. In turn, neuroinflammation promotes neurodegeneration and Aβ production. Hyperglycemia is also associated with neuroinflammation, leading to oxidative stress which could trigger the amyloid cascade of AD. Several other pathophysiological links exist between AD and glucose metabolism. In cognitively normal older adults, a high-glycemic diet was previously shown to be associated with cerebral Aβ burden (5). Very recently, in a longitudinal analysis over one year, the same research team found greater brain Aβ accumulation in participants with higher daily intake of sugar or carbohydrates (6). AD my also result from insulin resistance, which affects insulin signaling and favors the deposition of brain Aβ and pTau (7). Besides, type 2 diabetes which is an established risk factor for AD, shares in common the deleterious consequences of the expression of *apolipoprotein E allele 4* (*ApoE4*) (8). Carrying the *ApoE4* allele represents the main genetic risk factor for sporadic AD, indicating a likely common pathophysiological background with diabetes. As demonstrated by fluorodeoxyglucose positron emission tomography (FDG-PET), abnormal brain glucose metabolism in the temporal and parietal lobes occurs from the earliest stages, in AD animal models and AD patients, but also in asymptomatic individuals at risk of AD. Interestingly, these hypometabolic brain areas demonstrate impaired glucose utilization, whereas they may still uptake ketone bodies efficiently (KB) (9).

These findings have shed the light on the potential therapeutic use of KB in the field of AD therapeutic research. Two different strategies have been considered: first, the intake of medium-chain triglycerides (MCT) resulting in the production of KB after beta-oxidation in the liver. Thus, KB cross the blood brain barrier and fuel the brain. Second, when glucose is not readily available (e.g., starvation), a metabolic switch occurs in favor of KB usually released by the liver. The diets specifically designed for KB production are called ketogenic diets (KD) (10). The core characteristics of the KD are the association of a high amount of fat, with low carbohydrate intake: usually a macronutrient ratio of fat to protein and carbohydrate combined equal to 3 or 4:1. When ketosis is achieved, the main fuel used by the body shifts from glucose to favor KB, an adaptation that also occurs with extended fasting.

In this short review, we examined the current very recent (since May 2019) available evidence regarding KS/KD as therapeutic options for AD. As these nutritional interventions may raise major concerns in older adults or frail individuals suffering from AD, we also discuss the controversies and potential adverse effects due to KS or KD. Finally, we propose an overview of ongoing and future registered trials, in this promising field.

RECENT FINDINGS AND RESEARCH GAPS

As highlighted in previously published reviews in this field (11, 12), KD or KS in animal models have repeatedly shown improvements of cognitive performance, motor function or behavior. These clinical benefits were accompanied with decreased inflammatory markers, but also with alleviated brain Aβ load or tau (neurofibrillary tangles) burden. In humans, a growing body of evidence has also suggested the potential interest of KS or KD to slow down cognitive decline or to enhance cognition. Several studies have pointed out the change in brain metabolism assessed by PET imaging, after KS.

Since 2019, we found ten studies, in humans, aiming at improving cognitive performance or biomarkers of AD (See **Table 1**) (13–22). Among them, seven used KS vs. placebo and three KD vs. control diet. Of note, the most valuable clinical investigation regarding KS included 413 older participants (mean age = 77) with mild to moderate AD, followed-up over 6 months (15). The intervention consisted in 20 g of MCT supplementation, a standard daily dose to produce KB. Finally, Henderson et al. did not observe significant improvements regarding their two main endpoints i.e., cognitive performance and clinician's impression of change. On the other hand, in older adults with mild cognitive impairment (MCI) Fortier et al. showed that 30 g of MCT supplementation over a 6-month follow, significantly improved three major cognitive functions: episodic memory, executive function and language (19). Three studies in healthy participants used KS over very short intervention windows (0–5weeks) and reported improved cognitive functions and/or brain metabolism (14, 16, 22).

Three recent studies assessed the effect of a KD on cognition or AD biomarkers (13, 17, 18). In older adults with mild AD, Philipps et al. did not show any significant cognitive gain after 12 weeks of KD vs. CD. However, they reported significant functional improvement in complex activities of daily living, which was considered as clinically meaningful (13). The quality of life of participants under KD also raised of 3 points on the QoL-AD scale. Nagpal et al. and Neth et al. reported significant improvements in memory, brain metabolism and cerebrospinal fluid (CSF) amyloid and tau biomarkers, after only 6 weeks of KD, in adults without dementia (17, 18). The absence of long-term follow-up in these three studies prevented from giving insight into the persistence of cognitive gain (or perception of cognitive improvement) after discontinuation of the nutritional intervention. Adhesion to these nutritional interventions must be carefully examined, since meaningful changes in body

TABLE 1 | Studies of ketone supplementation or ketogenic diet with regards to cognition or AD biomarkers.

Study (ref)	Population	Mean age (SD)	Intervention	N	Follow-up (weeks)	Outcomes	Positive outcomes (intervention group)	Nutritional changes	Side effects
Philipps et al. (13)	Mild AD	70	RCT crossover, KD vs. CD	26	12 × 2	Cognition, IADL, QoL	Improved IADL performance and QoL	Weight loss	Mild. One (4%) withdrawal due to KD
Yomogida et al. (14)	Healthy adults	66	RCT crossover, MCT (50g vs. placebo)	20	–	Cognition, fMRI	Improved executive function and brain metabolism	Unassessed	Unassessed
Henderson et al. (15)	Mild to moderate AD	77	RCT, MCT 20g vs. placebo	413	26	Cognition, clinician's impression of change (ADCS-CGIC)	No cognitive change	No nutritional disorder reported	Minor digestive side effects
O'Neill et al. (16)	Healthy adults	60	RCT crossover, MCT (30 g) vs. placebo	80	2	Cognition, adverse effects	No cognitive change	Unassessed	Minor digestive side effects
Nagpal et al. (17)	Adults with MCI/CN	65	RCT crossover, KD vs. CD	17	6 × 2	CSF biomarkers, microbiome	CSF biomarkers improvement	Unassessed	Unassessed
Neth et al. (18)	Adults with SCI/MCI	64	RCT, KD vs. CD	20	6 × 2	Cognition, PET imaging, CSF biomarkers	Improved memory, brain metabolism and CSF biomarkers	Weight loss	None
Fortier et al. (19)	Adults with MCI	72	RCT MCT (30 g) vs. placebo	122	26	cognition	Improved cognition memory, executive function, language	Stable weight	Minor digestive adverse effects
Xu et al. (20)	Mild moderate AD	75	RCT crossover, MCT (17 g)-leucin vs. placebo	46	4 × 2	cognition	Improved cognition	Unassessed	None
Abe et al. (21)	Nursing home residents	85	RCT, MCT (6 g)-leucin vs. placebo	64	13	cognition	Improved cognition	Unassessed	None
Ashton et al. (22)	Healthy students	20	RCT, MCT (12 or 18 g) vs. placebo	30	5	cognition	Improved executive function/working memory	Unassessed	Minor digestive side effects

AD, Alzheimer's disease; ADCS-CGIC, Alzheimer's Disease Cooperative Study—Clinical Global Impression of Change; CD, control diet; GI, gastro-intestinal; KB, ketone bodies; KD, ketogenic diet; KS, ketone supplementation; MCI, mild cognitive impairment; N, number; PET, positon emission tomography; QoL, quality of life; RCT, randomized controlled trial.

composition may occur. More specifically, the monitoring of side effects is crucial in participants with AD, providing that nutritional status is an established predictor of accelerated cognitive or functional decline. In these three trials based on KD two reported weight loss under KD (Nagpal et al. did not follow weight change); in details: Philipps pointed out a mean weight loss of 2.6 kg in the intervention group after only 3 months. This weight loss represents a common adverse consequence of KD, due to drastic restriction in calorie-dense carbohydrates (23).

Several gaps in the available evidence limit our ability to reach a conclusion regarding those interventions in the field of AD. These recent studies display high heterogeneity regarding age, gender ratios or participants' cognitive status, which all influence the risk of subsequent cognitive decline. The limited follow-up durations (<3 months in all studies but two) as well as the repeated cognitive assessments could have induced a retest effect especially in healthy individuals or in adults with early MCI. In addition, the sustainability and the long-term expected benefits (after several years) associated with these interventions remain unknown, so far. Available evidence came from epileptic children monitoring: overall tolerability of KD and efficacy (regarding seizure incidence) were maintained after 6–12 years of treatment (24). Long-term studies are urgently needed in AD patients to assess the feasibility of such diets.

Another major concern for drawing conclusions regarding AD is the definition of the disease. Henderson et al. who included patients with mild to moderate AD, correctly acknowledged that up to 40% of clinically diagnosed (i.e., without CSF or PET biomarkers) patients with AD are likely to be amyloid-negative, hence misdiagnosed (25), which could have resulted in slower progression on cognitive performance under KS. Only two studies of this review (Nagpal, Neth) assessed the change on biological outcomes i.e., CSF biomarkers. Both showed significant improvement after 6 weeks of KD in adults without dementia. The role of *ApoE4* could mitigate the effect of ketone energy metabolism, given that *ApoE4* differentially impacts insulin resistance, glucose metabolism and microglia (26). Yet, KD or MCT intake appeared to be more efficient in non-*ApoE4* carriers (13, 27, 28).

ONGOING AND FUTURE TRIALS

Among 176 recruiting or active studies related to KD or MCT supplementation, targeting various conditions such as diabetes, epilepsy, glioblastoma, only five were consistent with this topic (see **Table 2**). The first one (NCT05012748) will describe circulating brain concentrations of free fatty acids under KD, using PET MRI. To be noted, this study will include obese patients between 50 and 70, unlikely to be affected by neurodegenerative conditions. The four other registered trials aim at enrolling AD patients (one of them will also include a subgroup of patients with Parkinson's disease). The kind of nutritional interventions are similar with those described above. Only one future study (NCT04701957) will assess the feasibility of KD in MCI due to AD (confirmed with biomarkers), over 1 year of nutritional follow-up.

DISCUSSION

Developing nutritional strategies to maintain cognitive abilities or improve quality of life of patients with AD or other neurodegenerative disorders has always been a research priority (1, 3). However, numerous nutritional interventions have failed to demonstrate significant effects on cognition (29–31). The Mediterranean diet represents an interesting preventive intervention but its efficacy is limited once diagnosed with AD (32). Saturated fat intake, which typically increases on KD, may also increase AD risk. In the Chicago Health and Aging Project, intake of high saturated fat was associated to a two- to three-fold increased risk of incident AD (33). However, 10 years later, the same authors acknowledged that the epidemiologic literature was seemingly inconsistent on this topic. For example, in the latter study, a decreased risk of AD was also related to consumption of monounsaturated and polyunsaturated fatty acids (34). Yet, high fat KD as well as MCT supplementation may positively influence the course of the disease, exerting a role on clinical symptoms (memory loss, executive function) but also on the pathophysiology of AD, in particular on inflammation or brain amyloid deposition (35).

During ketogenesis, the plasma concentration of KB (mainly β-hydroxybutyrate) gradually increases, and over 4 mmol/L, they become a source of energy for the central nervous system. In physiological ketosis, the concentration of KB does not exceed 8 mmol/L, blood pH remains stable, and glycemia, although decreasing, remains at its physiological concentration (36). Thus, KB can offset the absence of glucose act as an alternative fuel for the brain (37). In this context, KS or KD represent promising nutritional strategies to postpone cognitive decline due to AD, or even to *rescue brain energy*. As explained by Cunnane et al. while brain glucose uptake is compromised in MCI, brain ketone uptake and metabolism remain normal, in both MCI and mild-moderate AD (38). β-hydroxybutyrate and acetoacetate also demonstrated numerous benefic effects in neurological conditions, such as inhibit glutamatergic excitatory transmission in epilepsy and may also mitigate neuronal hyperexcitability resulting from AD neuropathology. The impact of these strategies even though symptomatic could be substantial given the number of people affected by AD. Besides, delaying the onset of behavioral symptoms or disability due to AD would lead to significant economic, public health and societal benefits (1).

Another original interest of the KD could be a reduction of carbohydrates consumption, which could trigger the brain amyloid accumulation. High glycemic diet was recently shown to influence change in amyloid levels, over the course of 1 year (6). In all participants, the association between change in amyloid retention and high glycemic diet was apparent in the precuneus. In prodromal AD, this brain area is specifically vulnerable to structural and functional alterations. Nevertheless, in participants with the highest amyloid status, this relationship

TABLE 2 | Ongoing trials examining ketone supplementation or ketogenic diet with cognitive outcomes (source: https://clinicaltrials.gov).

Team	CT identifier	Enrollment date	Design	Population	Number of participants	Follow-up (weeks)	Outcomes
Aarhus University Hospital, Denmark	NCT05012748	Aug 21→Nov 23	RCT crossover, KD vs. CD	Healthy obese patients 50–70 years	12	2 × 3	Circulating concentrations of free fatty acids (PET MRI)
Cognitive Neurology Center, Assistance Publique Hôpitaux de Paris France	NCT04701957	Feb 21→Feb 23	RCT, KD vs. CD	Adults with AD (biomarkers)	70	52	Feasibility, cognition, brain metabolism, IADL, side effects
Clinical and Translational Science Unit, University of Kansas Medical Center and NIH, USA	NCT03860792	Oct 19→Nov 23	RCT, KD + MCT vs. CD	Adults with AD	80	12	Cognition, cerebral concentration of N-Acetylaspartate, blood platelet mitochondrial function
Wake Forest University Health Sciences (USA)	NCT03130036	Jun 15→Feb 23	Non randomized parallel assignment	Healthy/at risk for AD/early AD	60	–	Brain biodistribution of ketone, [11C]Acetoacetate PET imaging
Université de Sherbrooke, Canada	NCT04322461	Mar 20→Dec 21	Non randomized MCT + physical activity	AD and Parkinson's disease	20	8	Cognition

AD, Alzheimer's disease; CD, control diet; KB, ketone bodies; KD, ketogenic diet; KS, ketone supplementation; NCT, National Clinical Trial; PET, positon emission tomography; RCT, randomized controlled trial.

extended into the posterior cingulate and lateral temporal lobe. These brain regions are highly susceptible to amyloid accumulation and metabolic impairment due to AD-related pathology. Thus, as demonstrated in animal models of AD, KD could play a direct protective role against the amyloid lesions of the disease (11).

However, an intriguing effect of KS is the cognitive improvement in healthy adults as well as in cognitively impaired patients, whereas the carbohydrates consumption remains equal. This effect could not be explained by the sole increase of energy supply to the brain, due to the rapid availability of KB following ingestion of MCT, since the doses used by several authors was low (22). The authors explained this gain in cognitive performance because of an increased rate of mitochondrial biogenesis. This phenomenon could be the benefic consequence of increased activation of PPARγ by KB.

Intermittent fasting has already been suggested as a therapeutic option for reducing the onset of neurodegenerative disorders (39). As in the KD, fasting induces a metabolic shift where energy results for KB. However, weight loss due to fasting is likely to accelerate cognitive decline, whereas maintaining stable weight and nutritional status are mandatory for patients with AD (23). Despite sufficient calorie intake, extreme carbohydrate restriction due to KD can profoundly affect diet quality. Low-carbohydrate diets are often low in thiamin, folates, vitamin A, vitamin E, vitamin B6, calcium, magnesium, iron or vitamin K. KD are typically low in fiber (40). In a recent prospective study, infants with drug-resistant epilepsy, who where started on KD (mostly 3:1), the incidence of adverse effects after one month of treatment was very high (91.7%), although none of them discontinued the diet for this reason (41). At diet initiation, the most common side effects were vomiting and hypoglycemia. After 1 month, the children mostly suffered from metabolic acidosis, constipation or vomiting. Non-compliance leading to diet interruption before 2 years of follow-up was reported in 21% of the infants. These findings confirm the long-term feasibility of such diet, in children. To determine whether similar results will be obtained with adults or older adults with AD remains a major challenge. In all cases, the monitoring of all adverse effects as well as the adherence to diet will be mandatory, in future longitudinal trials. Providing sufficient calorie and micronutrients is crucial for preventing the adverse consequences of nutrient deficiency. Therefore, an adequate long-term KD should also include appropriate vitamin supplementation. Finally, Bostock et al. described the potential adverse effects of self-administered KD from users of online forums: increased LDL-cholesterol levels, headache, fatigue, nausea, dizziness, digestive symptoms, and heart palpitations (42). Longer-term effects may lead to anemia, low bone mineral density, nephrolithiasis or cardiomyopathy (36).

CONCLUSION

KD or MCT supplementation may represent promising options to fight against the cognitive symptoms of AD, especially in the prodromal stage of the disease. A growing body of evidence has suggested that KD could enhance cognitive performance and hopefully postpone cognitive decline in AD. Large sampled randomized controlled trials with long-term follow-up and appropriate clinical and biological outcomes (such as CSF or brain metabolism biomarkers) are needed

to address the issues pointed out in this review. Special attention should be given to the onset of adverse effects (malnutrition and weight loss, digestive symptoms) and to the adherence to the diet, especially if these interventions are considered in the long term, as for the treatment of pharmacoresistant epilepsy. Whether cognitive gains would be maintained upon discontinuation of the KD (or KS) remains unknown so far.

The kind of KD suiting best for AD deserves further investigation: very restrictive diets may allow faster ketosis, but increase the risk of poor adherence. Recently, the Mediterranean diet has also been adapted to adhere to KD guidelines, and could represent a promising option to deal with the symptoms of neurodegenerative disorders.

AUTHOR CONTRIBUTIONS

ML and FM-L performed a review of the literature, analyzed and interpreted the data and drafted the manuscript. CP, MSO, and ED critically reviewed the first draft and revised the manuscript accordingly. All authors contributed to the article and approved the final submitted version.

REFERENCES

1. Winblad B, Amouyel P, Andrieu S, Ballard C, Brayne C, Brodaty H, et al. Defeating Alzheimer's disease and other dementias: a priority for European science and society. *Lancet Neurol.* (2016) 15:455–532. doi: 10.1016/S1474-4422(16)00062-4
2. Mahdiabadi S, Momtazmanesh S, Perry G, Rezaei N. Immune modulations and immunotherapies for Alzheimer's disease: a comprehensive review. *Rev Neurosci.* (2021) 2021:000010151520210092. doi: 10.1515/revneuro-2021-0092
3. Botchway BOA, Moore MK, Akinleye FO, Iyer IC, Fang M. Nutrition: review on the possible treatment for Alzheimer's disease. *J Alzheimers Dis.* (2018) 61:867–83. doi: 10.3233/JAD-170874
4. Heppner FL, Ransohoff RM, Becher B. Immune attack: the role of inflammation in Alzheimer disease. *Nat Rev Neurosci.* (2015) 16:358–72. doi: 10.1038/nrn3880
5. Taylor MK, Sullivan DK, Swerdlow RH, Vidoni ED, Morris JK, Mahnken JD, et al. A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults. *Am J Clin Nutr.* (2017) 106:1463–70. doi: 10.3945/ajcn.117.162263
6. Taylor MK, Sullivan DK, Morris JK, Vidoni ED, Honea RA, Mahnken JD, et al. High glycemic diet is related to brain amyloid accumulation over one year in preclinical Alzheimer's disease. *Front Nutr.* (2021) 27:741534. doi: 10.3389/fnut.2021.741534 eCollection 2021.
7. Matsuzaki T, Sasaki K, Tanizaki Y, Hata J, Fujimi K, Matsui Y, et al. Insulin resistance is associated with the pathology of Alzheimer disease: the Hisayama Study. *Neurology.* (2010) 75:764–70. doi: 10.1212/WNL.0b013e3181eee25f
8. Li X, Song D, Leng SX. Link between type 2 diabetes and Alzheimer's disease: from epidemiology to mechanism and treatment. *Clin Interv Aging.* (2015) 10:549–60. eCollection 2015. doi: 10.2147/CIA.S74042
9. Cunnane SC, Courchesne-Loyer A, Vandenberghe C, St-Pierre V, Fortier M, Hennebelle M, et al. Can ketones help rescue brain fuel supply in later life? Implications for cognitive health during aging and the treatment of Alzheimer's disease. *Front Mol Neurosci.* (2016) 9:53. doi: 10.3389/fnmol.2016.00053
10. Vidali S, Aminzadeh S, Lambert B, Rutherford T, Sperl W, Kofler B, et al. Mitochondria: the ketogenic diet—a metabolism-based therapy. *Int J Biochem Cell Biol.* (2015) 63:55–9. doi: 10.1016/j.biocel.2015.01.022
11. Lilamand M, Porte B, Cognat E, Hugon J, Mouton-Liger F, Paquet C. Are ketogenic diets promising for Alzheimer's disease? A translational review. *Alzheimers Res Ther.* (2020) 12:42. doi: 10.1186/s13195-020-00615-4
12. Lilamand M, Mouton-Liger F, Paquet C. Ketogenic diet therapy in Alzheimer's disease: an updated review. *Curr Opin Clin Nutr Metab Care.* (2021) 24:372–8. doi: 10.1097/MCO.0000000000000759
13. Phillips MC, Deprez LM, Mortimer GM, Murtagh DK, McCoy S, Mylchreest R, et al. Randomized crossover trial of a modified ketogenic diet in Alzheimer's disease. *Alzheimers Res Ther.* (2021) 13:51. doi: 10.1186/s13195-021-00783-x
14. Yomogida Y, Matsuo J, Ishida I, Ota M, Nakamura K, Ashida K, et al. An fMRI investigation into the effects of ketogenic medium-chain triglycerides on cognitive function in elderly adults: a pilot study. *Nutrients.* (2021) 13:2134. doi: 10.3390/nu13072134
15. Henderson ST, Morimoto BH, Cummings JL, Farlow MR, Walker J. A placebo-controlled, parallel-group, randomized clinical trial of AC-1204 in mild-to-moderate Alzheimer's disease. *J Alzheimers Dis.* (2020) 75:547–57. doi: 10.3233/JAD-191302
16. O'Neill BV, Dodds CM, Miller SR, Gupta A, Lawrence P, Bullman J, et al. The effects of GSK2981710, amedium-chain triglyceride, on cognitive function in healthy older participants:a randomised, placebo-controlled study. *Hum Psychopharmacol.* (2019) 34:e2694. doi: 10.1002/hup.2694
17. Nagpal R, Neth BJ, Wang S, Craft S, Yadav H. Modified Mediterranean-ketogenic dietmodulates gut microbiome and short-chain fatty acids in association withAlzheimer's disease markers in subjects with mild cognitive impairment. *EBio Med.* (2019) 47:529–42. doi: 10.1016/j.ebiom.2019.08.032
18. Neth BJ, Mintz A, Whitlow C, Jung Y, Solingapuram Sai K, Register TC, et al. Modified ketogenic diet is associated with improved cerebrospinal fluid biomarker profile, cerebral perfusion, and cerebral ketone body uptake in older adults at risk for Alzheimer's disease: a pilotstudy. *Neurobiol. Aging.* (2020) 86:54–63. doi: 10.1016/j.neurobiolaging.2019.09.015
19. Fortier M, Castellano CA, St-Pierre V, Myette-Côté E, Langlois F, Royet M. A ketogenic drink improves cognition in mild cognitive impairment: results of a 6-month RCT. *Alzheimers Dement.* (2020) 17:543–52. doi: 10.1002/alz.12206
20. Xu Q, Zhang Y, Zhang X, Liu L, Zhou B, Mo R, et al. Medium-chain triglycerides improved cognition and lipid metabolomics in mild to moderate Alzheimer's disease patients withAPOE4/: a double-blind, randomized, placebo-controlled crossover trial. *Clin Nutr.* (2020) 39:2092–105. doi: 10.1016/j.clnu.2019.10.017
21. Abe S, Ezaki O, Suzuki M. Medium-chain triglycerides (8:0 and 10:0) increase mini-mental state examination (MMSE) score in frail elderly adults in a randomized controlled trial. *J Nutr.* (2020) 150:2383–2390. doi: 10.1093/jn/nxaa186
22. Ashton JS, Roberts JW, Wakefield CJ, Page RM, MacLaren DPM, Marwood S, et al. The effects of medium chain triglyceride (MCT) supplementation using a C(8):C(10) ratio of 30:70 on cognitive performance in healthy young adults. *Physiol Behav.* (2021) 229:113252. doi: 10.1016/j.physbeh.2020.113252
23. Sanders CL, Wengreen HJ, Schwartz S, Behrens SJ, Corcoran C, Lyketsos CG, et al. Nutritional status is associated with severe dementia and mortality: the Cache County Dementia Progression Study. *Alzheimer Dis Assoc Disord.* (2018) 32:298–304. doi: 10.1097/WAD.0000000000000274
24. Groesbeck DK, Bluml RM, Kossoff EH. Long-term use of the ketogenic diet in the treatment of epilepsy. *Dev Med Child Neurol.* (2006) 48:978–81. doi: 10.1017/S0012162206002143
25. Mouton-Liger F, Wallon D, Troussière AC, Yatimi R, Dumurgier J, Magnin E, et al. Impact of cerebro-spinal fluid biomarkers of Alzheimer's disease in clinical practice: a multicentric study. *J Neurol.* (2014) 1:144–51. doi: 10.1007/s00415-013-7160-3
26. Norwitz NG, Saif N, Ariza IE, Isaacson RS. Precision nutrition for Alzheimer's prevention in ApoE4 carriers. *Nutrients.* (2021) 13:1362. doi: 10.3390/nu13041362
27. Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. *Nutr Metab.* (2009) 6:31. doi: 10.1186/1743-7075-6-31

28. Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, et al. Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiol Aging.* (2004) 25:311–4. doi: 10.1016/S0197-4580(03)00087-3
29. Vellas B, Coley N, Ousset PJ, Berrut G, Dartigues JF, Dubois B, et al. Long-term use of standardised Ginkgo biloba extract for the prevention of Alzheimer's disease (GuidAge): a randomised placebo-controlled trial. *Lancet Neurol.* (2012) 11:851–9. doi: 10.1016/S1474-4422(12)70206-5 Epub 2012 Sep 6.
30. Shah RC, Kamphuis PJ, Leurgans S, Swinkels SH, Sadowsky CH, Bongers A, et al. The S-Connect study: results from a randomized, controlled trial of Souvenaid in mild-to-moderate Alzheimer's disease. *Alzheimers Res Ther.* (2013) 5:59. doi: 10.1186/alzrt224
31. Andrieu S, Guyonnet S, Coley N, Cantet C, Bonnefoy M, Bordes S, et al. Effect of long-term omega 3 polyunsaturated fatty acid supplementation with or without multidomain intervention on cognitive function in elderly adults with memory complaints (MAPT): a randomised, placebo-controlled trial. *Lancet Neurol.* (2017) 16:377–89. doi: 10.1016/S1474-4422(17)30040-6
32. Sofi F, Cesari F, Abbate R, Gensini GF, Casini A. Adherence to Mediterranean diet and health status: meta-analysis. *BMJ.* (2008) 337:a1344. doi: 10.1136/bmj.a1344
33. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, et al. Dietary fats and the risk of incident Alzheimer disease. *Arch Neurol.* (2003) 60:194–200. doi: 10.1001/archneur.60.2.194
34. Morris MC, Tangney CC. Dietary fat composition and dementia risk. *Neurobiol Aging.* (2014) 35 Suppl 2:S59–64. doi: 10.1016/j.neurobiolaging.2014.03.038
35. Rusek M, Pluta R, Ułamek-Kozioł M, Czuczwar SJ. Ketogenic diet in Alzheimer's disease. *Int J Mol Sci.* (2019) 20:3892. doi: 10.3390/ijms20163892
36. Włodarek D. Role of ketogenic diets in neurodegenerative diseases (Alzheimer's Disease and Parkinson's Disease). *Nutrients.* (2019) 11:169. doi: 10.3390/nu11010169
37. Cunnane SC, Trushina E, Morland C, Prigione A, Casadesus G, Andrews ZB, et al. Brain energy rescue: an emerging therapeutic concept for neurodegenerative disorders of ageing. *Nat Rev Drug Discov.* (2020) 19:609–33. doi: 10.1038/s41573-020-0072-x
38. Cunnane SC, Sieber CC, Swerdlow RH, Cruz-Jentoft AJ. Mild cognitive impairment: when nutrition helps brain energy rescue-a report from the EuGMS 2020 Congress. *Eur Geriatr Med.* (2021) 12:1285–92. doi: 10.1007/s41999-021-00534-z
39. de Cabo R, Mattson MP. Effects of intermittent fasting on health, aging, and disease. *N Engl J Med.* (2019) 26:2541–51. doi: 10.1056/NEJMra1905136
40. Crosby L, Davis B, Joshi S, Jardine M, Paul J, Neola M, et al. Ketogenic diets and chronic disease: weighing the benefits against the risks. *Front Nutr.* (2021) 8:702802. eCollection 2021. doi: 10.3389/fnut.2021.702802
41. Armeno M, Verini A, Caballero E, Cresta A, Valenzuela GR, Caraballo R. Long-term effectiveness and adverse effects of ketogenic diet therapy in infants with drug-resistant epilepsy treated at a single center in Argentina. Epilepsy Res. (2021) 178:106793. doi: 10.1016/j.eplepsyres.2021.106793
42. Bostock ECS, Kirkby KC, Taylor BV, Hawrelak JA. Consumer reports of "keto flu" associated with the ketogenic diet. *Front Nutr.* (2020) 7:20. doi: 10.3389/fnut.2020.575713

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BRIEF RESEARCH REPORT
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Association of Vitamin K Insufficiency With Cognitive Dysfunction in Community-Dwelling Older Adults

*Kotaro Azuma[1], Yosuke Osuka[2], Narumi Kojima[2], Hiroyuki Sasai[2], Hunkyung Kim[2] and Satoshi Inoue[1]**

[1] Department of Systems Aging Science and Medicine, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan, [2] Research Team for Promoting Independence and Mental Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

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***Correspondence:**
Satoshi Inoue
sinoue@tmig.or.jp

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Vitamin K is a fat-soluble vitamin shown to be associated with several age-related diseases. Although a small number of epidemiological studies described the relationship between vitamin K status and cognitive impairment, vitamin K status was estimated by relatively special methods in previous reports. Here, we demonstrated the association of the concentration of undercarboxylated osteocalcin (ucOC) in serum, which is a biomarker for vitamin K insufficiency, with cognitive function in a cross-sectional study. A total of 800 community-dwelling older adults (mean age = 75.9) were invited to geriatric health examination, including a Mini-Mental State Examination (MMSE) and a blood test. By using binary logistic regression analysis, the risk of cognitive impairment equivalent or below the mild cognitive impairment level for each tertile of ucOC was examined, with the lowest tertile as the reference. We found a significant association of impaired cognitive function and concentration of ucOC in the highest tertile of ucOC, with the odds ratio of 1.65 (95% CI, 1.06 to 2.59, $P = 0.028$). When the analysis was repeated with each domain of MMSE, the highest tertile of ucOC was associated with impaired orientation, calculation, and language. As far as we know, this is the first report on the significant association of single ucOC measurement and cognitive impairment. Our analysis also suggests that vitamin K insufficiency could be associated with selected categories of cognitive function. Since the single measurement of ucOC in serum is a simple and widely available method for vitamin K evaluation, it could be useful as a biomarker of neurodegenerative diseases affecting the cognitive functions.

Keywords: Vitamin K, undercarboxylated osteocalcin (ucOC), mini-mental state examination (MMSE), mild cognitive impairment (MCI), orientation, calculation, language

INTRODUCTION

Vitamin K is a fat-soluble vitamin originally discovered as an essential factor in blood coagulation. Naturally existing vitamin K compounds are classified into two forms, namely vitamin K1 (phylloquinone) and K2 (menaquinone). Vitamin K1 is abundant in vegetables (1), while vitamin K2 is generated by bacteria and is contained in fermented foods (2, 3). For example, Japanese fermented soybeans (called "natto") contain high concentrations of vitamin K2 (2). It is also known that the intestinal microbiome is another important source of vitamin K2 production (4).

Functions of vitamin K can be explained by facilitating γ-carboxylation of some proteins, including coagulation factors, osteocalcin (OC), and matrix Gla protein (MGP), which are catalyzed by γ-glutamyl carboxylase (GGCX) (5). To date, other modes of vitamin K actions have been discovered, such as regulation of transcription by activating steroid and xenobiotic receptor (SXR) (6), physical association with 17β-Hydroxysteroid dehydrogenase type 4 (17β-HSD4) (7), and covalent modification of Bcl-2 antagonist killer 1 (Bak) (8).

Of note, several epidemiological studies revealed that vitamin K status is associated with aging-related diseases, including osteoporosis (9) and osteoarthritis (10). Meanwhile, there is a small number of epidemiological studies on the relationship between vitamin K status and cognitive impairment (11–17). In these epidemiological studies, vitamin K status was estimated by food-frequency questionnaires, measurement of dephosphorylated uncarboxylated MGP (dp-ucMGP), or direct measurement of vitamin K concentration by High Performance Liquid Chromatography (HPLC), which will require relatively special methods and/or facilities and may limit translation of these findings into clinical settings.

Osteocalcin (OC) is a protein produced and carboxylated by GGCX in the presence of vitamin K in osteoblastic cells. The concentration of undercarboxylated form of OC (ucOC) in serum is reported to be positively correlated with a fracture risk (18). Furthermore, a high ucOC concentration is used as a biomarker for vitamin K insufficiency for the bone tissue, and as the indicator of vitamin K treatment for osteoporosis in several countries including Japan. The measurement of ucOC is commercially available in relatively low cost compared with other methods, estimating vitamin K sufficiency. Here, we report a cross-sectional study in which the association of ucOC concentration with cognitive function is examined.

METHODS

Study Design

In 2020, a total of 800 people (mean age = 75.9) were recruited as a follow-up study of the baseline examinations conducted from 2017 to 2019 by mailing the community-dwelling older adults, who were randomly selected from the Basic Resident Register, residing in the Itabashi ward of metropolitan Tokyo. They were invited to a comprehensive geriatric health examination including a Mini Mental State Examination (MMSE) and a blood test. Serum ucOC concentration was measured by BML Inc. (Tokyo, Japan). This study was approved by the institutional ethical committee of Tokyo Metropolitan Institute of Gerontology (approval No.: H18-17, R1-20). All the participants provided written informed consent.

TABLE 1 | Characteristics of the study populations according to the levels of ucOC[a].

Characteristics	ucOC			*P* value [b]	Total
	T1	T2	T3		
ucOC (ng/mL)	< 2.37	2.37–4.00	> 4.00		
N	267	267	266		800
Age (year)	76.6 ± 4.7	75.0 ± 4.5	76.1 ± 5.3	**<0.001**	75.9 ± 4.9
Female	217 (81.3%)	253 (94.8%)	240 (90.2%)	**<0.001**	710 (88.8%)
Education (year)	12.7 ± 2.3	12.5 ± 2.3	12.9 ± 2.2	0.195	12.7 ± 2.3
Hypertension	111 (41.5%)	108 (40.4%)	122 (45.9%)	0.411	341 (42.6%)
Stroke	3 (1.1%)	6 (2.2%)	11 (4.1%)	0.079	20 (2.5%)
Heart Disease	40 (15.0%)	32 (12.0%)	39 (14.7%)	0.558	111 (13.9%)
Diabetes	50 (18.7%)	24 (9.0%)	29 (10.9%)	**0.002**	103 (12.9%)
Dyslipidemia	136 (50.9%)	116 (43.4%)	128 (48.1%)	0.202	380 (47.5%)
Osteoporosis	109 (40.8%)	55 (20.6%)	55 (20.7%)	**<0.001**	219 (27.4%)
Smokers	78 (29.2%)	54 (20.2%)	59 (22.2%)	**0.038**	191 (23.9%)
BMI (kg/m^2)	23.1 ± 3.3	23.0 ± 3.1	23.5 ± 3.5	0.175	23.2 ± 3.3
MMSE score	28.1 ± 2.4	28.1 ± 2.4	28.4 ± 1.9	0.254	28.2 ± 2.2

[a] *Values are means ± SDs for continuous variables or frequency (%) for categorical variables.*

[b] *P values are for one-way ANOVA (continuous variables) and chi square test (categorical variables).*

P < 0.05 are expressed in bold.

ucOC, undercarboxylated osteocalcin; BMI, body mass index; and MMSE, Mini-Mental State Examination.

Statistical Analyses

The levels of serum ucOC were divided into tertiles (T1, T2, and T3). Descriptive statistics were used to compare the characteristics of the patients according to each tertile of ucOC using one-way ANOVA for continuous variables and chi square test for categorical variables. Binary logistic regression analysis was performed to evaluate the association of cognitive impairment (total MMSE score < 27 or 28) (19, 20) with the following variables. Age (continuous), education (binary), sex (binary), hypertension (binary), stroke (binary), heart disease (binary), diabetes (binary), dyslipidemia (binary), osteoporosis (binary), smoking status (binary), body mass index (categorical; < 18.5, 18.5-25, 25–30, and 30+), and ucOC (categorical; T1, T2, T3). Binary logistic regression analysis was repeated to evaluate association of each category of MMSE (orientation, registration, calculation, recall, and language) with the same variables. The cut-off value of each category of MMSE was selected to yield the lowest *P* value in the analysis of the highest tertile of ucOC with the cognitive impairment. The IBM SPSS Statistics version 25 software (IBM Corporation, Armonk, NY, USA) was used for all statistical analyses.

RESULTS

The characteristics of the study population according to the levels of serum ucOC divided into tertiles (T1, T2, and T3) are

TABLE 2 | Association of characteristics with cognitive impairment.

Characteristics	*B*	OR (95% CI)	*P* value
Age	0.153	1.17 (1.12–1.22)	**<0.001**
Sex (women)	0.026	1.03 (0.55–1.90)	0.935
Education (>9 years)	−0.997	0.37 (0.23–0.59)	**<0.001**
Hypertension	0.018	1.02 (0.70–1.48)	0.925
Stroke	−0.120	0.89 (0.30–2.61)	0.827
Heart Disease	−0.258	0.77 (0.46–1.29)	0.323
Diabetes	0.311	1.37 (0.82–2.27	0.230
Dyslipidemia	−0.055	0.95 (0.66–1.37)	0.769
Osteoporosis	0.085	1.09 (0.71–1.66)	0.695
Smokers	0.317	1.73 (0.87–2.16)	0.170
BMI			
< 18.5	−0.115	0.89 (0.40–2.00)	0.781
18.5 - 25		Ref.	
25 - 30	0.254	1.29 (0.86–1.95)	0.225
30 <	−0.532	0.59 (0.17–1.99)	0.393
ucOC			
T1		Ref.	
T2	0.014	1.01 (0.64–1.60)	0.953
T3	0.502	1.65 (1.06–2.59)	**0.028**

B, logistic regression coefficient; BMI, body mass index; CI, confidence interval; MMSE, Mini-Mental State Examination; OR, odds ratio; Ref, reference; ucOC, undercarboxylated osteocalcin.
P < 0.05 are expressed in bold.

TABLE 3 | Associations between ucOC and categories of cognitive performances.

	ucOC		
	T1	T2	T3
ucOC (ng/mL)	< 2.37	2.37–4.00	>4.00
N	267	267	266
MMSE categories			
Orientation (score <9)			
OR (95% CI)	Ref.	0.75 (0.15–3.79)	7.46 (2.05–27.19)
P value		0.730	**0.002**
Registration (score <1)			
OR (95% CI)	Ref.	5.38 (0.58–66.23)	6.17 (0.38–22.81)
P value		0.184	0.133
Calculation (score < 5)			
OR (95% CI)	Ref.	1.36 (0.93–1.99)	1.52 (1.04–2.24)
P value		0.108	**0.031**
Recall (score < 3)			
OR (95% CI)	Ref.	0.76 (0.50–1.15)	0.78 (0.53–1.22)
P value		0.195	0.295
Language (score <8)			
OR (95% CI)	Ref.	0.53 (0.17–1.65)	2.44 (1.00–5.94)
P value		0.270	**0.049**

CI, confidence interval; MMSE, Mini-Mental State Examination; OR, odds ratio; Ref, reference; ucOC, undercarboxylated osteocalcin.
P < 0.05 are expressed in bold.
The data were adjusted for the following variables: age (continuous), education (binary), sex (binary), hypertension (binary), stroke (binary), heart disease (binary), diabetes (binary), dyslipidemia (binary), osteoporosis (binary), smoking status (binary), and body mass index (categorical; < 18.5, 18.5-25, 25-30, and 30+).

summarized in **Table 1**. The maximum score of MMSE is 30, while the mean MMSE score of the participants in this study was 28.2. In the present study, we focus on cognitive impairment equivalent or below the mild cognitive impairment (MCI) level. Since the cutoff value of MMSE score 27/28 is often employed as the definition of MCI in the study that was evaluating the cognitive function of the Japanese community-dwelling older adults (19), we mainly used this cutoff value. Meanwhile, there is a report proposing the cutoff value of 26/27 is the optimal balance of sensitivity and specificity (20). Among the participants in our study, 204 people (25.5%) had cognitive impairment defined as MMSE score < 28, while 129 people (16.1%) had cognitive impairment defined as MMSE score < 27.

Next, binary logistic regression analysis was performed to know the association of the cognitive impairment and each characteristic, including age, education, sex, hypertension, stroke, heart disease, diabetes, dyslipidemia, osteoporosis, smoking status, body mass index, and ucOC. Among them, the older age, the longer education years (more than 9 years), and the highest tertile (T3) of ucOC with the lowest tertile (T1) as the reference were significantly associated with cognitive impairment equivalent or below the MCI level defined as MMSE score < 28 (**Table 2**). The odds ratio (OR) of the highest tertile (T3) of ucOC was 1.65, and its 95% confidence interval (CI) was 1.06 to 2.59 (*P* value, 0.028). When the analysis was performed with another MCI cutoff value "MMSE score < 27," the results were basically the same; the older age, the longer education years, and the highest tertile of ucOC (T3) were significantly associated with cognitive impairment equivalent or below the MCI level defined as MMSE score < 27. When this cutoff value was employed, the OR of the highest tertile of ucOC (T3) was 1.73, and its 95% CI was 1.01–2.96 (*P* value, 0.045). These results indicated older age, shorter education (<10 years), and vitamin K insufficiency are associated with cognitive impairment.

The MMSE is composed of five categories, including orientation (maximum, 10 points), registration (maximum, 3 points), calculation (maximum, 5 points), recall (maximum, 3 points), and language (maximum, 9 points). When the analysis was repeated with each category of MMSE, the highest tertile of ucOC was associated with impaired orientation (OR, 7.16; 95% CI, 2.05–27.19; *P* value, 0.002), calculation (OR, 1.52; 95% CI, 1.04–2.24; *P* value, 0.031), and language (OR, 2.44; 95% CI, 1.00–5.94; *P* value,0.049), with adjustment for age, education, sex, hypertension, stroke, heart disease, diabetes, dyslipidemia, osteoporosis, smoking status, and body mass index (**Table 3**).

DISCUSSION

In the present study, we demonstrated the association of vitamin K insufficiency as evaluated by serum ucOC with cognitive impairment in the community-dwelling older adult population. As far as we know, this is the first report that focuses on the significant association of single ucOC measurement and

cognitive impairment. Furthermore, our analysis suggests that vitamin K insufficiency could be associated with selected categories of cognitive function. Although validation in independent studies will be required, these findings can be a clue to generate hypothesis that vitamin K has important roles in specific areas of brain responsible for several categories of functions.

The finding of the present study was in line with the previous epidemiological studies, showing that lower vitamin K intake is associated with cognitive impairment (11, 12, 14, 16, 17). Among those studies, Kiely and colleagues employed the %ucOC as one of the indicators to evaluate the vitamin K status by measuring both carboxylated OC and ucOC (16). Unfortunately, the relationship between %ucOC and cognitive function was not significant in the study ($P = 0.06$), and the usefulness of ucOC was not discussed in the report. Meanwhile, we showed significant association of vitamin K insufficiency as evaluated by serum ucOC with cognitive impairment, suggesting the possibility that a single measurement of ucOC could be a biomarker for evaluating a vitamin K insufficiency in some epidemiological studies. Since the measurement of ucOC is relatively simple and widely available compared with other methods of vitamin K evaluation, our study would inspire studies on other diseases, as well as cognitive dysfunction, in which vitamin K insufficiency could be involved. In this sense, a protein induced by vitamin K absence-II (PIVKA II), which is an abnormal prothrombin without γ-carboxylation, would be another candidate for efficient evaluation of vitamin K status in the epidemiological studies (21).

It remains unknown whether the undercarboxylated form of osteocalcin itself affects the cognitive function. It is also possible to consider that a high concentration of ucOC merely reflected a low vitamin K intake, and that other substrates of GGCX are responsible for the mechanism. Previous studies with an animal model suggested Gas6 and Protein S, both of which are substrates of GGCX and have neuroprotective effect. The Gas6 was reported to prevent apoptosis of neuronal cells (22), oligodendrocyte loss, and microglial activation (23). Also, Gas6 is reported to prevent amyloid beta protein-induced apoptosis of cortical neuron (24), suggesting its protective role against Alzheimer's disease. Protein S is shown to be associated with neuronal protection against ischemic injury (25), suggesting its beneficial role for cognitive impairment due to vascular dementia. It is also possible that other γ-carboxylated proteins including unidentified ones expressed within the brain or expressed outside the brain, which enable to pass through a blood brain barrier, would be important for brain protective effect. In human, a single nucleotide polymorphism (SNP) of *GGCX* gene is shown to be associated with risk susceptibility of stroke when combined with SNP of NAD(P)H:quinone oxidoreductase (*NQO1*) gene, which encodes a protein protective against oxidative stress (26). The SNP of GGCX is responsible for the difference of an amino acid (Gln325Arg), while the protective SNP against stroke encodes GGCX with higher activity (325Gln) (26, 27).

Since vitamin K has multiple modes of action (5), other mechanisms independent of GGCX could be involved in its effects on cognitive impairment. A nuclear receptor, SXR, for which vitamin K functions as a ligand (6), is shown to have anti-inflammatory roles (28). Among vitamin K compounds, the vitamin K2 form is known to activate SXR (6). In a study analyzing the postmortem brains of centenarians, MK-4 form of vitamin K2 was detected in human brains (17). Meanwhile, expression of SXR was reported in the capillaries in rat brains (29), which supports the existence of SXR-dependent vitamin K function in the brain. The relationship of inflammation and cognitive impairment is proposed including the indirect immune pathways from the gut microbiome and systemic circulation (30). Considering the SXR knockout mice display intestinal inflammation (28), SXR might mediate beneficial functions of vitamin K on cognitive functions by suppressing inflammatory response of the intestine. Vitamin K in the body is partially derived from intestinal microbiome (4), and the relationship of several forms of microbiome-derived vitamin K, and the cognitive function emerged as a new study topic (11).

It is an intriguing question to know the blood concentration of vitamin K is correlated with vitamin K concentration in brain. Although we can assume function of GGCX or SXR outside the brain could affect brain function *via* circulating γ-carboxylated proteins or inflammatory mediators, some hypothesis may depend on the GGCX or SXR functions within the specific area of the brain. In the study analyzing postmortem brains of centenarians, the concentration of vitamin K in the blood did not correlate with that in the brain (17). Meanwhile, in other reports, measurement of vitamin K concentration in the brain decreased with the storage time of the brain (31), which may affect the study using centenarians.

In summary, the epidemiological studies, including the present study, will promote future studies of vitamin K on a higher brain function including basic studies and clinical intervention studies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Ethical Committee of Tokyo Metropolitan Institute of Gerontology (approval number: H18-17, R1-20). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KA, YO, NK, and HS acquired the data. KA conducted the data analyses. All authors contributed to the interpretation of data, drafted the manuscript, approved the final

version for publication, and conceived and designed the study.

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REFERENCES

1. Suttie JW, Booth SL. Vitamin K. *Adv Nutr*. (2011) 2:440–1. doi: 10.3945/an.111.000786
2. Tarvainen M, Fabritius M, Yang B. Determination of vitamin K composition of fermented food. *Food Chem.* (2019) 275:515–22. doi: 10.1016/j.foodchem.2018.09.136
3. Fu X, Harshman SG, Shen X, Haytowitz DB, Karl JP, Wolfe BE, et al. Multiple vitamin K forms exist in dairy foods. *Curr Dev Nutr*. (2017) 1:e000638. doi: 10.3945/cdn.117.000638
4. Zhang Z, Liu L, Liu C, Sun Y, Zhang D. New aspects of microbial vitamin K2 production by expanding the product spectrum. *Microb Cell Fact*. (2021) 20:84. doi: 10.1186/s12934-021-01574-7
5. Azuma K, Inoue S. Multiple modes of vitamin K actions in aging-related musculoskeletal disorders. *Int J Mol Sci.* (2019) 20:2844. doi: 10.3390/ijms20112844
6. Tabb MM, Sun A, Zhou C, Grün F, Errandi J, Romero K, et al. Vitamin K2 regulation of bone homeostasis is mediated by the steroid and xenobiotic receptor SXR. *J Biol Chem.* (2003) 278:43919–27. doi: 10.1074/jbc.M303136200
7. Otsuka M, Kato N, Ichimura T, Abe S, Tanaka Y, Taniguchi H, et al. *Vitamin K2 binds 17beta-hydroxysteroid dehydrogenase 4 and modulates estrogen metabolism Life Sci.* (2005) 76:2473–82. doi: 10.1016/j.lfs.2004.12.020
8. Karasawa S, Azuma M, Kasama T, Sakamoto S, Kabe Y, Imai T, et al. Vitamin K2 covalently binds to Bak and induces Bak-mediated apoptosis. *Mol Pharmacol.* (2013) 83:613–20. doi: 10.1124/mol.112.082602
9. Booth SL, Tucker KL, Chen H, Hannan MT, Gagnon DR, Cupples LA, et al. Dietary vitamin K intakes are associated with hip fracture but not with bone mineral density in elderly men and women. *Am J Clin Nutr.* (2000) 71:1201–8. doi: 10.1093/ajcn/71.5.1201
10. Neogi T, Booth SL, Zhang YQ, Jacques PF, Terkeltaub R, Aliabadi P, et al. Low vitamin K status is associated with osteoarthritis in the hand and knee. *Arthritis Rheum.* (2006) 54:1255–61. doi: 10.1002/art.21735
11. Presse N, Shatenstein B, Kergoat MJ, Ferland G. Low vitamin K intakes in community-dwelling elders at an early stage of Alzheimer's disease. *J Am Diet Assoc.* (2008) 108:2095–9. doi: 10.1016/j.jada.2008.09.013
12. Presse N, Belleville S, Gaudreau P, Greenwood CE, Kergoat MJ, Morais JA, et al. Vitamin K status and cognitive function in healthy older adults. *Neurobiol Aging.* (2013) 34:2777–83. doi: 10.1016/j.neurobiolaging.2013.05.031
13. van den Heuvel EG, van Schoor NM, Vermeer C, Zwijsen RM, den Heijer M, Comijs HC. Vitamin K status is not associated with cognitive decline in middle aged adults. *J Nutr Health Aging.* (2015) 19:908-12. doi: 10.1007/s12603-015-0579-8
14. Chouet J, Ferland G, Féart C, Rolland Y, Presse N, Boucher K, et al. Dietary vitamin K intake is associated with cognition and behaviour among geriatric patients: The CLIP study. *Nutrients.* (2015) 7:6739–50. doi: 10.3390/nu7085306
15. McCann A, Jeffery IB, Ouliass B, Ferland G, Fu X, Booth SL, et al. Exploratory analysis of covariation of microbiota-derived vitamin K and cognition in older adults. *Am J Clin Nutr*. (2019) 110:1404–15. doi: 10.1093/ajcn/nqz220
16. Kiely A, Ferland G, Ouliass B, O'Toole PW, Purtill H, O'Connor EM. Vitamin K status and inflammation are associated with cognition in older Irish adults. *Nutr Neurosci*. (2020) 23:591–9. doi: 10.1080/1028415X.2018.1536411
17. Tanprasertsuk J, Ferland G, Johnson MA, Poon LW, Scott TM, Barbey AK, et al. Concentrations of circulating phylloquinone, but not cerebral menaquinone-4, are positively correlated with a wide range of cognitive measures: Exploratory findings in centenarians. *J Nutr*. (2020) 150:82–90. doi: 10.1093/jn/nxz200
18. Szulc P, Chapuy MC, Meunier PJ, Delmas PD. Serum undercarboxylated osteocalcin is a marker of the risk of hip fracture in elderly women. *J Clin Invest*. (1993) 91:1769–74. doi: 10.1172/JCI116387
19. Kinoshita K, Otsuka R, Takada M, Tsukamoto-Yasui M, Nishita Y, Tange C, et al. The association between dietary amino acid intake and cognitive decline 8 years later in Japanese community-dwelling older adults. *J Nutr Health Aging.* (2021) 25:165–71. doi: 10.1007/s12603-020-1470-9
20. O'Bryant SE, Humphreys JD, Smith GE, Ivnik RJ, Graff-Radford NR, Petersen RC, et al. Detecting dementia with the mini-mental state examination in highly educated individuals. *Arch Neurol*. (2008) 65:963–7. doi: 10.1001/archneur.65.7.963
21. Torii S, Ikari Y, Tanabe K, Kakuta T, Hatori M, Shioi A, et al. *Plasma phylloquinone, menaquinone-4 and menaquinone-7 levels and coronary artery calcification. J Nutr Sci.* (2016) 5:e48. doi: 10.1017/jns.2016.20
22. Funakoshi H, Yonemasu T, Nakano T, Matumoto K, Nakamura T. Identification of Gas6, a putative ligand for Sky and Axl receptor tyrosine kinases, as a novel neurotrophic factor for hippocampal neurons. *J Neurosci Res*. (2002) 68:150–60. doi: 10.1002/jnr.10211
23. Binder MD, Cate HS, Prieto AL, Kemper D, Butzkueven H, Gresle MM, et al.Gas6 deficiency increases oligodendrocyte loss and microglial activation in response to cuprizone-induced demyelination. *J Neurosci.* (2008) 28:5195–206. doi: 10.1523/JNEUROSCI.1180-08.2008
24. Yagami T, Ueda K, Asakura K, Sakaeda T, Nakazato H. Kuroda T, et al. Gas6 rescues cortical neurons from amyloid beta protein-induced apoptosis. *Neuropharmacology*. (2002) 43:1289–96. doi: 10.1016/S0028-3908(02)00333-7
25. Liu D, Guo H, Griffin JH, Fernández JA, Zlokovic BV. Protein S confers neuronal protection during ischemic/hypoxic injury in mice. *Circulation*. (2003) 107:1791–6. doi: 10.1161/01.CIR.0000058460.34453.5A
26. Shyu HY, Fong CS, Fu YP, Shieh JC, Yin JH, Chang CY, et al. *Genotype polymorphisms of GGCX, NQO1, and VKORC1 genes associated with risk susceptibility in patients with large-artery atherosclerotic stroke. Clin Chim Acta.* (2010) 411:840–5. doi: 10.1016/j.cca.2010.02.071
27. Kinoshita H, Nakagawa K, Narusawa K, Goseki-Sone M, Fukushi-Irie M, Mizoi L, et al.A functional single nucleotide polymorphism in the vitamin-K-dependent gamma-glutamyl carboxylase gene (Arg325Gln) is associated with bone mineral density in elderly Japanese women. *Bone*. (2007) 40:451–6. doi: 10.1016/j.bone.2006.08.007
28. Zhou C, Tabb MM, Nelson EL, Grün F, Verma S, Sadatrafiei A, et al. Mutual repression between steroid and xenobiotic receptor and NF-kappaB signaling pathways links xenobiotic metabolism and inflammation. *J Clin Invest*. (2006) 116:2280–9. doi: 10.1172/JCI26283
29. Bauer B, Hartz AM, Fricker G, Miller DS. Pregnane X receptor up-regulation of P-glycoprotein expression and transport function at the blood-brain barrier. *Mol Pharmacol.* (2004) 66:413–9.
30. McGrattan AM, McGuinness B, McKinley MC, Kee F, Passmore P, Woodside JV, et al. Diet and inflammation in cognitive ageing and Alzheimer's disease. *CurrNutr Rep*. (2019) 8:53–65. doi: 10.1007/s13668-019-0271-4

31. Fu X, Shea MK, Dolnikowski GG, Patterson WB, Dawson-Hughes B, Holland TM, et al. Vitamin D and vitamin K concentrations in human brain tissue are influenced by freezer storage time: the memory and aging project. *J Nutr.* (2021) 151:104–8. doi: 10.1093/jn/nxaa336

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Preventive Vitamin A Supplementation Improves Striatal Function in 6-Hydroxydopamine Hemiparkinsonian Rats

Anaïs Marie[1], *Julien Leroy*[1], *Morgane Darricau*[1,2†], *Serge Alfos*[1], *Veronique De Smedt-Peyrusse*[1], *Emmanuel Richard*[3], *Sylvie Vancassel*[1] *and Clementine Bosch-Bouju*[1*]

[1] Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE), Institut Polytechnique de Bordeaux (INP), NutriNeuro, University of Bordeaux, Bordeaux, France, [2] Institut des Maladies Neurodégénératives, University of Bordeaux, Bordeaux, France, [3] Institut National de la Santé et de la Recherche Médicale (INSERM), Centre Hospital-Universitaire (CHU) Bordeaux, University of Bordeaux, Bordeaux, France

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Gorazd Drevenšek,
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Alexander Hawlitschka,
University Hospital Rostock, Germany

***Correspondence:**
Clementine Bosch-Bouju
clementine.bosch-bouju@inrae.fr

†Present address:
Morgane Darricau,
Institut des Maladies Neurodégénératives, University of Bordeaux, Bordeaux, France

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Background: The mechanisms leading to a loss of dopaminergic (DA) neurons from the substantia nigra *pars compacta* (SNc) in Parkinson's disease (PD) have multifactorial origins. In this context, nutrition is currently investigated as a modifiable environmental factor for the prevention of PD. In particular, initial studies revealed the deleterious consequences of vitamin A signaling failure on dopamine-related motor behaviors. However, the potential of vitamin A supplementation itself to prevent neurodegeneration has not been established yet.

Objective: The hypothesis tested in this study is that preventive vitamin A supplementation can protect DA neurons in a rat model of PD.

Methods: The impact of a 5-week preventive supplementation with vitamin A (20 IU/g of diet) was measured on motor and neurobiological alterations induced by 6-hydroxydopamine (6-OHDA) unilateral injections in the striatum of rats. Rotarod, step test and cylinder tests were performed up to 3 weeks after the lesion. Post-mortem analyses (retinol and monoamines dosages, western blots, immunofluorescence) were performed to investigate neurobiological processes.

Results: Vitamin A supplementation improved voluntary movements in the cylinder test. In 6-OHDA lesioned rats, a marked decrease of dopamine levels in striatum homogenates was measured. Tyrosine hydroxylase labeling in the SNc and in the striatum was significantly decreased by 6-OHDA injection, without effect of vitamin A. By contrast, vitamin A supplementation increased striatal expression of D2 and RXR receptors in the striatum of 6-OHDA lesioned rats.

Conclusions: Vitamin A supplementation partially alleviates motor alterations and improved striatal function, revealing a possible beneficial preventive approach for PD.

Keywords: vitamin A, dopamine, Parkinson's disease, 6-hydroxydopamine (6-OHDA), aldehyde dehydrogenase ALDH1A1, substantia nigra *pars compacta* (SNc), striatum

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease with multifactorial origins that involves interactions between genetic and environmental factors (1, 2). PD mainly results from the degeneration of dopamine (DA) neurons from the substantia nigra *pars compacta* (SNc) innervating the striatum (3). PD is characterized by disabling motor symptoms, such as akinesia, bradykinesia and tremor at rest, but these motor symptoms generally appear when a large proportion of DA neurons have already degenerated (4). In this context, nutrition has recently attracted attention as a potent modifiable environmental factor for the prevention of neurodegeneration in PD (5, 6).

Vitamin A (retinol) is a lipophilic vitamin that is critical for brain development and function along life (7). Vitamin A acts through its active metabolite, retinoic acid, that binds to nuclear receptors and modulate gene transcription (8). Supporting the relevance of vitamin A for PD, vitamin A-deficient rats display motor alterations (9) and conversely, DA neurons degeneration in mouse and rat PD models is prevented by pharmacological administration of retinoic acid or derivatives (10–13). Further supporting an implication of vitamin A signaling in PD is the observation that the sub-population of DA SNc neurons that is more prone to degenerate expresses the enzyme aldehyde dehydrogenase 1 subtype A1 (ALDH1A1) (8, 14–16), the synthesis enzyme of retinoic acid. Interestingly, mice lacking ALDH1A1 enzyme or ALDH1A1$^+$ neurons, exhibit motor impairments along with alterations of DA metabolism (17, 18). This evidence highlights a role for vitamin A signaling in dopamine-related motor behaviors. However, the relevance of vitamin A nutritional supplementation for the prevention of PD, which can be easier to implement in humans compared to synthetic retinoids, has been overlooked.

Here, we hypothesized that preventive vitamin A supplementation can protect DA neurons, especially those expressing ALDH1A1 enzyme. We modeled DA fibers degeneration in rats with unilateral 6-hydroxydopamine (6-OHDA) lesion in the striatum (19). We investigated the impact of preventive vitamin A supplementation with dietary vitamin A (20 IU/g of diet) for 5 weeks before 6-OHDA or sham lesion. Motor behaviors were analyzed 1 to 3 weeks after the lesion. Post-mortem analyses were performed to study retinoid metabolism and DA transmission, with a focus on ALDH1A1$^+$ DA neurons. This work shows that vitamin A supplementation in 6-OHDA rats improves striatal function, with a mild protective effect measured on ALDH1A1$^+$ DA neurons.

Abbreviations: 6-OHDA, 6-hydroxydopamine; TH, tyrosine hydroxylase; ALDH1A1, aldehyde dehydrogenase 1 subtype A1; SNc, substantia nigra *pars compacta;* PD, Parkinson's disease; DA, dopamine; RXR, retinoid X receptor; RAR, retinoic acid receptor; D2R, dopamine D2 receptor; IBA1, Ionized calcium binding adapter molecule 1; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HPLC, high performance liquid chromatography; Ctrl, control; PBS, phosphate buffer saline.

MATERIALS AND METHODS

Animals and Diet

Animals experiments were performed according to criteria of the European Communities Council Directive (2010/63/UE) and the French National Committee (authorization 16476-2018052314372190). All efforts were made to reduce the number of animals used and to minimize their suffering. A total of 66 male Wistar rats (Janvier Labs, France) was used (6-weeks old, 180–200g). Rats were housed 2 per cage and maintained in enriched and controlled environment (22°C ± 2°C, 40% of humidity), with a 12-h light/dark cycle (light on at 7 a.m.) with *ad libitum* access to water and food. Upon their arrival, rats were randomly allocated to two groups with different diet. One group received a diet with sufficient amount of retinol (5 IU retinol/g, INRAE, Jouy-en-Josas, 1 IU = 0.3 μg retinol), the second group received a similar diet but supplemented with vitamin A, with 20 IU retinol/g (INRAE, Jouy-en-Josas). The two diets differed only by the amount of retinol (retinyl acetate). Rats were weighted weekly and no body weight difference existed between the two groups at experiment onset. Animals were handled daily throughout the duration of the experiment.

Surgery

After 5 weeks of dietary treatment (5 or 20 IU/g of diet), rats were subjected to unilateral stereotaxic injections in the striatum. Animals in both groups were randomly assigned to sham (0.9% saline containing 0.05 % of ascorbic acid, ref 95210 Sigma, St.Louis, MO, USA) or 6-hydroxydopamine (6-OHDA, 2 μg/μl in 0.9% saline and 0.05% of ascorbic acid solution, Sigma, St.Louis, MO, USA) injections. 6-OHDA is a neurotoxic dopaminergic analog carried out via monoamines transporters which allows the selective loss of catecholaminergic neurons, including dopaminergic and noradrenergic neurons (20). Unilateral injections in the striatum consisted in 2 injections of 3.5 μl each (7 μl/hemisphere in total). Briefly, Animals received carprofen (5 mg/kg, i.p, CARPROX VET®, Virbac, France) for analgesia and desipramine (15 mg/kg, i.p, ref D3900, Sigma, St.Louis, MO, USA) to protect noradrenergic neurons from 6-OHDA injection. Then, rats were placed on a stereotaxic apparatus (RWD life science) under body-temperature control and constant isoflurane anesthesia (3.5 % for induction, 1.5–2 % for maintenance, in 0.2% in air at 400 ml/min). The injected hemisphere (right or left striatum) was assigned randomly for each rat and the following coordinates for the 2 injection sites were chosen (19, 21): anteroposterior from bregma (AP) +1.2 and +0.2 mm; mediolateral (ML), ± 3.0–3.8 mm; dorsoventral (DV) −5 mm. Delivery rate for injections was 1.5 μl/min (10 μl 36G nanofil syringe, World Precision Instruments, USA, and ultra-micro pump UMP3, World Precision Instruments, USA) and the syringe was left in place for 5 min after each injection before slow removal. At the end of the injections, animals were sutured and lidocaine gel (Xylocaine ® 2 % Gel, AstraZeneca, UK) was applied on the sutures. Animals were kept on a heating pad until recovery and they were monitored twice a day for 4 days following the surgery.

Behavioral Tests

Stepping Test

In order to quantify the akinesia of the contralateral forepaw induced by 6-OHDA injection, we performed stepping test as previously described (19, 22). For this test, animals were habituated for two consecutive days, three days before the first test. Briefly, rats were hold by a trained experimenter over a 90 cm-long bench and only one forepaw was placed on the bench. Rats were slowly moved along the bench (right to left and reverse) and the number of adjusted steps made by each paw on the bench was counted. The procedure was performed 3 times for each paw, on both directions. The stepping test was performed twice, the first test occurring before 6-OHDA (or sham) injection (named "pre-test" thereafter), and 3 weeks after for the second test. The result for each paw is the average of 3 consecutive trials. Results are presented as the percentage of steps made by the paw contralateral to the injected hemisphere (named "lesioned paw" thereafter) compared to the total number of steps. To assess the effect of 6-OHDA injection, the result of the test 3 weeks after the injection is normalized to pre-test.

Cylinder Test

Spontaneous forelimb use was tested in the cylinder test. In a clear plexiglass cylinder (diameter: 16 cm; height: 31 cm), we quantify the number of touches made by the rat with each forepaw on the cylinder wall while rearing during 3 min-sessions (23). The cylinder test was made at only one time point, 3 weeks after stereotaxic surgery. Results are presented as the percentage of touches made by the lesioned paw, compared to the total number of touches.

Rotarod Test

Motor coordination and balance of rats were tested with rotarod test (Rota-Rod ENV-578(R), MedAssociates) as previously described (24). Briefly, rats were placed on a rotative axis and their latency to fall was measured. Each session had a maximum duration of 5 min. Three days before the first test, training sessions were performed on three consecutive days, with two sessions per day at constant speed (day 1 at 12 rpm, day 2 at 10 rpm, day 3 morning at 12 rpm and day 3 afternoon 14 rpm). For test sessions, the rotative axis followed a linear acceleration from 5 to 45 rpm. Test sessions were performed 1 week before, and 1, 2 and 3 weeks after striatal unilateral injections. Data are presented as latency to fall (s) for each animal, as automatically registered by the apparatus.

Sample Collection

Three weeks after stereotaxic injections, animals were euthanized following 2 different procedures. On one side, 36 rats were deeply anesthetized with isoflurane (5 %) and decapitated. Blood was collected from the trunk and brain was immediately frozen in isopentane and stored at −80°C. The liver was also collected and stored at −80°C for retinol assay. On the other side, 30 rats were euthanized with intraperitoneal injection of hexagon (150 mg/Kg)/lidocaine (230 mg/Kg). Blood was collected *via* an intracardiac puncture and then rats were transcardially perfused with paraformaldehyde (PFA) 4% solution in order to fix brain tissues. Brains were quickly extracted, post-fixed in PFA 4% for 24 h and plunged in 30 % sucrose solution for 48 h before storage at −80°C. All Blood samples were centrifuged (10 min, 10 000 x g, 4°C) and plasma was stored at −80°C until used.

Retinol Assays

Retinol was measured in liver extracts (the storage organ of vitamin A), that have been homogenized in sodium phosphate-EDTA (0.05 M, pH 7.8) buffer, and in the plasma. Retinol was extracted with a solution of tocopherol acetate (1.057 mmol/L, T3376, Sigma St.Louis, MO, USA) used as internal standard, added with a solution of hexane and butyl-hydroxy-toluene (20 mg/mL, ref B1378, Sigma St.Louis, MO, USA). Homogenates were centrifuged (2 min, 20°C, 16,000 × *g*) and the supernatant containing retinol was recovered and evaporated under nitrogen. Following evaporation, residues were treated with methanol and assayed by high performance liquid chromatography (HPLC) as previously described (25). Two vitamin A calibration standard (Chromsystems 34004) were processed in parallel with samples. Results are given in μmol/g for the liver and in μmol/L for the plasma.

Monoamines Measures

HPLC analyses were performed on 1 mm-punches of the striatum from brains frozen in isopentane and then stored at −80°C. To do so, 200 μm-coronal brain sections were performed using a cryostat tissue slicer (Leica Biosystems, Germany). According to rat brain atlas (21), punches of the striatum were taken between 2.20 and −1.20-mm from bregma. Punches were immediately stored at −80°C.

As previously described (26) monoamines were extracted from striatum punches using a TissuLyser system (Qiagen, Courtaboeuf, France) in extraction buffer containing 12 mM perchloric acid, 56 μM EDTA, 0.26 mM sodium disulfite and 3 mM octanesulfonic acid. One part of the supernatant (containing proteins) was frozen at −80°C for subsequent western-blot analysis, the other part (300 μL) was used for measure of monoamines content. Total protein level for each sample was quantified using bicinchoninic acid (BCA) assay, in order to normalize HPLC results.

The monoamines of interest, DA and its main metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were identified using HPLC coupled with an electrochemical detector as previously described (26). Results for the lesioned side were normalized to the control side for each animal.

Western Blot

Expression levels of proteins (RXR, D2R and ALDH1A1) were measured by western-blot in striatum punches. Briefly, 10 μg of proteins with 2 μL of NaOH 1 M were added to loading buffer to basify the extraction medium used for monoamines extraction. Proteins samples were loaded on 12 % sodium polyacrylamide-dodecyl sulfate gel and then transferred to nitrocellulose membranes. Membranes were saturated with a solution of PBS-tween (0.1%, TWEEN®20, P1379, Sigma St.Louis, MO, USA) milk (5%) and incubated overnight at

TABLE 1 | Reagents and resources used for western blot experimentation.

	Antibodies	Host species	Dilution	Reference
Primary	ALDH1A1	Rabbit	1:2000	HPA002123, Sigma
Secondary	Anti-rabbit IgG-HRP conjugated	Donkey	1:5000	711-035-152, Jackson ImmunoResearch
Primary	RXRγ	Rabbit	1:1500	ab53162, Sigma
Secondary	Anti-rabbit IgG-HRP conjugated	Donkey	1:5000	711-035-152, Jackson ImmunoResearch
Primary	D2R	Rabbit	1:2000	AB5084P, Merck
Secondary	Anti-rabbit IgG-HRP conjugated	Donkey	1:5000	711-035-152, Jackson ImmunoResearch
Primary	GAPDH	Rabbit	1:15 000	D16H11, Cell Signaling
Secondary	Anti-rabbit IgG-HRP conjugated	Donkey	1:20 000	711-035-152, Jackson ImmunoResearch
Primary	α-tubuline	Mouse	1:10 000	T5168, Sigma
Secondary	Anti-mouse IgG-HRP conjugated	Donkey	1:10 000	715-035-151, Jackson ImmunoResearch

TABLE 2 | Reagents and resources used for immunostaining experimentation.

	Antibodies	Host species	Dilution	Blocking solution	Reference
Primary	TH	Mouse	1:1000	3% bovine serum albumine,	mab 318, Merck
Secondary	Anti-mouse IgG-Alexa 488	Donkey	1:1000	0.3% Triton X-100, PBS	ab150105, Abcam
Primary	ALDH1A1	Rabbit	1:1000	3% bovine serum albumine,	HPA002123, Sigma
Secondary	Anti-rabbit IgG-Alexa 568	Donkey	1:1000	0.3% Triton X-100, PBS	ab175470, Abcam
Primary	IBA1	Rabbit	1:500	10% normal donkey serum,	019-19741, Fujifilm
Secondary	Anti-rabbit IgG-Alexa 488	Goat	1:1000	0.5% Triton X-100, PBS	A11008, Invitrogen

4°C with different primary antibodies (**Table 1**). After 1 h-incubation with secondary antibody solution (**Table 1**) coupled to horseradish peroxidase (HRP), chemiluminescence has been detected with peroxidase revealing solution (SuperSignal West Dura, ThermoFisher, Waltham, MA, USA) and were revealed using ChemiDoc MP (Biorad, Hercules, CA, USA). Signal intensity was quantified and normalized on GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) or α-tubuline protein expression for each sample. Results are given in % of the lesioned side, normalized to the control side.

Immunofluorescence Staining

Coronal sections of 40-μm thickness were made using a cryostat tissue slicer (Leica Biosystems, Germany) from brains post-fixed in PFA and stored at −80°C. According to (27), we selected 3 levels of interest for striatum and SNc. The 3 levels were designated as anterior (bregma striatum: +2.2, SNC: −5.2), intermediate (bregma striatum: +1.6, SNC: −5.3) and posterior (bregma striatum: +0.7, SNC: −5.8). Sections were processed for simple or double-fluorescent immunohistochemistry. After washes with PBS, sections were blocked with appropriate blocking solution depending on primary antibodies (**Table 2**) for 1 h at room temperature. Sections were then incubated with primary antibodies diluted in blocking solution, overnight at 4°C. The next day, sections were washed with blocking solution [or PBS for IBA1 (Ionized calcium binding adapter molecule 1) labeling] and incubated with the secondary antibody conjugated to a fluorochrome for 2 h at room temperature, protected from light. Sections were washed with PBS and then mounted in mounting medium with DAPI (sc-359850, CliniSciences, France).

Image Analysis

All striatum sections were scanned using a widefield microscope (Hamamatsu Nanozoomer 2.0 HT) with 20X (20X, NA 0.75) objective to visualize the whole striatum. SNc sections were scanned using a laser scanning confocal microscope (Leica DM5500 TCS SPE) with 40X oil-immersion objective to visualize dopaminergic neurons in the tissue. Setting parameters for acquisition, such as laser power or photomultipliers gain, were kept constant between all animals for a given labeling. Digital images obtained were processed with Image J software. In order to limit analysis bias, we used semi-automated quantification with macros on Image J.

For the striatum, image of each brain section was divided in two parts, the lesioned side, corresponding to the injected hemisphere (with 6-OHDA or saline solution) and the control side. Each side has been analyzed. For TH and ALDH1A1 staining in the striatum, we quantified the intensity of staining. We assigned green color to TH-associated fluorescence channel and magenta color to ALDH1A1-associated fluorescence channel. From TH channel, we selected the striatum from the whole brain section using the "wand" set tool in Image J, which defines the region of interest (ROI). Area and staining intensity for TH and ALDH1A1 were measured based on this ROI.

For IBA1 labeling, only the lesioned side was analyzed. We assigned green color to IBA1-associated fluorescence and we first delimited the striatum using the "freehand" set tool in image J. Then we defined parameters for "subtract background" and "rolling" that we applied to all brain sections analyzed. These tools allowed us to better isolate activated microglia labeled by IBA1 and count them afterwards. To select each activated microglia, we used the tool "process find maxima" and "analyze

TABLE 3 | Summary of statistical analysis.

Figure 2	**Statistical test**	***n***	**Outcome measure**	**6-OHDA effect**		**Vitamin A effect**		**Time effect**		
Figure 2A: Body weight	3-way ANOVA	16–18	Body weight (g)	$F_{(1,62)} = 0.2926$	$p = 0.591$	$F_{(1,62)} = 0,0802$	$p = 0.778$	$F_{(1.494,92.65)} = 3.778$	$p < 0.001^{***}$	
				6-OHDA effect		**Vitamin A effect**		**Interaction**		***post-hoc* test**
Figure 2B: liver retinol level	2-way ANOVA	7–9	Retinol level (μmol/g)	$F_{(1,29)} = 0.6026$	$p = 0.443$	$F_{(1,29)} = 15,19$	$p < 0.001^{***}$	$F_{(1,29)} = 0,03328$	$p = 0.856$	
Figure 2C: Plasma retinol level	2-way ANOVA	8–9	Retinol level (μmol/l)	$F_{(1,31)} = 4.661$	$p = 0.038^{*}$	$F_{(1,31)} = 2,687$	$p = 0.111$	$F_{(1,31)} = 0,9726$	$p = 0.331$	
Figure 2D: RXRγ expression	2-way ANOVA	5–8	RXRγ/GAPDH (AU)	$F_{(1,23)} = 0.3524$	$p = 0.558$	$F_{(1,23)} = 7,447$	$p = 0.012^{*}$	$F_{(1,23)} = 0,03963$	$p = 0.843$	
Figure 3				**6-OHDA effect**		**Vitamin A effect**		**Interaction**		
Figure 3A: Step test - Pre-test	2-way ANOVA	16–18	Total # steps	$F_{(1,62)} = 0.1438$	$p = 0.706$	$F_{(1,62)} = 0.0255$	$p = 0.874$	$F_{(1,62)} = 0.6536$	$p = 0.422$	
Figure 3A: Step test	2-way ANOVA	16–18	step test score	$F_{(1,62)} = 15.21$	$p < 0.001^{***}$	$F_{(1,62)} = 0.5599$	$p = 0.457$	$F_{(1,62)} = 0.2593$	$p = 0.612$	
Figure 3B: Cylinder test number total of steps	2-way ANOVA	16–18	Total # touches	$F_{(1,62)} = 4.704$	$p = 0.033^{*}$	$F_{(1,62)} = 0,05835$	$p = 0.809$	$F_{(1,62)} = 0,07746$	$p = 0.781$	
Figure 3B: Cylinder test	2-way ANOVA	16–18	Touches (% ctrl paw)	$F_{(1,62)} = 27.83$	$p < 0.001^{***}$	$F_{(1,62)} = 0,8238$	$p = 0.367$	$F_{(1,62)} = 7,439$	$p = 0.008^{**}$	5 IU sham vs 5 IU 6-OHDA $p < 0.001$; 20 IU sham vs 5 IU 6-OHDA $p = 0.006$; 20 IU 6-OHDA vs 5 IU 6-OHDA $p = 0.007$
Figure 3C: Rotarod week−1	2-way ANOVA	16–18	Time on rotarod (s)	$F_{(1,62)} = 0.1225$	$p = 0.727$	$F_{(1,62)} = 0,0441$	$p = 0.834$	$F_{(1,62)} = 0,04134$	$p = 0.839$	
				6-OHDA effect		**Vitamin A effect**		**Time effect**		
Figure 3C: Rotarod	3-way ANOVA	16–18	Time on rotarod (s)	$F_{(1,62)} = 3.687$	$p = 0.059^{\#}$	$F_{(1,62)} = 0.01228$	$p = 0.912$	$F_{(1.938,120.2)} = 1.860$	$p = 0.161$	
Figure 4				**6-OHDA effect**		**Vitamin A effect**		**Interaction**		**PostHoc test**
Figure 4A: DA	2-way ANOVA	6–9	DA Level (% ctrl side)	$F_{(1,28)} = 57.82$	$p < 0.001^{***}$	$F_{(1,28)} = 0,141$	$p = 0.710$	$F_{(1,28)} = 0,5414$	$p = 0.468$	
Figure 4B: DOPAC	2-way ANOVA	6–9	DOPAC Level (% ctrl side)	$F_{(1,28)} = 17.9$	$p < 0.001^{***}$	$F_{(1,28)} = 0,6695$	$p = 0.420$	$F_{(1,28)} = 0,03669$	$p = 0.849$	
Figure 4C: HVA	2-way ANOVA	6–9	HVA Level (% ctrl side)	$F_{(1,28)} = 30.74$	$p < 0.001^{***}$	$F_{(1,28)} = 0,7256$	$p = 0.401$	$F_{(1,28)} = 0,4844$	$p = 0.492$	
Figure 4D: DA/DOPAC	2-way ANOVA	7–9	Ratio	$F_{(1,29)} = 7.079$	$p = 0.012^{*}$	$F_{(1,29)} = 0,3332$	$p = 0.568$	$F_{(1,29)} = 1,024$	$p = 0.319$	
Figure 4E: DA/HVA	2-way ANOVA	7–9	Ratio	$F_{(1,29)} = 4.185$	$p = 0.050^{*}$	$F_{(1,29)} = 0,2515$	$p = 0.619$	$F_{(1,29)} = 0,02674$	$p = 0.871$	
Figure 5				**6-OHDA effect**		**Vitamin A effect**		**Interaction**		***post-hoc* test**

(Continued)

TABLE 3 | Continued

Figure 5B: TH intermediate striatum	2-way ANOVA	6–7	TH Intensity (%ctrl side)	$F_{(1,23)} = 207.2$	$p < 0.001^{***}$	$F_{(1,23)} = 0,02812$	$p = 0.868$	$F_{(1,23)} = 3,779$	$p = 0.064^{\#}$	5 IU sham vs. 5 IU 6-OHDA $p < 0.001$; 20 IU sham vs. 20 IU 6-OHDA $p < 0.001$; 5 IU sham vs. 20 IU 6-OHDA $p < 0.001$; 20 IU sham vs 5 IU 6-OHDA $p < 0.001$
Figure 5C: TH posterior striatum	2-way ANOVA	5–8	TH Intensity (%ctrl side)	$F_{(1,22)} = 210.5$	$p < 0.001^{***}$	$F_{(1,22)} = 1,224$	$p = 0.280$	$F_{(1,22)} = 5,718$	$p = 0.025^{*}$	5 IU sham vs. 20 IU sham $p = 0.031$; 5 IU sham vs. 5 IU 6-OHDA $p < 0.001$; 20 IU sham vs. 20 IU 6-OHDA $p < 0.001$; 5 IU sham vs. 20 IU 6-OHDA $p < 0.001$; 20 IU sham vs. 5 IU 6-OHDA $p < 0.001$
Figure 5E: TH intermediate SNc	2-way ANOVA	6–9	# TH neurons	$F_{(1,25)} = 17.66$	$p < 0.001^{***}$	$F_{(1,25)} = 0,1169$	$p = 0.735$	$F_{(1,25)} = 0,2052$	$p = 0.654$	
Figure 5F: TH posterior SNc	2-way ANOVA	6–8	# TH neurons	$F_{(1,24)} = 5.407$	$p = 0.028^{*}$	$F_{(1,24)} = 0,2853$	$p = 0.598$	$F_{(1,24)} = 0,5264$	$p = 0.475$	
Figure 6	**Statistical test**	***n***	**Outcome measure**	**6-OHDA effect**		**Vitamin A effect**		**Interaction**		***post-hoc* test**
Figure 6: D2R expression	2-way ANOVA	8–9	D2R/α-tubuline	$F_{(1,29)} = 8.001$	$p = 0.008^{**}$	$F_{(1,29)} = 2,031$	$p = 0.164$	$F_{(1,29)} = 6,87$	$p = 0.013^{*}$	5 IU sham vs. 5 IU 6-OHDA $p < 0.001$; 20 IU sham vs. 5 IU 6-OHDA $p = 0.006$; 5 IU 6-OHDA vs. 20 IU 6-OHDA $p = 0.007$
Figure 7				**6-OHDA effect**		**Vitamin A effect**		**Interaction**		***post-hoc* test**
Figure 7B: ALDH1A1 intermediate SNc	2-way ANOVA	6–9	# ALDH1A1 neurons	$F_{(1,25)} = 26.38$	$p < 0.001^{***}$	$F_{(1,25)} = 3,186$	$p = 0.086^{\#}$	$F_{(1,25)} = 0,4866$	$p = 0.491$	5 IU sham vs. 20 IU sham $p = 0.09$ #; 5 IU sham vs. 5 IU 6-OHDA $p = 0.006$; 20 IU sham vs. 20 IU 6-OHDA $p < 0.001$; 5 IU sham vs. 20 IU 6-OHDA $p = 0.019$; 20 IU sham vs. 5 IU 6-OHDA $p < 0.001$
Figure 7C: ALDH1A1 posterior SNc	2-way ANOVA	6–8	# ALDH1A1 neurons	$F_{(1,24)} = 13.25$	$p = 0.001^{**}$	$F_{(1,24)} = 1,248$	$p = 0.275$	$F_{(1,24)} = 0,7636$	$p = 0.390$	
Figure 7E: Merge intermediate SNc	2-way ANOVA	5–9	% ALDH1A1 neurons	$F_{(1,24)} = 5.675$	$p = 0.025^{*}$	$F_{(1,24)} = 1,247$	$p = 0.275$	$F_{(1,24)} = 0,1778$	$p = 0.677$	
Figure 7F: Merge posterior SNc	2-way ANOVA	6–8	% ALDH1A1 neurons	$F_{(1,24)} = 11.09$	$p = 0.002^{**}$	$F_{(1,24)} = 1,921$	$p = 0.178$	$F_{(1,24)} = 0,0028$	$p = 0.958$	

(Continued)

TABLE 3 | Continued

Figure 8			6-OHDA effect		Vitamin A effect	Interaction	*post-hoc* test
Figure 8A: ALDH1A1 expression	2-way ANOVA	8–9	ALDH1A1/ GAPDH (AU)	$F_{(1,29)} = 19.28$	$p < 0.001$***	$F_{(1,29)} = 0,05594$	$p = 0.814$	$F_{(1,29)} = 0,3644$	$p = 0.550$	
Figure 8C: ALDH1A1 intermediate striatum	2-way ANOVA	6–7	ALDH1A1 Intensity (AU)	$F_{(1,23)} = 58.71$	$p < 0.001$***	$F_{(1,23)} = 0,6587$	$p = 0.425$	$F_{(1,23)} = 1,466$	$p = 0.238$	
Figure 8D: ALDH1A1 posterior striatum	2-way ANOVA	5–8	ALDH1A1 Intensity (AU)	$F_{(1,22)} = 87.44$	$p < 0.001$***	$F_{(1,22)} = 3,488$	$p = 0,075$ #	$F_{(1,22)} = 2,146$	$p = 0.157$	5 IU sham vs. 20 IU sham $p = 0.039$ 5 IU sham vs. 5 IU 6-OHDA $p < 0.001$ 20 IU sham vs. 20 IU 6-OHDA $p < 0.001$ 5 IU sham vs 20 IU 6-OHDA $p < 0.001$ 20 IU sham vs 5 IU 6-OHDA $p < 0.001$

*Statistical significance was assessed as #$p < 0.09$, *$p < 0.05$,**$p < 0.01$, ***$p < 0.001$.*

particles" with common thresholds for all cuts. Finally, we measured the area of the striatum previously delimited and we used the function "count" to determine the number of IBA1 positive microglia in the striatum. Results are expressed in number of $IBA1^+$/ striatum area (mm^2) which correspond to a density of activated microglia. We also quantified the total intensity of staining for IBA1 in the striatum contralateral to the lesion, using the ROI previously defined for the counting.

For SNc, final images of the whole SNc correspond to a mosaic of several images taken individually at 40X. Both sides of SNc, lesioned and control, were imaged and analyzed. For TH and ALDH1A1 labeled in SNc, we quantified the number of positive neurons. Similar to the striatum, we assigned green color for TH staining and magenta color for ALDH1A1 staining. The silver color on figures corresponds to the merge of both colors and indicates TH and ALDH1A1 positive neurons. From TH channel, we isolated neurons from the background with a median filter and applied a threshold with common parameters for all cuts. Then, we selected labeled neurons with the tool "analyze particles" and we used the function "count" to determine the number of TH positive neurons in the SNc. The process was repeated for ALDH1A1 and merge channels, in order to determine the number of TH+ALDH1A1 positive neurons. Note that all ALDH1A1 positive neurons were also TH positive neurons.

These analyses were carried out for the anterior, intermediate and posterior level of striatum and SNc. For the striatum, results of the lesioned side are expressed as normalized to the staining intensity of the control side.

Statistical Analysis

Data were analyzed using GraphPad prism 7.0 an 9.2.0 (Graphpad software). Two-way ANOVA were used when the effects of 2 factors (factors "vitamin A diet" and "6-OHDA injection") were tested. Three-way ANOVA (mixed-effects model) were performed for repeated data across time points (factors "diet", "6-OHDA" and "time"). In case of significant interaction for 2-way and 3-way ANOVAs ($p \leq 0.06$), a *post-hoc* test was performed based on the two-stage step-up method of Benjamini. All data are presented as means ± SEM. Details of the statistical analysis are summarized in **Table 3**. Statistical significance in statistics tables is expressed as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. The significance on the figure is expressed with letter a, b, c and d, which differed from each other. The notation ab indicates that the experimental group does not differ from either group with notation a or notation b.

RESULTS

Impact of 6-OHDA Injection and Dietary Vitamin A on Retinol Levels

Adult male rats were subjected to 5 weeks of dietary treatment, either sufficient diet (5 IU retinol/g of diet, referred to as 5 IU thereafter) or supplemented diet (20 IU retinol/g of diet, referred to as 20 IU thereafter). Rats then received a sham or 6-OHDA unilateral injection in the striatum, leading to 4 experimental groups (**Figure 1**). Dietary treatments were continued for 3

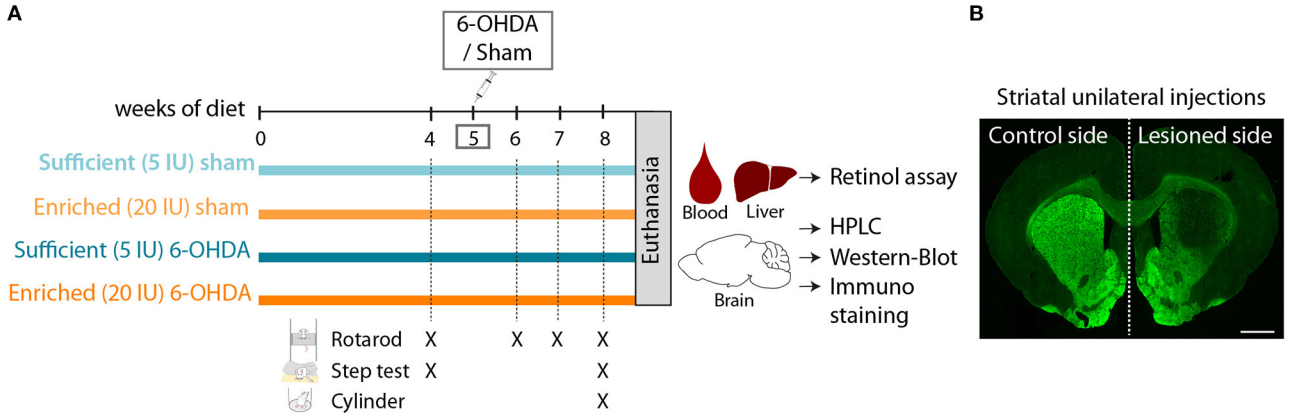

FIGURE 1 | Experimental design. **(A)** Schematic overview of experimental design. 5 IU and 20 IU stand for 5 IU/g of diet and 20 IU /g of diet, respectively. **(B)** Representative image of rat striatum (bregma +1.6) injected with 6-OHDA (lesioned side), labeled with TH immunofluorescence (green). Scale bar: 1000 μm.

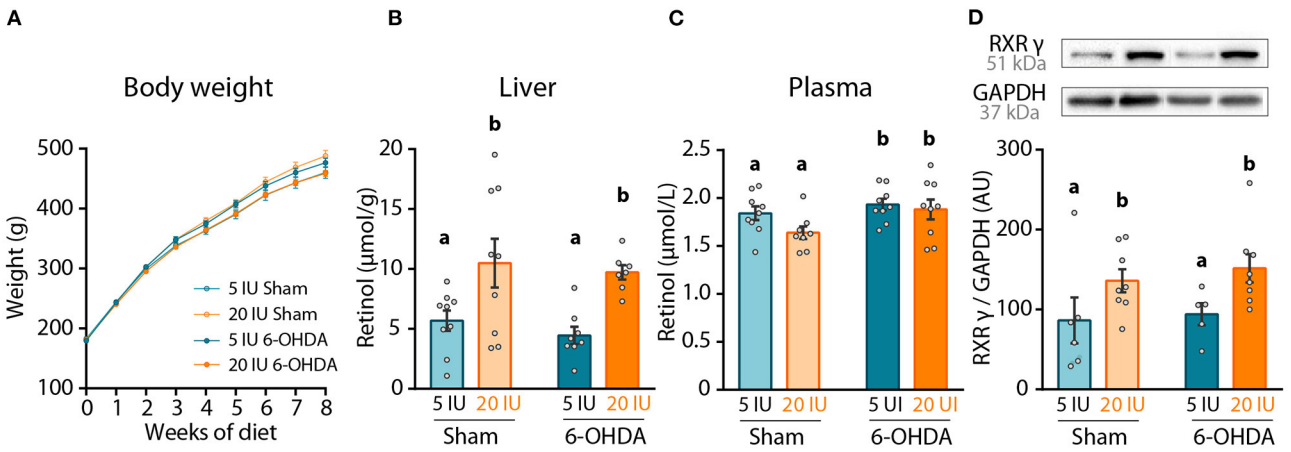

FIGURE 2 | Impact of dietary vitamin A and 6-OHDA injection on retinol levels and striatum RXRγ expression. **(A)** Measure of rats' body weight (g) throughout experiment. **(B,C)** Levels of retinol in the liver (μmol/g) **(B)**, and in the plasma (μmol/L) **(C)**. **(D)** Representative western blot for retinoid X receptor gamma (RXRγ) in striatum homogenates (upper inset) and quantification (graph). Protein expression levels were normalized with GAPDH for each sample. Results are expressed as means ± SEM, with individual data points for histograms. a, b: values significantly different ($p < 0.005$). Details of the statistical analysis are summarized in **Table 3**.

additional weeks during which, behavioral tests were performed. Body weight curves were not different between the 4 groups (6-OHDA effect $p = 0.591$; diet effect: $p = 0.778$), indicating that dietary treatment and intrastriatal injections had no effect on body weight (**Figure 2A**).

The effect of dietary treatment was first assessed on retinol levels, both in the plasma and the liver. Vitamin A supplemented diet almost doubled retinol levels in the liver ($p < 0.001$), but did not alter retinol levels in the plasma ($p = 0.111$) (**Figures 2B,C**). These results confirm the impact of dietary vitamin A treatment on retinol metabolism. Of note, 6-OHDA injection slightly but yet significantly increased retinol levels in the plasma ($p = 0.038$), but not in the liver ($p = 0.443$).

In the brain, the best proxy for retinoid signaling is the measure of retinoids receptors, since their expression is directly controlled by retinoic acid levels (28, 29). Thus, we quantified in the striatum the expression of retinoid X receptor (RXRγ), an isoform highly expressed in this structure (30). Vitamin A enriched diet significantly increased RXRγ expression in both sham and 6-OHDA-injected rats ($p = 0.012$) (**Figure 2D**). Of note, 6-OHDA injection did not modify expression of RXRγ in the striatum ($p = 0.558$).

Additionally, we quantified microglia in the striatum, as reflected by IBA1 staining, since 6- OHDA and dietary vitamin A can act on neuro-inflammatory processes (8, 31). In our conditions, quantification of the number of IBA1$^+$ cells, as well as total IBA1 intensity in the striatum did not reveal any significant effect of 6-OHDA or vitamin A supplementation (**Supplementary Figure 1**).

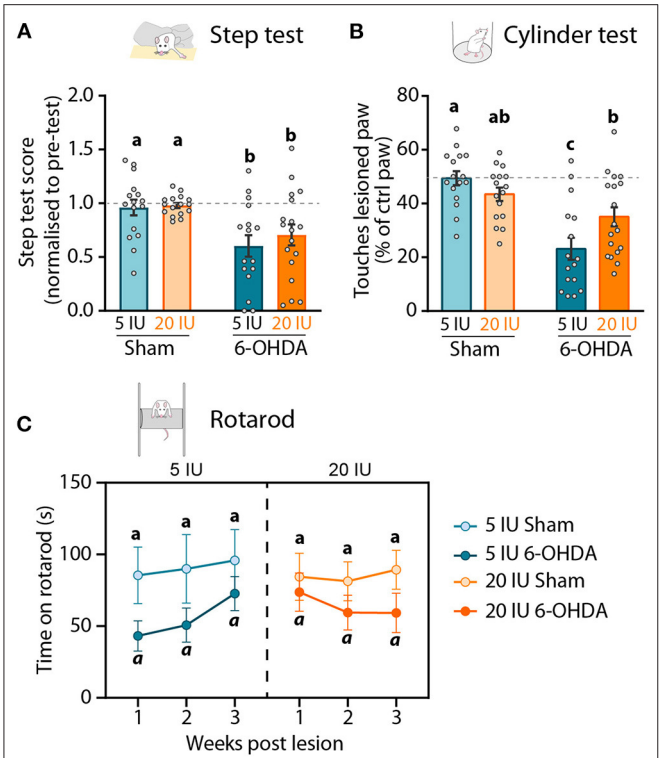

FIGURE 3 | Impact of 6-OHDA injection and dietary vitamin A on motor behavior. **(A)** Step test: Step test score 3 weeks after the lesion represents the number of steps made by the lesioned paw as the percent (%) of the total number of steps, normalized to the step test performed before the lesion. **(B)** Cylinder test: number of touches made by the lesioned paw expressed as % of total touches made by the control paw the cylinder test 3 weeks after the lesion. **(C)** Rotarod test: latency to fall (s) of rats from the apparatus 1, 2 and 3 weeks after the lesion. Results are expressed as means ± SEM, with individual data points for histograms. a–c: values significantly different ($p < 0.005$, *a*: $p = 0.059$). Details of the statistical analysis are summarized in **Table 3**.

Altogether these results validate the positive impact of 8-week dietary vitamin A supplementation on retinol function at the periphery and in the striatum.

Impact of 6-OHDA Injection and Dietary Vitamin A on Motor Behavior

The effect of preventive vitamin A supplementation and 6-OHDA injection on motor behavior was measured with three classical tests: the stepping test, the cylinder test and the rotarod test.

The stepping test was used to validate the impact of unilateral injection of 6-OHDA on the mobility of the paw contralateral to the lesion. The stepping test training and the first stepping session were performed before the unilateral injection, and the number of steps made by both paws was not different between groups (**Table 3**). Three weeks after 6-OHDA injection, the number of steps made by the paw contralateral to the lesion (named 'lesioned paw' thereafter) was reduced by half compared to sham rats (6-OHDA effect: $p < 0.001$) (**Figure 3A**). No significant effect of vitamin A supplementation was observed.

In the cylinder test, rats were freely moving and the number of touches made by each paw on the wall of the cylinder while rearing was quantified. This test was used to measure forelimb akinesia. In order to avoid habituation, this test was performed only once, 3 weeks after the lesion. In total, sham rats made significantly more paw touches than 6-OHDA lesioned rats (6-OHDA effect: $p = 0.033$). Under sufficient diet, 6-OHDA rats used significantly less their lesioned paw, compared to sham rats (5 IU sham vs. 5 IU 6-OHDA: $p < 0.001$). By contrast, the use of the lesion paw in vitamin A supplemented rats was not significantly different between sham and 6-OHDA rats (20 IU sham vs. 20 IU 6-OHDA: $p = 0.072$) (**Figure 3B**). Proportion of touches made with the lesioned paw was significantly higher for 6-OHDA rats under vitamin A supplementation, compared to 6-OHDA rats without supplementation (5 IU 6-OHDA vs. 20 IU 6 OHDA: $p = 0.007$).

In the rotarod test, we measured the latency to fall of rats placed on an accelerating rotative axis (4–40 rpm). The rotarod training and the first test were performed before the lesion, in order to ensure the absence of initial difference between groups (**Table 3**). Rotarod tests were then performed 1, 2 and 3 weeks after the lesion. In sham rats under sufficient or enriched diet, latency to fall remained stable across sessions (**Figure 3C**). In 6-OHDA rats, the 3-way ANOVA analysis revealed a strong tendency for shorter latency to fall, compared to sham rats ($p = 0.059$). Despite statistical analysis did not reveal significant effect, we observed that the impact of the lesion was not stable across time, and differ between 6-OHDA rats under sufficient or supplemented diet. In 6-OHDA rats under sufficient diet, latency to fall was more affected 1 week after the lesion that 3 weeks later. Conversely, in 6-OHDA rats with supplemented diet, latency to fall was close to sham rats 1 week after the lesion, but was shorter at 2 and 3 weeks after the lesion.

Taken together, these results reveal that beneficial effect of vitamin A supplementation on motor impairments induced by unilateral 6-OHDA injection in the striatum is only observable in the cylinder test.

Impact of 6-OHDA Lesion and Dietary Vitamin A on Dopamine Transmission in the Nigro-Striatal Pathway

We then focused on the integrity of the DA system, in order to investigate the mechanisms by which vitamin A supplementation improved motor function. First, we measured the levels of DA and metabolites in striatal homogenates. Since no difference was observed in the contralateral side of the lesion between groups, results were normalized to the contralateral side. As expected, 6-OHDA lesion significantly reduced DA levels in the lesioned hemisphere ($p < 0.001$) compared to sham rats. Similar results were observed for two major degradation products of DA, namely 3,4-Dihydroxyphenylacetic acid (DOPAC) ($p < 0.001$) and homovanillic acid (HVA) ($p < 0.001$) (**Figures 4A–C**). Similarly, DA/DOPAC and DA/HVA ratios were significantly reduced by 6-OHDA lesion ($p = 0.012$ and $p = 0.05$ respectively) (**Figures 4D,E**), suggesting increased metabolism of DA. However, no effect of vitamin A

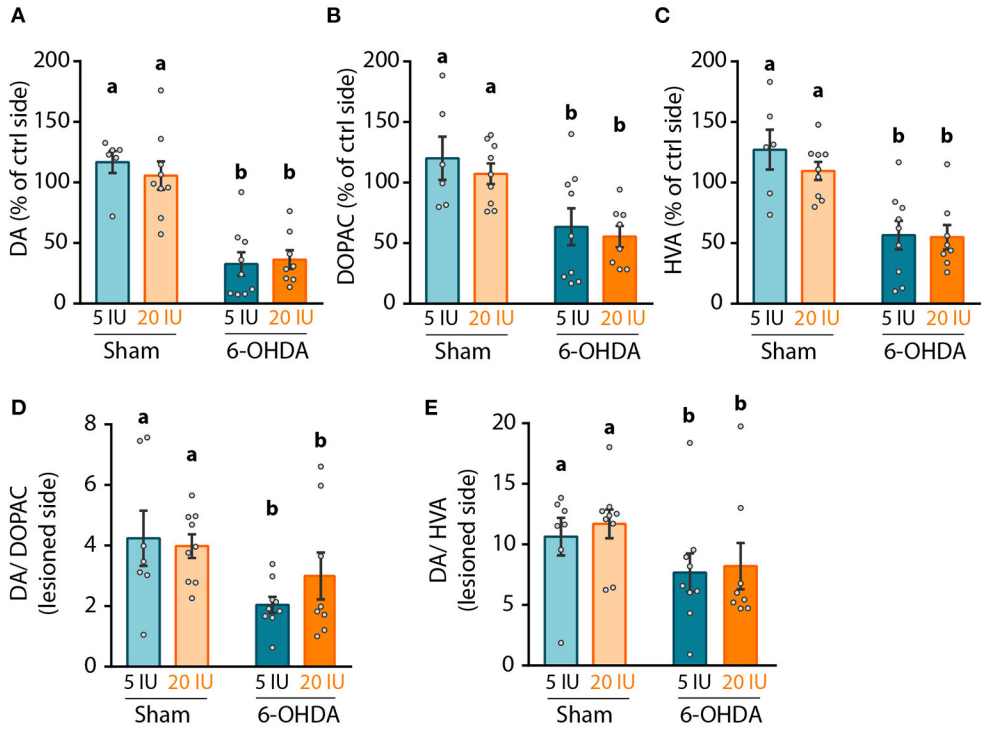

FIGURE 4 | Impact of 6-OHDA lesion and dietary vitamin A on monoamines levels in the striatum. **(A–C)** Quantification of dopamine (DA) using HPLC coupled to electrochemical detection **(A)**, 3,4-Dihydroxyphenylacetic acid (DOPAC) **(B)**, and homovanillic acid (HVA) **(C)** in the striatum. Results are expressed in pmoles/ug of total proteins. **(D,E)** Expression of DA/DOPAC **(D)** and DA/HVA **(E)** ratios. Results are expressed as means ± SEM with individual data points. a, b: values significantly different ($p < 0.005$). Details of the statistical analysis are summarized in **Table 3**.

supplementation was detected on monoamines measurements (**Figures 4A–E**).

Next, we quantified the intensity of the TH signal as a proxy for the density of DA fibers in the striatum by immunofluorescence, and the number of TH^+ neurons in the SNc. Analyses were performed on three antero-posterior levels for the striatum and the SNc: anterior (bregma striatum: +2.2, SNC: −5.2); intermediate (bregma striatum: +1.6, SNC: −5.3); and posterior (bregma striatum: +0.7, SNC: −5.8). The anterior levels are presented in **Supplementary Figures 2–4**. In the striatum (**Figure 5A**, **Supplementary Figures 2A,B**), TH intensity was clearly reduced at the injection site, in 6-OHDA rats but not in sham rats, for both intermediate (5 IU sham vs. 5 IU 6-OHDA: $p < 0.001$; 20 IU sham vs. 20 IU 6-OHDA: $p < 0.001$) (**Figure 5B**), and posterior (5 IU sham vs. 5 IU 6-OHDA: $p < 0.001$; 20 IU sham vs. 20 IU 6-OHDA: $p < 0.001$) (**Figure 5C**) levels. This is in accordance with measurements of DA in the striatum (**Figure 4**). Of note, TH intensity in the posterior striatum was increased by vitamin A supplementation in sham rats, but not in 6-OHDA rats (5 IU sham vs 20 IU sham: $p = 0.031$). In the SNc (**Figure 5D**, **Supplementary Figures 2A,C**), the number of positive neurons significantly decreased in rats injected with 6-OHDA, in both intermediate ($p < 0.001$) (**Figure 5E**) and posterior ($p = 0.028$) (**Figure 5F**) levels, with no difference between dietary groups.

Finally, dopamine D2 receptor (D2R) expression was measured in the striatum by western blot, since its expression is under the control of retinoic acid (32). We found that 6-OHDA injection significantly reduced D2R protein level in the striatum (**Figure 6**). However, this effect was not observed in 6-OHDA rats under supplemented diet, for which D2R expression levels were similar to sham rats (5 IU sham vs. 5 IU 6-OHDA: $p < 0.001$; 20 IU sham vs. 20 IU 6-OHDA: $p = 0.882$) (**Figure 6**). This suggests that increased retinoid signaling induced by vitamin A supplementation (**Figure 2**) prevented the decrease in striatal D2R expression in 6-OHDA rats. Of note, the decrease of D2R expression observed in 6-OHDA rats under sufficient diet is not usual, since studies have mainly found increased binding of D2R (33) or no change in D2R expression (34, 35). This discrepancy may be due to technical reasons, such as protein quantification vs. binding assays, or the conditions of the lesion.

Impact of Vitamin A Supplementation on $ALDH1A1^+$ Dopaminergic Neurons

Analysis of global DA transmission in the nigro-striatal pathway did not reveal a clear effect of vitamin A supplementation, in comparison to motor improvement. This may be due to a specific impact of vitamin A on the sub-population of DA neurons expressing ALDH1A1. To test whether $ALDH1A1^+$ DA neurons were specifically protected by vitamin A supplementation, we quantified the number of $ALDH1A1^+$ DA neurons in

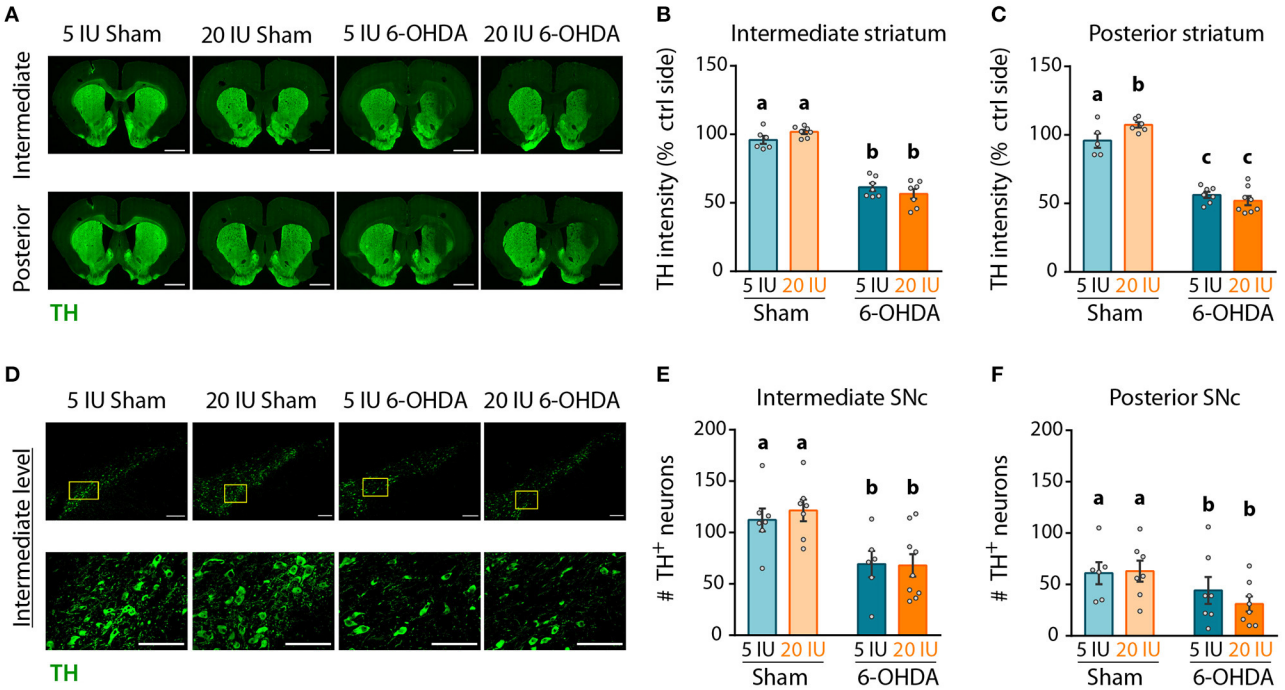

FIGURE 5 | Impact of 6-OHDA lesion and dietary vitamin A on extent of DA fibers in the striatum. **(A)** Representative images for the 4 experimental groups of intermediate and posterior striatum (lesioned and control sides) immunostained for tyrosine hydroxylase (TH) (green). Scale bar: 1000 μm. **(B,C)** Quantification of fluorescence intensity of TH staining in intermediate **(B)** and posterior **(C)** striatum. TH intensity was normalized to the ipsilateral side. **(D)** Representative images for the 4 experimental groups of intermediate SNc (lesioned side) immunostained for TH (green). Top images correspond to a confocal mosaic of multiple images taken individually at 40X, scale bar: 200 μm. Bottom images correspond to a zoom from mosaic images (yellow squares in top images), scale bar: 100 μm. **(E,F)** Quantification of the number (#) of TH^+ neurons in intermediate **(E)** and posterior **(F)** SNc. Results are expressed as means ± SEM with individual data points. a–c: values significantly different ($p < 0.005$). Details of the statistical analysis are summarized in **Table 3**.

the anterior, intermediate and posterior SNc (**Figure 7A**, **Supplementary Figure 3**). Analysis revealed that 6-OHDA lesion significantly decreased the number of $ALDH1A1^+$ neurons in intermediate ($p < 0.001$) (**Figure 7B**) and posterior SNc ($p = 0.001$) (**Figure 7C**). Of note, we generally observed a tendency for more $ALDH1A1^+$ neurons with vitamin A supplementation, in both sham and 6-OHDA rats. However, statistics only revealed a trend ($p = 0.086$) for the intermediate level of the SNc (**Figure 7B**).

Since $ALDH1A1^+$ neurons are also TH^+ neurons, we quantified the proportion of TH^+ neurons that expressed ALDH1A1 (**Figure 7D**), in order to identify this sub-population of DA neurons. In sham rats fed with sufficient diet, the proportion of TH^+ neurons that expressed ALDH1A1 was 36, 36, and 20%, for the anterior, intermediate and posterior levels of the SNc, respectively. These proportions are smaller than previous reports in the literature, with about 70% in mice as in humans (15, 16, 18). This difference may be due to the rat model, as well as the analysis methods. Proportions of $ALDH1A1^+$ DA neurons were significantly reduced by 6-OHDA lesion, at every level of the SNc (**Figures 7E,F**, **Supplementary Figure 3**) (8E: $p = 0.025$; 8F: $p = 0.002$). Of note, a trend for a higher proportion of TH^+ neurons expressing ALDH1A1 was observed in vitamin A supplemented rats.

Lastly, we quantified ALDH1A1 expression in the striatum by western-blot, as a reflect of the amount of DA afferents from $ALDH1A1^+$ DA neurons. As for $ALDH1A1^+$ neurons in SNc, 6-OHDA injection significantly reduced ALDH1A1 levels in the striatum compared to sham-injected rats ($p < 0.001$) (**Figure 8A**). However, no effect of vitamin A supplementation was observed.

We refined the analysis with ALDH1A1 staining, in the anterior, intermediate and posterior parts of the striatum (**Figure 8B**, **Supplementary Figure 4**). Confirming western blot measurements, 6-OHDA lesion significantly reduced ALDH1A1 staining in the striatum, at the anterior ($p = 0.033$, **Supplementary Figure 4B**), intermediate ($p < 0.001$, **Figure 8C**) and posterior ($p < 0.001$, **Figure 8D**) levels. Vitamin A supplementation induced no effect on ALDH1A1 staining in the striatum of 6-OHDA rats. By contrast, sham rats supplemented with vitamin A showed increased ALDH1A1 staining in the posterior striatum, compared to sham rats fed sufficient diet (5 IU sham vs. 20 IU sham: $p = 0.039$).

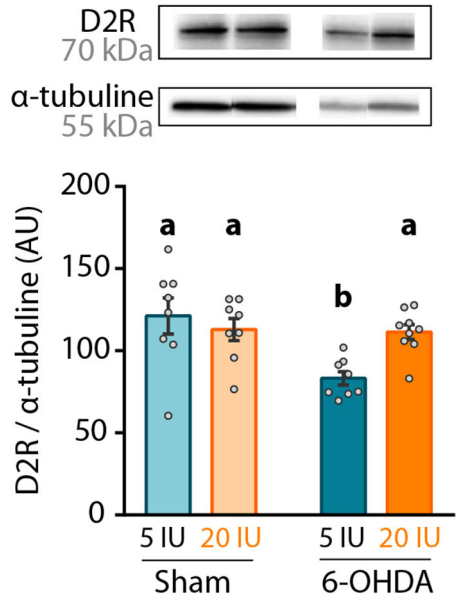

FIGURE 6 | Impact of 6-OHDA lesion and dietary vitamin A on D2R protein levels in the striatum. Representative western blot for dopamine D2 receptor (D2R) in striatum homogenates (upper inset) and quantification (graph). Protein expression levels were normalized with α-tubuline for each sample. Results are expressed as means ± SEM with individual data points. a, b: values significantly different ($p < 0.005$). Details of the statistical analysis are summarized in **Table 3**.

DISCUSSION

In this study, we examined the impact of preventive vitamin A supplementation in a PD rat model on motor behavior and dopamine transmission with the initial hypothesis that vitamin A would protect ALDH1A1$^+$ DA neurons.

First of all, when one is studying vitamin A supplementation, the levels of vitamin A intake have to be precisely controlled since chronic high doses of vitamin A can lead to oxidative stress and toxicity for the liver (8, 36, 37). In rats, common levels for sufficient retinol intakes range between 5 and 8 IU/g of diet, while vitamin A supplementation ranges from 20 to ∼50 IU/g (31, 38, 39). In the current study, we used a dose at the bottom range of supplementation, with 20 IU/g of diet, which corresponds to ∼600 IU/ day or 180μg retinol/day. This level of vitamin A supplementation is considered as comparable to therapeutic doses used in humans (40, 41). However, the toxicity of this dose is unclear. Indeed, oxidative stress has been observed (40, 41), but other reports showed no deleterious effect on behavior and inflammatory status (31, 38). In our conditions, vitamin A supplementation (20 IU/g of diet) for 8 weeks was sufficient to increase retinol in the liver, the storage organ for retinol, but we did not identify any adverse effect of vitamin A supplementation, as illustrated by measures of body weight, motor function and DA transmission. Thus, we conclude that in our conditions, vitamin A supplementation was adequate.

Second, we explored the impact of 6-OHDA lesion and vitamin A supplementation on motor function using 3 complementary motor tests (23, 42). We did not observe strong effect of 6-OHDA and vitamin A supplementation in the rotarod test, and only an effect of the injection in the step test. In both tests, the animal was forced to move to maintain its equilibrium. By contrast, our data revealed positive effects of vitamin A supplementation in the cylinder test, where the animals were free to make voluntary movements, in accordance with their natural instinct of exploration (43). Voluntary movements are controlled by the implicit motivation of producing movement, which is modulated by nigrostriatal DA neurons (16, 44). Yet, motor motivation is reduced in PD patients, as measured by reduced vigor of movement (45, 46). Here, our data suggests that vitamin A supplementation in 6-OHDA lesioned rats has a beneficial impact on motor function through the improvement of motivation for voluntary movements.

In order to identify neurobiological mechanisms by which vitamin A supplementation improved motor function in 6-OHDA lesion rats, we focused our analyses on ALDH1A1 expressing DA neurons. These neurons constitute the sub-population of DA neurons that are preferentially degenerating in PD patients (15, 16), and their role in motor learning and motor vigor has been recently revealed in mice (18). Intriguingly, these neurons lose their ability to express ALDH1A1 before degenerating, suggesting that ALDH1A1 enzyme may have a protective effect for these neurons (15, 47). Yet, ALDH1A1 expression is controlled by retinoic acid, the active metabolite of vitamin A, therefore, we initially hypothesized that vitamin A intake was able to modulate ALDH1A1 expression in SNc neurons.

In humans, ALDH1A1 is also an enzyme that raises interest, since it has been identified as a potential biomarker for PD (15, 48–50). Indeed, mRNA levels of ALDH1A1 in peripheral blood are significantly decreased in PD patients compare to control cases (50). Moreover, clinical studies showed that the genetic variability of *ALDH1A1* is a good predictive factor for PD diagnosis and the progress rate of the disease (51–53). Yet, if vitamin A is necessary for ALDH1A1 expression, a decreased vitamin A bioavailability may constitute a risk factor for the disease. However, no clear link has been established yet in PD patients between vitamin A function, ALDH1A1 expression and PD symptoms (8).

Here, we show that in sham rats, vitamin A supplementation tended to increase the number of ALDH1A1$^+$ DA neurons in the SNc, which leads to a significant increase of ALDH1A1 fibers in the posterior striatum. This supports the fact that dietary vitamin A supplementation can sustain ALDH1A1 expression in DA neurons. However, this increase of ALDH1A1$^+$ neurons and fibers in the striatum with vitamin A supplementation was not significant in 6-OHDA rats. This may be explained by the delay after the lesion chosen here. Indeed, it is possible that protection of ALDH1A1$^+$ neurons with vitamin A supplementation was effective after the lesion but not maintained in time. In addition,

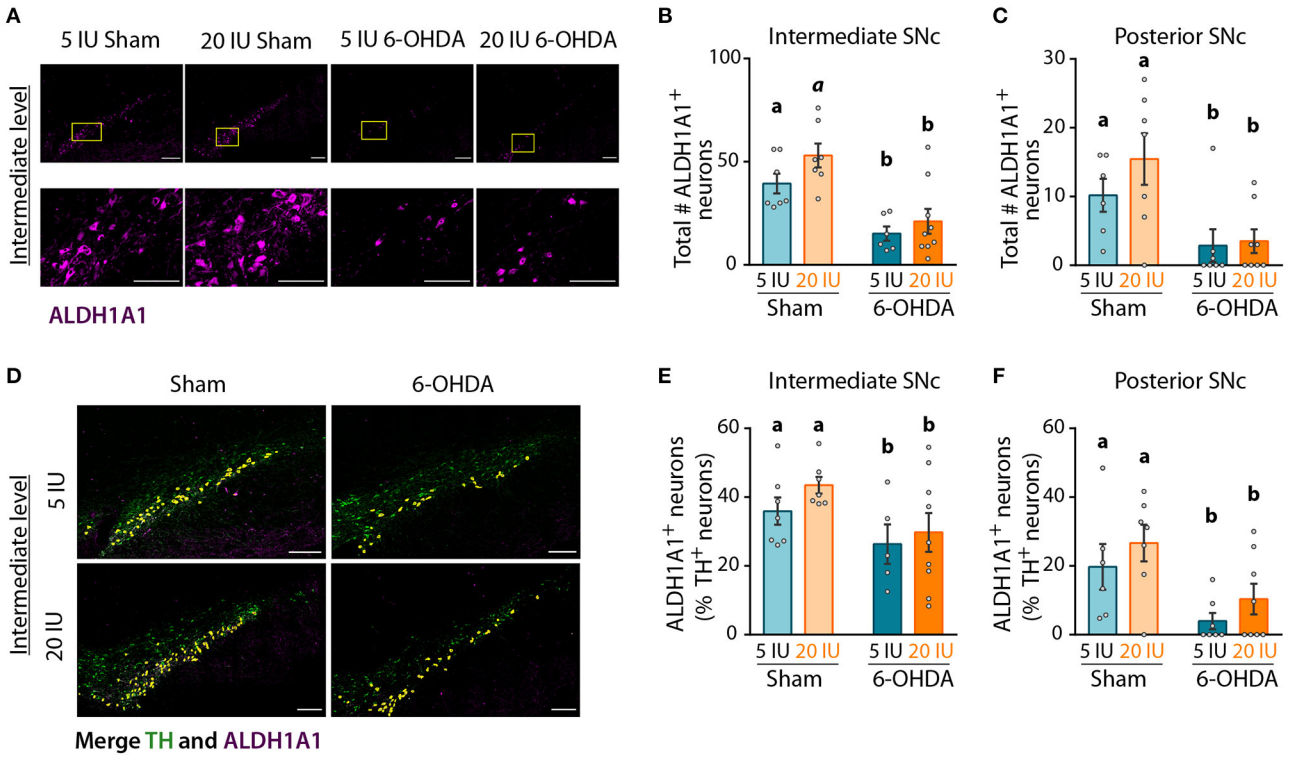

FIGURE 7 | Impact of 6-OHDA lesion and dietary vitamin A on ALDH1A1$^+$ DA neurons. **(A)** Representative images for the 4 experimental groups of intermediate SNc (lesioned side) immunostained for aldehyde dehydrogenase A subtype a1 (ALDH1A1) (magenta). Top images correspond to a mosaic of multiple images taken individually at 40X, scale bar: 200 μm. Bottom images correspond to a zoom from mosaic images (yellow squares in top images), scale bar: 100 μm. **(B,C)** Quantification of the number (#) of ALDH1A1$^+$ neurons in intermediate **(B)** and posterior **(C)** SNc. **(D)** Representative images for the 4 experimental groups of intermediate SNc (lesioned side) immunostained for tyrosine hydroxylase (TH) (green) and ALDH1A1 (magenta). Co-staining is highlighted in yellow. Scale bar: 200 μm. **(E,F)** Quantification of the number (#) of ALDH1A1$^+$ and TH$^+$ neurons expressed in percent (%) of TH$^+$ neurons in intermediate **(E)** and posterior **(F)** SNc. Results are expressed as means ± SEM with individual data points. a, b: values significantly different ($p < 0.005$); *a* indicate a trend for statistical significance ($p = 0.09$). Details of the statistical analysis are summarized in **Table 3**.

we used a mild supplementation (20 IU/g of diet), therefore, a higher vitamin A intake, still in therapeutic range (e.g. 40 IU/g of diet) may exhibit more pronounced effects on 6-OHDA-induced motor and neurobiological alterations. Furthermore, ALDH1A1$^+$ DA neurons are distributed in certain territories of the SNc and ALDH1A1$^+$ fibers are preferentially innervating striosomes compartments of the striatum (16, 54–56). Therefore, a more detailed analysis of ALDH1A1$^+$ DA neurons, accounting for these sub-territories may reveal a more specific impact of vitamin A on ALDH1A1$^+$ neurons.

Finally, this work revealed that the stronger improvement induced by vitamin A supplementation in 6-OHDA rats in addition to voluntary movement, concerned RXRγ and D2R expression in the striatum. RXRγ is a sub-type of RXR; its expression is directly controlled by retinoic acid levels and its most potent endogenous ligand is 9-cis-dihydro retinoic acid (57). The striatum is the brain structure containing the highest levels of RXRγ (58), indicating its crucial role for striatum function. Through dimerization with other nuclear receptors, such as RARβ or Nur77, RXRγ has an important function for the development of the striatum and the nigro-striatal pathway (59–61), and for dopamine transmission at adult age (62). As a consequence, mice lacking RARβ and/or RXRγ exhibit strong motor impairments along with alteration of DA transmission, analog to PD mice models (58). Of note, RXRγ controls the expression of D2R in the striatum (32, 63). Here, our data shows that increased retinol level in the liver is reflected in the striatum by the increased expression of RXRγ. Consequently, D2R expression is also significantly increased in 6-OHDA rats under vitamin A supplementation, compared to 6-OHDA rats under sufficient diet. This increase in D2R expression may explain improved motor function in 6-OHDA rats under vitamin A supplementation. For future studies, a deeper analysis of retinoid and dopamine receptors may help to better understand the role of retinoid signaling in improving striatal function with vitamin A supplementation.

In conclusion, our study revealed beneficial impact of vitamin A supplementation on striatal function, with improved voluntary movements, increased expression of RXRγ and

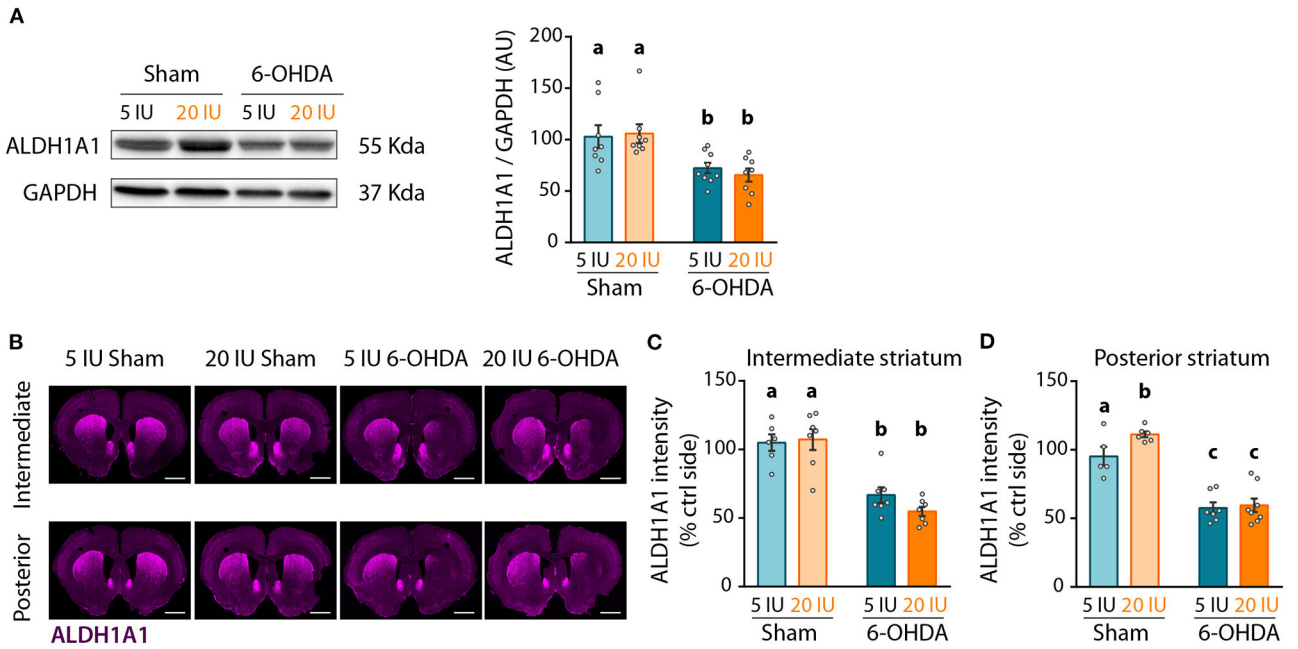

FIGURE 8 | Impact of 6-OHDA lesion and dietary vitamin A on extent of ALDH1A1$^+$ fibers in the striatum. **(A)** Representative western blot for aldehyde dehydrogenase A subtype a1 (ALDH1A1) in striatum homogenates (left inset) and quantification (graph). Protein expression levels were normalized with GAPDH for each sample. **(B)** Representative images for the 4 experimental groups of intermediate and posterior striatum (lesioned and control sides) immunostained for ALDH1A1 (magenta). Scale bar: 1000 μm. **(C,D)** Quantification of fluorescence intensity of ALDH1A1 staining in intermediate **(C)** and posterior **(D)** striatum. ALDH1A1 intensity is normalized to the ipsilateral side. Results are expressed as means ± SEM with individual data points. a–c: values significantly different ($p < 0.005$). Details of the statistical analysis are summarized in **Table 3**.

D2R in the striatum, and a trend for more ALDH1A1$^+$ DA neurons and fibers. A more sustained supplementation (amount and/or duration) with a more degenerative rat model, such as expression of human alpha-synuclein in SNc (64) may help to better understand the role of dietary vitamin A in the survival of DA neurons in the context of PD. In addition, this work may trigger future research to investigate the beneficial effect of preventive vitamin A supplementation in patients, particularly for those with low vitamin A metabolism.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Comité d'éthique de Bordeaux - CEEA 50.

AUTHOR CONTRIBUTIONS

CB-B: conceptualization and project administration. CB-B and SV: funding and supervision of staff. AM, JL, MD, SA, VS-P, ER, SV, and CB-B: experiments. AM and CB-B: statistical analyses and writing—original draft. AM: visualization. FD, RG, CB, AM, SA, CB-B, and VP: writing—review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.811843/full#supplementary-material

REFERENCES

1. Chen H. The changing landscape of parkinson epidemiologic research. *J Park Dis.* (2018) 8:1–12. doi: 10.3233/JPD-171238
2. Chen Y, Sun X, Lin Y, Zhang Z, Gao Y, Wu IXY. Non-genetic risk factors for Parkinson's disease: an overview of 46 systematic reviews. *J Park Dis.* (2021) 11:919–35. doi: 10.3233/JPD-202521
3. Gupta A, Dawson VL, Dawson TM. What causes cell death in Parkinson's disease? *Ann Neurol.* (2009) 64:S3–S15. doi: 10.1002/ana.21573
4. von Linstow CU, DeLano-Taylor M, Kordower JH, Brundin P. Does developmental variability in the number of midbrain dopamine neurons affect individual risk for sporadic Parkinson's disease? *J Park Dis.* (2020) 10:405–11. doi: 10.3233/JPD-191877
5. Boulos C, Yaghi N, El Hayeck R, Heraoui GN, Fakhoury-Sayegh N. Nutritional risk factors, microbiota and Parkinson's disease: what is the current evidence? *Nutrients.* (2019) 11:1896. doi: 10.3390/nu11081896
6. Mischley LK, Lau RC, Bennett RD. Role of diet and nutritional supplements in Parkinson's disease progression. *Oxid Med Cell Longev.* (2017) 2017:1–9. doi: 10.1155/2017/6405278
7. Shearer KD, Stoney PN, Morgan PJ, McCaffery PJ. A vitamin for the brain. *Trends Neurosci.* (2012) 35:733–41. doi: 10.1016/j.tins.2012.08.005
8. Marie A, Darricau M, Touyarot K, Parr-Brownlie LC, Bosch-Bouju C. Role and mechanism of vitamin A metabolism in the pathophysiology of Parkinson's disease. *J Park Dis.* (2021) 11:949–70. doi: 10.3233/JPD-212671
9. Carta M, Stancampiano R, Tronci E, Collu M, Usiello A, Morelli M, et al. Vitamin A deficiency induces motor impairments and striatal cholinergic dysfunction in rats. *Neuroscience.* (2006) 139:1163–72. doi: 10.1016/j.neuroscience.2006.01.027
10. Friling S, Bergsland M, Kjellander S. Activation of Retinoid X Receptor increases dopamine cell survival in models for Parkinson's disease. *BMC Neurosci.* (2009) 10:146. doi: 10.1186/1471-2202-10-146
11. Yin L-H, Shen H, Diaz-Ruiz O, Bäckman CM, Bae E, Yu S-J, et al. Early post-treatment with 9-cis retinoic acid reduces neurodegeneration of dopaminergic neurons in a rat model of Parkinson's disease. *BMC Neurosci.* (2012) 13:120. doi: 10.1186/1471-2202-13-120
12. Esteves M, Cristóvão AC, Saraiva T, Rocha SM, Baltazar G, Ferreira L, et al. Retinoic acid-loaded polymeric nanoparticles induce neuroprotection in a mouse model for Parkinson's disease. *Front Aging Neurosci.* (2015) 7:20. doi: 10.3389/fnagi.2015.00020
13. Spathis AD, Asvos X, Ziavra D, Karampelas T, Topouzis S, Cournia Z, et al. Nurr1:RXRα heterodimer activation as monotherapy for Parkinson's disease. *Proc Natl Acad Sci.* (2017) 114:3999–4004. doi: 10.1073/pnas.1616874114
14. Damier P, Hirsch EC, Agid Y, Graybiel AM. The substantia nigra of the human brain II Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain J Neurol.* (1999) 122:1437–48. doi: 10.1093/brain/122.8.1437
15. Liu G, Yu J, Ding J, Xie C, Sun L, Rudenko I, et al. Aldehyde dehydrogenase 1 defines and protects a nigrostriatal dopaminergic neuron subpopulation. *J Clin Invest.* (2014) 124:3032–46. doi: 10.1172/JCI72176
16. Carmichael K, Evans RC, Lopez E, Sun L, Kumar M, Ding J, et al. Function and regulation of ALDH1A1-positive nigrostriatal dopaminergic neurons in motor control and Parkinson's disease. *Front Neural Circuits.* (2021) 15:644776. doi: 10.3389/fncir.2021.644776
17. Wey MC-Y, Fernandez E, Martinez PA, Sullivan P, Goldstein DS, Strong R. Neurodegeneration and motor dysfunction in mice lacking cytosolic and mitochondrial aldehyde dehydrogenases: implications for Parkinson's disease. *PLoS ONE.* (2019) 7:e31522. doi: 10.1371/journal.pone.0031522
18. Wu J, Kung J, Dong J, Chang L, Xie C, Habib A, et al. Distinct connectivity and functionality of aldehyde dehydrogenase 1a1-positive nigrostriatal dopaminergic neurons in motor learning. *Cell Rep.* (2012) 28:1167–81.e7. doi: 10.1016/j.celrep.2019.06.095
19. Decressac M, Mattsson B, Björklund A. Comparison of the behavioural and histological characteristics of the 6-OHDA and α-synuclein rat models of Parkinson's disease. *Exp Neurol.* (2012) 235:306–15. doi: 10.1016/j.expneurol.2012.02.012
20. Hernandez-Baltazar D, Zavala-Flores LM, Villanueva-Olivo A. The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model. *Neurol Engl Ed.* (2017) 32:533–9. doi: 10.1016/j.nrleng.2015.06.019
21. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates.* Ed 6. London: Elsevier. (2007)
22. Olsson M, Nikkhah G, Bentlage C, Bjorklund A. Forelimb akinesia in the rat Parkinson model: differential effects of dopamine agonists and nigral transplants as assessed by a new stepping test. *J Neurosci.* (1995) 15:3863–75. doi: 10.1523/JNEUROSCI.15-05-03863.1995
23. Winkler C, Kirik D, Björklund A, Dunnett SB. Chapter 11 Transplantation in the rat model of Parkinson's disease: ectopic versus homotopic graft placement. In: *Progress in Brain Research Elsevier*, (2000). p. 233–265. doi: 10.1016/S0079-6123(00)27012-X
24. Sarukhani M, Haghdoost-Yazdi H, Sarbazi Golezari A, Babayan-Tazehkand A, Dargahi T, Rastgoo N. Evaluation of the antiparkinsonism and neuroprotective effects of hydrogen sulfide in acute 6-hydroxydopamine-induced animal model of Parkinson's disease: behavioral, histological and biochemical studies. *Neurol Res.* (2018) 40:525–33. doi: 10.1080/01616412.2017.1390903
25. Biesalski HK, Ehrenthal W, Gross M, Hafner G, Harth O. Rapid determination of retinol (vitamin A) in serum by high pressure liquid chromatography (HPLC). *Int J Vitam Nutr Res Int Z Vitam- Ernahrungsforschung J Int Vitaminol Nutr.* (1983) 53:130–7.
26. Monchaux De Oliveira C, Pourtau L, Vancassel S, Pouchieu C, Capuron L, Gaudout D, et al. Saffron extract-induced improvement of depressive-like behavior in mice is associated with modulation of monoaminergic neurotransmission. *Nutrients.* (2021) 13:904. doi: 10.3390/nu13030904
27. Favier M, Carcenac C, Drui G, Vachez Y, Boulet S, Savasta M, et al. Implication of dorsostriatal D3 receptors in motivational processes: a potential target for neuropsychiatric symptoms in Parkinson's disease. *Sci Rep.* (2017) 7:41589. doi: 10.1038/srep41589
28. Etchamendy N, Enderlin V, Marighetto A, Pallet V, Higueret P, Jaffard R. Vitamin A deficiency and relational memory deficit in adult mice: relationships with changes in brain retinoid signalling. *Behav Brain Res.* (2003) 145:37–49. doi: 10.1016/S0166-4328(03)00099-8
29. Husson M, Enderlin V, Alfos S, Boucheron C, Pallet V, Higueret P. Expression of neurogranin and neuromodulin is affected in the striatum of vitamin A-deprived rats. *Mol Brain Res.* (2004) 123:7–17. doi: 10.1016/j.molbrainres.2003.12.012
30. Krezel W, Kastner P, Chambon P. Differential expression of retinoid receptors in the adult mouse central nervous system. *Neuroscience.* (1999) 89:1291–300. doi: 10.1016/S0306-4522(98)00342-X
31. Kunzler A, Ribeiro CT, Gasparotto J, Petiz LL, da Rosa Silva HT, da Silva JD, et al. The effects of retinol oral supplementation in 6-hydroxydopamine dopaminergic denervation model in Wistar rats. *Neurochem Int.* (2019) 125:25–34. doi: 10.1016/j.neuint.2019.02.002
32. Samad TA, Krezel W, Chambon P, Borrelli E. Regulation of dopaminergic pathways by retinoids: Activation of the D2 receptor promoter by members of the retinoic acid receptor–retinoid X receptor family. *Proc Natl Acad Sci USA.* (1997) 6. doi: 10.1073/pnas.94.26.14349
33. Mann T, Zilles K, Dikow H, Hellfritsch A, Cremer M, Piel M, et al. Dopamine, noradrenaline and serotonin receptor densities in the striatum of hemiparkinsonian rats following botulinum neurotoxin-A injection. *Neuroscience.* (2018) 374:187–204. doi: 10.1016/j.neuroscience.2018.01.053

34. Martres MP, Sokoloff P, Giros B, Schwartz JC. Effects of dopaminergic transmission interruption on the d_2 receptor isoforms in various cerebral tissues. *J Neurochem.* (1992) 58:673–9. doi: 10.1111/j.1471-4159.1992.tb09770.x
35. Mishra A, Singh S, Tiwari V, Chaturvedi S, Wahajuddin M, Shukla S. Dopamine receptor activation mitigates mitochondrial dysfunction and oxidative stress to enhance dopaminergic neurogenesis in 6-OHDA lesioned rats: a role of Wnt signalling. *Neurochem Int.* (2019) 129:104463. doi: 10.1016/j.neuint.2019.104463
36. Jick SS, Kremers HM, Vasilakis-Scaramozza C. Isotretinoin use and risk of depression, psychotic symptoms, suicide, and attempted suicide. *Arch Dermatol.* (2000) 136:1231–6. doi: 10.1001/archderm.136.10.1231
37. Klamt F, Roberto de. Oliveira M, Moreira JCF. Retinol induces permeability transition and cytochrome c release from rat liver mitochondria. *Biochim Biophys Acta BBA - Gen Subj.* (2005) 1726:14–20. doi: 10.1016/j.bbagen.2005.07.016
38. Touyarot K, Bonhomme D, Roux P, Alfos S, Lafenêtre P, Richard E, et al. mid-life vitamin A supplementation prevents age-related spatial memory deficits and hippocampal neurogenesis alterations through CRABP-I. *PLoS ONE.* (2013) 8:e72101. doi: 10.1371/journal.pone.0072101
39. Létondor A, Buaud B, Vaysse C, Richard E, Layé S, Pallet V, et al. EPA/DHA and vitamin A supplementation improves spatial memory and alleviates the age-related decrease in hippocampal RXRγ and kinase expression in rats. *Front Aging Neurosci.* (2016) 8:103. doi: 10.3389/fnagi.2016.00103
40. de Oliveira MR, Moreira JCF. Acute and chronic vitamin A supplementation at therapeutic doses induces oxidative stress in submitochondrial particles isolated from cerebral cortex and cerebellum of adult rats. *Toxicol Lett.* (2007) 173:145–50. doi: 10.1016/j.toxlet.2007.07.002
41. de Oliveira MR, Oliveira MWS, Behr GA, Hoff MLM, da Rocha RF, Moreira JCF. Evaluation of the effects of vitamin A supplementation on adult rat substantia nigra and striatum redox and bioenergetic states: Mitochondrial impairment, increased 3-nitrotyrosine and α-synuclein, but decreased D2 receptor contents. *Prog Neuropsychopharmacol Biol Psychiatry.* (2009) 33:353–62. doi: 10.1016/j.pnpbp.2008.12.020
42. Carter R, Morton J, Dunnet S. Motor coordination and balance in rodents. *Curr Protoc Neurosci.* (2001) 8:8–12.
43. Su R, Zhen J, Wang W, Zhang J, Zheng Y, Wang X. Time-course behavioral features are correlated with Parkinson's disease-associated pathology in a 6-hydroxydopamine hemiparkinsonian rat model. *Mol Med Rep.* (2017) 17:3356–63. doi: 10.3892/mmr.2017.8277
44. Dudman JT, Krakauer JW. The basal ganglia: from motor commands to the control of vigor. *Curr Opin Neurobiol.* (2016) 37:158–66. doi: 10.1016/j.conb.2016.02.005
45. Mazzoni P, Hristova A, Krakauer JW. Why Don't We Move Faster? *Parkinson's Disease, Movement Vigor, and Implicit Motivation J Neurosci.* (2007) 27:7105–16. doi: 10.1523/JNEUROSCI.0264-07.2007
46. Colón-Semenza C, Fulford D, Ellis T. Effort-Based Decision-Making for Exercise in People with Parkinson's Disease. *J Park Dis.* (2021) 11:725–35. doi: 10.3233/JPD-202353
47. Cai H, Liu G, Sun L, Ding J. Aldehyde Dehydrogenase 1 making molecular inroads into the differential vulnerability of nigrostriatal dopaminergic neuron subtypes in Parkinson's disease. *Transl Neurodegener.* (2014) 3:27. doi: 10.1186/2047-9158-3-27
48. Galter D, Buervenich S, Carmine A, Anvret M, Olson L. ALDH1 mRNA: presence in human dopamine neurons and decreases in substantia nigra in Parkinson's disease and in the ventral tegmental area in schizophrenia. *Neurobiol Dis.* (2003) 14:637–47. doi: 10.1016/j.nbd.2003.09.001
49. Grünblatt E, Zehetmayer S, Jacob CP, Müller T, Jost WH, Riederer P. Pilot study: peripheral biomarkers for diagnosing sporadic Parkinson's disease. *J Neural Transm Vienna Austria.* (2010) 117:1387–93. doi: 10.1007/s00702-010-0509-1
50. Fan H-H, Guo Q, Zheng J, Lian Y-Z, Huang S-S, Sun Y, et al. ALDH1A1 Genetic Variations May Modulate Risk of Parkinson's Disease in Han Chinese Population. *Front Neurosci.* (2021) 15:620929. doi: 10.3389/fnins.2021.620929
51. Molochnikov L, Rabey JM, Dobronevsky E, Bonuccelli U, Ceravolo R, Frosini D, et al. A molecular signature in blood identifies early Parkinson's disease. *Mol Neurodegener.* (2012) 7:26. doi: 10.1186/1750-1326-7-26
52. Salas-Leal AC, Sandoval-Carrillo A, Romero-Gutiérrez E, Castellanos-Juárez FX, Méndez-Hernández EM, La Llave-León O, et al. rs3764435 Associated With Parkinson's Disease in Mexican Mestizos: Case-Control Study Reveals Protective Effects Against Disease Development and Cognitive Impairment. *Front Neurol.* (2019) 10:1066. doi: 10.3389/fneur.2019.01066
53. Rabey JM, Yarden J, Dotan N, Mechlovich D, Riederer P, Youdim MBH. Creation of a gene expression classifier for predicting Parkinson's disease rate of progression. *J Neural Transm.* (2020) 127:755–62. doi: 10.1007/s00702-020-02194-y
54. Sgobio C, Wu J, Zheng W, Chen X, Pan J, Salinas AG, et al. Aldehyde dehydrogenase 1–positive nigrostriatal dopaminergic fibers exhibit distinct projection pattern and dopamine release dynamics at mouse dorsal striatum. *Sci Rep.* (2017) 7:5283. doi: 10.1038/s41598-017-05598-1
55. Poulin J-F, Caronia G, Hofer C, Cui Q, Helm B, Ramakrishnan C, et al. Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches. *Nat Neurosci.* (2018) 21:1260–71. doi: 10.1038/s41593-018-0203-4
56. Pan J, Yu J, Sun L, Xie C, Chang L, Wu J, et al. ALDH1A1 regulates postsynaptic μ-opioid receptor expression in dorsal striatal projection neurons and mitigates dyskinesia through transsynaptic retinoic acid signaling. *Sci Rep.* (2019) 9:3602. doi: 10.1038/s41598-019-40326-x
57. Rühl R, Krezel W, de Lera AR. 9-Cis-13,14-dihydroretinoic acid, a new endogenous mammalian ligand of retinoid X receptor and the active ligand of a potential new vitamin A category: vitamin A5. *Nutr Rev.* (2018) 76:929–41. doi: 10.1093/nutrit/nuy057
58. Krezel W, Ghyselinck N, Samad TA, Dupé V, Kastner P, Borrelli E, et al. Impaired locomotion and dopamine signaling in retinoid receptor mutant mice. *Science.* (1998) 279:863–7. doi: 10.1126/science.279.352.863
59. Rataj-Baniowska M, Niewiadomska-Cimicka A, Paschaki M, Szyszka-Niagolov M, Carramolino L, Torres M, et al. Retinoic acid receptor β controls development of striatonigral projection neurons through FGF-dependent and meis1-dependent mechanisms. *J Neurosci Off J Soc Neurosci.* (2015) 35:14467–75. doi: 10.1523/JNEUROSCI.1278-15.2015
60. Niewiadomska-Cimicka A, Krzyzosiak A, Ye T, Podleśny-Drabiniok A, Dembélé D, Dollé P, et al. Genome-wide analysis of RARβ transcriptional targets in mouse striatum links retinoic acid signaling with Huntington's disease and other neurodegenerative disorders. *Mol Neurobiol.* (2017) 54:3859–78. doi: 10.1007/s12035-016-0010-4
61. Podleśny-Drabiniok A, Sobska J, de Lera AR, Gołembiowska K, Kamińska K, Dollé P, et al. Distinct retinoic acid receptor (RAR) isotypes control differentiation of embryonal carcinoma cells to dopaminergic or striatopallidal medium spiny neurons. *Sci Rep.* (2017) 7:13671. doi: 10.1038/s41598-017-13826-x
62. Lévesque D, Rouillard C. Nur77 and retinoid X receptors: crucial factors in dopamine-related neuroadaptation. *Trends Neurosci.* (2007) 30:22–30. doi: 10.1016/j.tins.2006.11.006
63. Krzyzosiak A, Szyszka-Niagolov M, Wietrzych M, Gobaille S, Muramatsu S, Krezel W. Retinoid x receptor gamma control of affective behaviors involves dopaminergic signaling in mice. *Neuron.* (2010) 66:908–20. doi: 10.1016/j.neuron.2010.05.004
64. Bourdenx M, Dovero S, Engeln M, Bido S, Bastide MF, Dutheil N, et al. Lack of additive role of ageing in nigrostriatal neurodegeneration triggered by α-synuclein overexpression. *Acta Neuropathol Commun.* (2015) 3:46. doi: 10.1186/s40478-015-0222-2

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An Increased Anticholinergic Drug Burden Index Score Negatively Affect Nutritional Status in Older Patients Without Dementia

Esra Ates Bulut[1], *Neziha Erken*[2], *Derya Kaya*[2], *Fatma Sena Dost*[2] *and Ahmet Turan Isik*[2]*

[1] *Department of Geriatric Medicine, Adana City Training and Research Hospital, Adana, Turkey,* [2] *Unit for Aging Brain and Dementia, Department of Geriatric Medicine, Faculty of Medicine, Dokuz Eylul University, Izmir, Turkey*

Introduction/Aim: Anticholinergic drugs, which have severe central and peripheric side effects, are frequently prescribed to older adults. Increased anticholinergic drug burden is associated with poor physical and cognitive functions. On the other side, the impact of anticholinergics on nutritional status is not elaborated in the literature. Therefore, this study was aimed to investigate the effect of the anticholinergic burden on nutrition.

Materials and Methods: Patients who underwent comprehensive geriatric assessment (CGA) 6 months apart were included in the study. Patients diagnosed with dementia were excluded because of the difference in the course of cognition, physical performance and nutrition. Nutritional status and global cognition were evaluated using Mini Nutritional Assessment-short form (MNA-SF), Mini-Mental State Examination (MMSE). Anticholinergic drug burden was assessed with the Drug Burden Index (DBI), enabling a precise dose-related cumulative exposure. Patients were divided into three groups according to DBI score: 0, no DBI exposure; 0–1, low risk; and ≥1, high risk. Regression analysis was performed to show the relationship between the difference in CGA parameters and the change in DBI score at the sixth month.

Results: A total of 423 patients were included in the study. Participants' mean age was 79.40 ± 7.50, and 68.6% were female. The DBI 0 score group has better MMSE and MNA-SF scores and a lower rate of falls, polypharmacy, malnutrition, and risk of malnutrition in the baseline. Having malnutrition or risk of malnutrition is 2.21 times higher for every one-unit increase in DBI score. Additionally, during the 6-month follow-up, increased DBI score was associated with decreased MNA-SF and MMSE score, albumin.

Conclusions: The harmful effects of anticholinergics may be prevented because anticholinergic activity is a potentially reversible factor. Therefore, reducing exposure to drugs with anticholinergic activity has particular importance in geriatric practice.

Keywords: anticholinergic activity, cognitive functions, drug burden index, malnutrition, older adults

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Mona E. Aboutabl,
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University of Alberta, Canada

***Correspondence:**
Ahmet Turan Isik
atisik@yahoo.com

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INTRODUCTION

Older adults are vulnerable to adverse drug reactions and drug-drug interactions because they frequently experience multiple systemic diseases, leading to multiple drug use. Hence, polypharmacy is a significant concern in the management of older patients, and several tools have been developed to review medications, such as Beers Criteria (1), STOPP (Screening Tool of Older Persons' Prescriptions) and START (Screening Tool to Alert to Right Treatment) criteria (2). Basically, anticholinergic effect determines the safety of the drugs. Anticholinergics inhibit acetylcholine action, which has a crucial role in the regulation of several central and peripheral nervous system functions. Significant anticholinergic effects include dry mouth, constipation, tachycardia, urinary retention, drowsiness, and confusion (3).

Mainly, immobilization, urinary incontinence, neurologic and psychiatric comorbidities (dementia, depression, Parkinson's disease, epilepsy) were reported as the most significant risk factors for the anticholinergic prescription (4). This group is also at risk of malnutrition. Due to the effects on gastrointestinal motility and secretions, as well as sedative potency, anticholinergics were reported to be related to gait imbalance, dysphagia, delirium (5, 6). A dry mouth may cause difficulty in swallowing, and decreased gastric motility and constipation may contribute to satiety and anorexia. Drowsiness and confusion may cause dehydration, swallowing problems, and aspiration (5), each of which poses a severe risk for malnutrition.

Moreover, older adults are more susceptible to anticholinergic agents because of physiological and pathological changes with aging, including decreased physiological reserve, pharmacodynamic and pharmacokinetic alterations. The anticholinergic burden is termed the cumulative effect of anticholinergic agents and reported as a predictor of cognitive decline, poorer physical performance, falls, and even mortality (7–11). Currently, the optimal scale has not been determined to qualify total anticholinergic drug burden (12). The Drug Burden Index (DBI) is one of the most commonly used validated risk assessment scales to estimate cumulative exposure to anticholinergic medications (13). Additionally, the DBI enables dose-related measurement of each drug and offers an extensive evaluation of several drug classes. Accumulating evidence indicates DBI is a useful indicator of adverse health outcomes and functionality in older adults (14, 15).

Many studies have investigated the relationship between anticholinergic drug burden and cognitive, physical functions. However, the information about the impact of anticholinergics on the nutritional status is limited. We aimed to show the association between DBI score and malnutrition in older adults.

MATERIALS AND METHODS

Patients and Procedures

The records of patients who applied to the geriatric outpatient clinic between January 2017 and March 2020 were retrospectively reviewed. A total of 1,805 patients' files were screened. Patients who were older than 65 years old and underwent comprehensive geriatric assessment (CGA) (16) two times at 6-month intervals were included in the study. The number of patients who were evaluated twice at the end of 6 months was 811. Exclusion criteria were determined as a severe illness that may impair general health status during the follow-up period, such as acute coronary syndrome, sepsis, acute renal failure, gastrointestinal bleeding, and diagnosis of cancer, immobility, substance and alcohol abuse. Additionally, patients diagnosed with major neurocognitive disorders, including Alzheimer's disease, vascular dementia, Lewy body dementia, and frontotemporal dementia, which cause progressive deterioration of cognitive domains, were excluded from the study because many medications used in the treatment of those patients, including antipsychotics, sedatives, and antidepressants, also increase DBI scores, and the trajectory of dementia differs in functionality, cognition, nutritional status from cognitive intact older adults. As a result, a total of 423 patients, who did not have exclusion criteria and whose records were eligible, were included in the study.

The study was designed as a retrospective observational cohort study. The primary outcome was defined to show the difference of nutritional parameters including Mini-Nutritional Assessment short-form, weight, albumin between patient groups with increased or decreased DBI score at the end of 6 months, and secondary outcomes were global cognitive performance and functionality.

The investigation was conformed to the Declaration of Helsinki and approved by the local ethics committee.

Comprehensive Geriatric Assessment and Laboratory Measurements

Detailed medication history (including dosage and duration), sociodemographic characteristics, chronic systemic diseases, comprehensive geriatric assessment parameters including Mini-Mental State Examination (MMSE), Geriatric Depression Scale (GDS) (17), Basic (Barthel) and Instrumental (Lawton) Activities of Daily Living (BADL and IADL), Mini-Nutritional Assessment short-form (MNA-SF) were obtained from records of the patients. Falls history in the last year was recorded. Dementia and depression were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition (DSM-5) criteria. Comorbidity scores of the patients were calculated using the Charlson Comorbidity Index. Polypharmacy was determined as concurrent five or more drug usage (18). Urinary incontinence was considered positive in having involuntary urinary leakage in the last 3 months except for urinary tract infection (18). Chronic non-cancer pain is accepted as lasting beyond the expected healing time or at least 3–6 months (19).

Laboratory tests including hemogram, albumin, 25-hydroxy vitamin D, vitamin B12, low-density lipoprotein (LDL), high-density lipoprotein (HDL), estimated glomerular filtration rate according to Modification of Diet in Renal Disease (MDRD) Study equation (20) were evaluated.

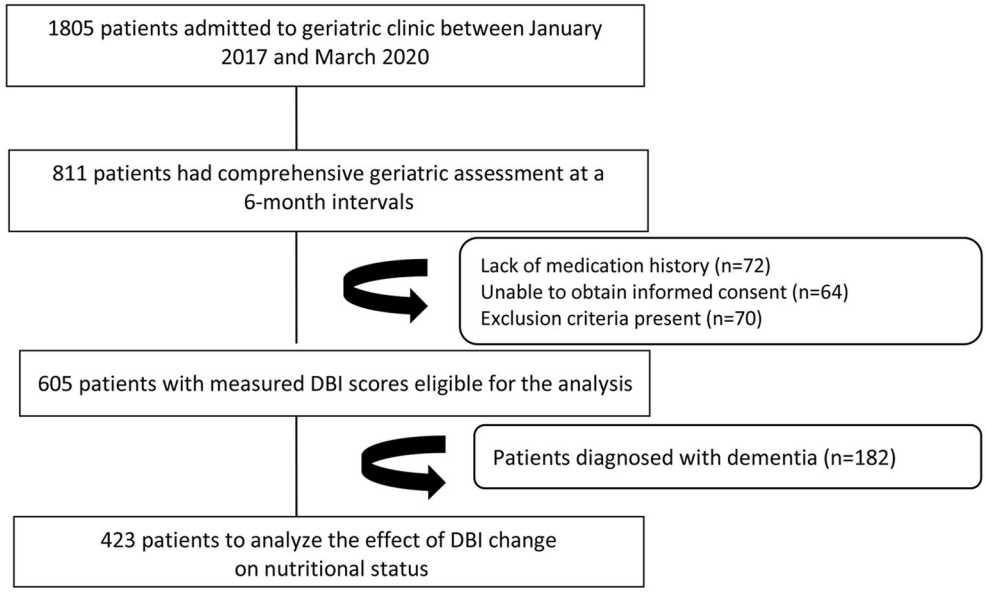

FIGURE 1 | Flowchart of the participants in the study.

Drug Burden Index and Anticholinergic Risk Groups

DBI is calculated for each regularly used drug using a formula calculating the ratio between the prescribed daily dose and the sum of the dose that gives 50% of the maximal effect and the prescribed dose. The total score was achieved by summing the score of each sedative drug separately (21). The calculation was carried out by the web portal software program "Anticholinergic Burden Calculator" (www.anticholinergicscales.es/) (22). The main drug classes that were evaluated for their anticholinergic drug burden were antihistamines, benzodiazepines, antipsychotics, selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs), alpha blockers, metoclopramide, bladder antimuscarinics and muscle relaxants. Scores of the participants were calculated as a continuous variable. Accordingly, three groups were identified: DBI score 0 (no DBI exposure), DBI score 0–1 (low), and DBI score $\geq$ 1 (high) (23).

Nutritional Assessment and Malnutrition

The patients were grouped according to MNA-SF scores: malnourished (<7 points), risk of malnutrition (8–11 points), or well-nourished ($\geq$12 points) (24). Nutritional assessment was conducted twice apart 6-months to the patients such as other CGA parameters.

Statistical Analysis

Categorical variables were evaluated with chi-square tests. Normal distribution was checked using the Kolmogorov-Smirnov test. Categorical and continuous variables are expressed as percentages (%) and mean $\pm$ standard deviations, respectively. The baseline difference of continuous variables between the three groups according to the DBI score was evaluated with the Kruskal-Wallis test and nominal. Categorical variables were evaluated with chi-square test. The relationship between nutritional status and DBI risk groups in the baseline was assessed with logistic regression analysis. In the 6th month patients without dementia were reorganized into three groups: Patients with no DBI exposure from the beginning, patients with decreased DBI score, patients with increased DBI scores. **Figure 1** shows a flowchart of the participants in the study. The change in scores was obtained by subtracting the baseline value from the 6th month follow-up value, and the delta (Δ) value was specified for each test score [(Δ) value (6th month evaluation-Baseline evaluation)]. Logistic regression analysis was performed to show the effect of change in DBI score on CGA parameters. Multiple linear regression analysis was conducted to show relationship between change in DBI score and laboratory values. *p*-values lower than 0.05 are accepted as statistically significant. All statistical analyses were performed using the SPSS 25.0 (SPSS Inc.) package program.

RESULTS

A total of 423 patients were included in the study. Participants' mean age was 79.40 $\pm$ 7.50, and 68.6% were female. When the patients were divided into three according to DBI risk score in the baseline, 225 patients had DBI 0 score, 135 had low risk, and 33 had high risk. Age, sex, and educational status were similar between the groups. Patients diagnosed with diabetes mellitus and depression were common in the low and high-risk groups ($p < 0.05$). Compared with patients with a DBI score 0, those in the risk groups had a higher rate of polypharmacy, falls and

TABLE 1 | Patients' characteristics according to basal DBI risk status.

	DBI score			
	Zero (0) ***n*: 225**	**Low risk (0-1)** ***n*: 135**	**High risk (≥1)** ***n*: 33**	***p***
Demographics				
Sex (female%)	68.2	68.9	69.7	0.98
Age (mean ± std deviation)	78.88 ± 7.43	80.51 ± 7.44	78.87 ± 8.03	0.08
Marital status (married) (%)	62.8	60.2	56.3	0.94
Education (years)	8.17 ± 4.93	8.08 ± 4.80	6.38 ± 4.81	0.16
Comorbidities (%)				
Depression	22.4	42.2	48.5	**<0.01**
Diabetes mellitus	23.5	28.9	54.5	**<0.01**
Hypertension	65.9	71.1	72.7	0.48
Ischemic cardiac disease	14.5	17.8	21.9	0.46
Charlson comorbidity index	0.82 ± 1.06	1.18+1.38	1.27 ± 1.28	**0.01**
Geriatric syndromes (%)				
Polypharmacy	35.3	65.2	87.9	**<0.01**
Urinary Incontinence	38.8	40.0	39.4	0.97
Falls	21.2	34.8	42.4	**<0.01**
Pain	46.6	50.7	59.4	0.34
Comprehensive geriatric assessment parameters				
MMSE	27.02 ± 3.28	25.87 ± 4.22	24.50 ± 4.96	**0.01**
GDS	2.52 ± 3.12	3.15 ± 3.02	5.76 ± 4.43	**<0.01**
MNA-SF	12.49 ± 1.96	11.85 ± 2.28	11.70 ± 2.08	**<0.01**
BADL	96.08 ± 6.95	93.70 ± 9.75	88.42 ± 17.20	**<0.01**
IADL	19.99 ± 4.04	18.80 ± 5.00	17.06 ± 6.15	**<0.01**
Laboratory values				
Hemoglobin (g/dL)	13.03 ± 1.48	12.73 ± 1.50	12.51 ± 1.52	**0.02**
Albumin (mg/dL)	4.19 ± 0.27	4.07 ± 0.37	4.09 ± 0.41	**0.02**
HDL (mg/dL)	58.63 ± 13.95	55.95 ± 13.65	52.11 ± 14.11	0.06
LDL (mg/dL)	133.85 ± 37.98	133.95 ± 41.44	118.64 ± 34.40	0.27
MDRD (mL/dk)	75.69 ± 18.01	72.67 ± 19.71	73.44 ± 17.65	0.32
Vitamin D (ng/mL)	20.72 ± 14.42	19.25 ± 13.08	22.62 ± 15.60	0.27
Vitamin B12 (pg/mL)	447.01 ± 330.19	537.97 ± 408.66	485.68 ± 375.56	0.25

MDRD, Modification of Diet in Renal Disease; MMSE, Mini Mental State Examination; GDS, Geriatric Depression Scale (0–15); MNA-SF, Mini Nutritional Assessment (0–12); BADL, Basic Activities of Daily Living (0–100); IADL, Instrumental Activities of Daily Living (0–23). Bold values denote statistical significance at the $p < 0.05$ level.

malnutrition, and higher Charlson Comorbidity Score, whereas lower baseline albumin, and hemoglobin levels. Moreover, the DBI 0 score group has the best values in regards to CGA parameters. **Table 1** summarizes the baseline characteristics of the patients.

According to the MNA-SF scores, normal nutritional status was statistically higher in the patients with DBI 0 score ($p < 0.01$) compared to both low and high DBI risk groups in the baseline. The total rate of malnutrition and malnutrition risk was similar in the DBI low and high risk groups. **Figure 2** shows the nutritional status of the groups in the baseline.

When the relationship between nutritional status and DBI score and number of drugs routinely used was evaluated, it was found that both parameters were associated with increased malnutrition and malnutrition risk. However, the DBI score was found to have a more substantial contribution to the risk. The odds of having malnutrition or risk of malnutrition is 2.21 times greater for every one-unit increase in DBI score. The anticholinergic characteristics of drugs is more substantial impact on the nutritional status instead of total number of drugs used. **Table 2** shows the relationship between nutritional status and drug number, and DBI scores.

Accordingly, the follow-up data of 414 patients whose DBI score of 0 from the beginning or DBI score changed was analyzed. According to the change in DBI score, three groups were obtained: no DBI exposure from the beginning, decreased DBI scores, and increased DBI scores. Delta scores did not differ between the three groups (**Table 3**).

When the change in CGA parameters was analyzed, it was shown that every one-unit increase in the DBI score decreases the positive change in nutrition score by 37%. Similarly, the MMSE score decreases by almost 40%. Moreover, a one-unit increase

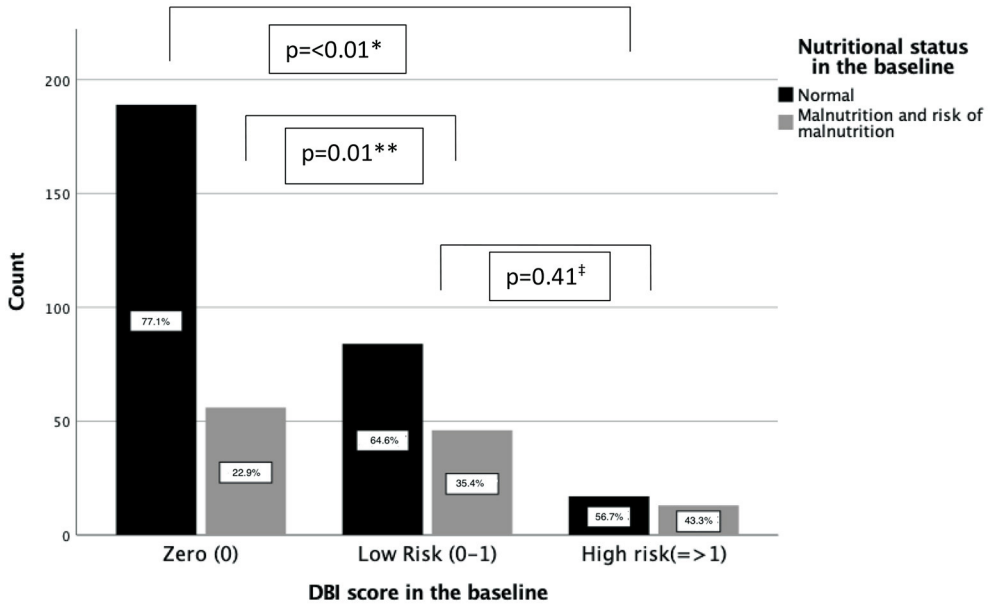

FIGURE 2 | Patients with normal nutritional status within the DBI risk groups. *indicates the *p*-value between DBI high risk and zero groups in terms of malnutrition and risk of malnutrition. **indicates the *p*-value between DBI low risk and zero groups in terms of malnutrition and risk of malnutrition. ‡indicates the *p*-value between DBI low risk and high risk groups in terms of malnutrition and risk of malnutrition.

TABLE 2 | Relationship between total number of drugs routinely used, DBI score and nutritional status of patients in the baseline.

	Malnutrition and malnutrition risk	
	OR (95%CI)	***p***
DBI score	2.21(1.31–3.72)	**0.003**
Drug number	1.13(1.06–1.20)	**<0.001**

Bold values denote statistical significance at the p < 0.05 level.

in DBI score also associated with the decrease in albumin level (**Table 4**).

DISCUSSION

The study shows that increased DBI scores are related to geriatric syndromes such as falls, malnutrition, and polypharmacy. Additionally, an increase in the DBI score during the sixth-month follow-up duration is associated with the lower cognitive and nutritional status in cognitively intact older patients. Notably, the DBI score seems more influential on nutritional status than the total drug number.

Parkinson's disease, cognitive impairment, genitourinary conditions, depression, and institutionalization are major predictors of the higher use of anticholinergic drugs. Although older patients are more vulnerable to the side effects of these drugs, anticholinergic drugs are frequently prescribed to older adults. Anticholinergic use prevalence is reported from 22.8 to 55.9% in community-dwelling older adults (12) and more than 79% of inpatients (25). Several tools and guidelines designed to review drugs accept anticholinergic drugs as potentially inappropriate drugs (26). Higher anticholinergic burden is blamed for adverse health outcomes, such as cognitive decline, poorer physical performance, falls, longer length of stay and mortality (11, 12, 27–29). The present study results also support the negative effect of the anticholinergic burden on cognitive functions. The anticholinergic effects on the central nervous system (CNS) areas responsible for movement control, balance, learning, and memory were blamed for the worse outcomes. On the other hand, it is a debate whether increased anticholinergic burden leads to worse outcomes or whether the diseases being treated by the anticholinergic drugs, including neurodegenerative diseases, are responsible for the decline in cognition and physical performance. Therefore, we excluded patients diagnosed with dementia to mitigate the confounding effect of the underlying condition on the course of patients.

Moreover, malnutrition and risk of malnutrition are significant conditions that are reported between 10 and 40% in community dwelling older adults (30). Malnutrition also causes serious health consequences such as falls, osteoporosis, orthostatic hypotension, and mortality (31). Many risk factors, including increased age, dementia, decreased social support, dysphagia, depression, polypharmacy, anorexia were specified to develop inadequate nutrition (32). However, to our knowledge, the relationship between malnutrition and anticholinergic drug burden is not elaborated in literature so far. In this longitudinal study, it was observed that increased anticholinergic

TABLE 3 | Delta changes of cognition, nutrition and functionality parameters in patients without dementia.

Δ variables (6th month – baseline score)	Zero (0) from beginning (Group1) *N*: 100	Decreased score (Group2) *N*: 125	Increased score (Group3) *N*: 189	*P*
Comprehensive geriatric assessment parameters				
ΔMMSE	−0.24 ± 2.23	0.47 ± 3.02	−0.18 ± 2.43	0.24
ΔGDS	−0.83 ± 2.82	−0.24 ± 3.31	−0.36 ± 2.22	0.87
ΔBADL	−0.12 ± 3.46	−0.69 ± 6.48	−0.62 ± 6.37	0.77
ΔIADL	−1.01 ± 2.76	−1.08 ± 2.91	−1.33 ± 3.48	0.95
ΔMNA-SF	0.55 ± 2.10	0.51 ± 2.28	0.36 ± 1.52	0.51
Laboratory values				
ΔHemoglobin	0.04 ± 0.93	0.12 ± 1.26	−0.05 ± 0.95	0.13
ΔAlbumin	−0.00 ± 0.25	0.04 ± 0.27	−0.08 ± 0.38	0.07
ΔLDL	−0.02 ± 42.43	1.49 ± 47.92	4.96 ± 42.22	0.64
ΔMDRD	0.24 ± 10.37	−1.53 ± 14.36	0.58 ± 15.27	0.79
ΔWeight	−0.10 ± 2.83	−0.91 ± 3.07	−0.36 ± 2.75	0.22

MDRD, Modification of Diet in Renal Disease; MMSE, Mini Mental State Examination; GDS, Geriatric Depression Scale (0–15); MNA-SF, Mini Nutritional Assessment (0–12); BADL, Basic Activities of Daily Living (0–100); IADL, Instrumental Activities of Daily Living (0–23).

TABLE 4 | The relationship between increase in the DBI score and changes in laboratory and comprehensive geriatric assessment parameters.

	Beta	OR (confidence interval 95%)	*p*
Comprehensive geriatric assessment parameters			
MMSE	−0.483	0.617 (0.418–0.911)	**0.015**
GDS	−0.115	0.891 (0.574–1.385)	0.610
MNA-SF	−0.460	0.631 (0.450–0.884)	**0.008**
BADL	−0.347	0.707 (0.466–1.072)	0.102
IADL	−0.104	0.902 (0.585–1.389)	0.639
	Beta	**Beta upper–lower level**	***p***
Laboratory values			
MDRD	1.491	(−1.044)–(4.026)	0.248
Hemoglobin	−0.127	(−0.313)–(0.060)	0.183
LDL	4.351	(−4.455)–(13.158)	0.331
Albumin	−0.086	(−0.159)–(−0.014)	**0.020**
Weight (kg)	0.035	(−0.489)–(0.560)	0.895

MDRD, Modification of Diet in Renal Disease; MMSE, Mini Mental State Examination; GDS, Geriatric Depression Scale; MNA-SF, Mini Nutritional Assessment; BADL, Basic Activities of Daily Living; IADL, Instrumental Activities of Daily Living. Bold values denote statistical significance at the $p < 0.05$ level.

load was associated with worsening nutritional status and decreased albumin level which is also an indirect marker for malnutrition (33). The impact of the anticholinergic burden on nutrition may be explained by the gastrointestinal system and cognitive effects of anticholinergic agents (5). One of the well-known peripheral side effects, dry mouth (xerostomia), leads to poor dentition, altered taste, difficulty in deglutition, and digestion (34). Dysphagia makes the development of aspiration more likely and causes serious complications such as aspiration pneumonia. Reduced gastrointestinal motility and secretion contribute to constipation, anorexia, and early satiety. Additionally, central side effects including cognitive impairment, drowsiness, confusion, poor attention, restlessness may lead to swallowing difficulties, dehydration, and aspiration. Sedative effects of the drugs contribute to anorexia (3).

Although the best scale evaluates the cholinergic load is not determined, DBI is a feasible and validated scale (29, 35). Using the DBI offers an assessment of anticholinergic activity with a dose-response model. Thus, the exposure of drugs being administered to a patient could precisely be estimated. Previous studies support that DBI is related to worse cognitive and physical function (6, 36–39). Accordingly, DBI is selected in the present study to evaluate the anticholinergic burden. High and low exposure risk groups were identified to show the impact of cholinergic burden change on nutrition, cognition, and functionality in the longitudinal analysis. Increased DBI scores negatively influence cognitive and nutritional scores.

The study has many strength aspects. First, the anticholinergic burden was evaluated based on medications at the baseline, and changes take into consideration during the 6-month follow-up. Second, all patients were examined in a detailed manner with CGA, including functionality, global cognition, nutritional status, and most of the geriatric syndromes. Third, the study has a large sample size, and a validated scale was used to quantify the anticholinergic burden objectively. Fourth, to the best of our knowledge, the study is the first longitudinal study investigating the relationship between DBI rated anticholinergic burden and nutritional status in older adults. On the contrary, there are limited features of the study. Although patients with major neurocognitive disorders were excluded from the study, we could not adjust all confounding factors such as depression, increasing the risk of exposure to drugs with anticholinergic properties. In addition, the follow-up duration may be shorter to assess long-term effects of anticholinergic drugs. Thus, larger size, long term follow-up studies are needed to clarify the complex relationship between anticholinergic burden and nutritional status in older adults.

CONCLUSION

The serious adverse effects may be prevented because the anticholinergic activity is a potentially reversible factor. Therefore, reducing exposure to drugs with anticholinergic activity has particular importance in geriatric practice. Withdrawal of unnecessary drugs (to avoid potential drug interactions) or decrease anticholinergic agents are the main strategies that should be implemented in clinical settings to improve nutritional and cognitive outcomes in older adults. Such tiny interventions provide essential contributions in the management of geriatric cases. Further evidence is warranted to explain the mechanisms that effects of anticholinergic medications on nutrition.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Non-interventional Studies Ethics Committee of Dokuz Eylül University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

AI and EA made the study concept and design. NE and FD helped acquisition of data. EA and DK performed analysis, interpretation of data, and draft the manuscript. AI established critical revision of the manuscript for important intellectual content. All authors contributed to the article and approved the submitted version.

REFERENCES

1. American Geriatrics Society 2019 updated AGS beers criteria® for potentially inappropriate medication use in older adults. *J Am Geriatr Soc.* (2019) 67:674–94. doi: 10.1111/jgs.15767
2. O'Mahony D, O'Sullivan D, Byrne S, O'Connor MN, Ryan C, Gallagher P. STOPP/START criteria for potentially inappropriate prescribing in older people: version 2. *Age Ageing.* (2015) 44:213–8. doi: 10.1093/ageing/afu145
3. Feinberg M. The problems of anticholinergic adverse effects in older patients. *Drugs Aging.* (1993) 3:335–48. doi: 10.2165/00002512-199303040-00004
4. Wawruch M, Macugova A, Kostkova L, Luha J, Dukat A, Murin J, et al. The use of medications with anticholinergic properties and risk factors for their use in hospitalised elderly patients. *Pharmacoepidemiol Drug Saf.* (2012) 21:170–6. doi: 10.1002/pds.2169
5. Castejón-Hernández S, Latorre-Vallbona N, Molist-Brunet N, Cubí-Montanyà D, Espaulella-Panicot J. Association between anticholinergic burden and oropharyngeal dysphagia among hospitalized older adults. *Aging Clin Exp Res.* (2020) 33:1981–5. doi: 10.1007/s40520-020-01707-9
6. Wilson NM, Hilmer SN, March LM, Cameron ID, Lord SR, Seibel MJ, et al. Associations between drug burden index and falls in older people in residential aged care. *J Am Geriatr Soc.* (2011) 59:875–80. doi: 10.1111/j.1532-5415.2011.03386.x
7. Fox C, Smith T, Maidment I, Chan WY, Bua N, Myint PK, et al. Effect of medications with anti-cholinergic properties on cognitive function, delirium, physical function and mortality: a systematic review. *Age Ageing.* (2014) 43:604–15. doi: 10.1093/ageing/afu096
8. Bishara D, Perera G, Harwood D, Taylor D, Sauer J, Stewart R, et al. The anticholinergic effect on cognition (AEC) scale-Associations with mortality, hospitalisation and cognitive decline following dementia diagnosis. *Int J Geriatr Psychiatry.* (2020) 35:1069–77. doi: 10.1002/gps.5330
9. Cao YJ, Mager DE, Simonsick EM, Hilmer SN, Ling SM, Windham BG, et al. Physical and cognitive performance and burden of anticholinergics, sedatives, and ACE inhibitors in older women. *Clin Pharmacol Ther.* (2008) 83:422–9. doi: 10.1038/sj.clpt.6100303
10. Hilmer SN, Mager DE, Simonsick EM, Ling SM, Windham BG, Harris TB, et al. Drug burden index score and functional decline in older people. *Am J Med.* (2009) 122:1142–9.e1–2. doi: 10.1016/j.amjmed.2009.02.021
11. Stewart C, Yrjana K, Kishor M, Soiza RL, Taylor-Rowan M, Quinn TJ, et al. Anticholinergic burden measures predict older people's physical function and quality of life: a systematic review. *J Am Med Direct Assoc.* (2021) 22:56–64. doi: 10.1016/j.jamda.2020.05.065
12. Salahudeen MS, Duffull SB, Nishtala PS. Anticholinergic burden quantified by anticholinergic risk scales and adverse outcomes in older people: a systematic review. *BMC Geriatr.* (2015) 15:31. doi: 10.1186/s12877-015-0029-9
13. Hilmer SN, Mager DE, Simonsick EM, Cao Y, Ling SM, Windham BG, et al. A drug burden index to define the functional burden of medications in older people. *Arch Intern Med.* (2007) 167:781–7. doi: 10.1001/archinte.167.8.781
14. Salahudeen MS, Hilmer SN, Nishtala PS. Comparison of anticholinergic risk scales and associations with adverse health outcomes in older people. *J Am Geriatr Soc.* (2015) 63:85–90. doi: 10.1111/jgs.13206
15. Okudur SK, Dokuzlar O, Aydin AE, Kocyigit SE, Soysal P, Isik AT. The evaluation of relationship between polypharmacy and anticholinergic burden scales. *Northern Clin Istanbul.* (2021) 8:139–44. doi: 10.14744/nci.2020.17136
16. Unutmaz GD, Soysal P, Tuven B, Isik AT. Costs of medication in older patients: before and after comprehensive geriatric assessment. *Clin Interv Aging.* (2018) 13:607–13. doi: 10.2147/CIA.S159966
17. Durmaz B, Soysal P, Ellidokuz H, Isik AT. Validity and reliability of geriatric depression scale-15 (short form) in Turkish older adults. *Northern Clin Istanbul.* (2018) 5:216–20. doi: 10.14744/nci.2017.85047
18. Ates Bulut E, Soysal P, Isik AT. Frequency and coincidence of geriatric syndromes according to age groups: single-center experience

in Turkey between 2013 and 2017. *Clin Interv Aging.* (2018) 13:1899–905. doi: 10.2147/CIA.S180281
19. Pharmacological management of persistent pain in older persons. *J Am Geriatr Soc.* (2009) 57:1331–46. doi: 10.1111/j.1532-5415.2009.02376.x
20. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Inter Med.* (2006) 145:247–54. doi: 10.7326/0003-4819-145-4-200608150-00004
21. Wouters H, Hilmer SN, Twisk J, Teichert M, Van Der Meer HG, Van Hout HPJ, et al. Drug burden index and cognitive and physical function in aged care residents: a longitudinal study. *J Am Med Directors Assoc.* (2020) 21:1086–92.e1. doi: 10.1016/j.jamda.2020.05.037
22. Villalba-Moreno Á, Alfaro-Lara ER, Sánchez-Fidalgo S, Nieto-Martín MD, Santos-Ramos B. Development of the anticholinergic burden calculator web tool. *Farm Hosp.* (2017) 41:647–8. doi: 10.1136/ejhpharm-2017-000640.421
23. Gnjidic D, Hilmer SN, Hartikainen S, Tolppanen AM, Taipale H, Koponen M, et al. Impact of high risk drug use on hospitalization and mortality in older people with and without Alzheimer's disease: a national population cohort study. *PLoS ONE.* (2014) 9:e83224. doi: 10.1371/journal.pone.0083224
24. Rubenstein LZ, Harker JO, Salva A, Guigoz Y, Vellas B. Screening for undernutrition in geriatric practice: developing the short-form mini-nutritional assessment (MNA-SF). *J Gerontol Series A Biol Sci Med Sci.* (2001) 56:M366–72. doi: 10.1093/gerona/56.6.M366
25. Pasina L, Colzani L, Cortesi L, Tettamanti M, Zambon A, Nobili A, et al. Relation between delirium and anticholinergic drug burden in a cohort of hospitalized older patients: an observational study. *Drugs Aging.* (2019) 36:85–91. doi: 10.1007/s40266-018-0612-9
26. Idil E, Aydin AE, Ates Bulut E, Isik AT. Rationally decreasing the number of drugs seems to be a useful therapeutic approach in older adults: 6-month follow-up study. *Arch Gerontol Geriatr.* (2021) 96:104472. doi: 10.1016/j.archger.2021.104472
27. Ruxton K, Woodman RJ, Mangoni AA. Drugs with anticholinergic effects and cognitive impairment, falls and all-cause mortality in older adults: a systematic review and meta-analysis. *Br J Clin Pharmacol.* (2015) 80:209–20. doi: 10.1111/bcp.12617
28. Ali S, Peterson GM, Bereznicki LR, Salahudeen MS. Association between anticholinergic drug burden and mortality in older people: a systematic review. *Eur J Clin Pharmacol.* (2020) 76:319–35. doi: 10.1007/s00228-019-02795-x
29. Welsh TJ, van der Wardt V, Ojo G, Gordon AL, Gladman JRF. Anticholinergic drug burden tools/scales and adverse outcomes in different clinical settings: a systematic review of reviews. *Drugs Aging.* (2018) 35:523–38. doi: 10.1007/s40266-018-0549-z
30. Kocyigit SE, Soysal P, Ates Bulut E, Isik AT. Malnutrition and Malnutrition risk can be associated with systolic orthostatic hypotension in older adults. *J Nutr Health Aging.* (2018) 22:928–33. doi: 10.1007/s12603-018-1032-6
31. Kocyigit SE, Ates Bulut E, Aydin AE, Isik AT. Improvement of nutritional status enhances cognitive and physical functions in older adults with orthostatic hypotension. *Nutrition.* (2021) 90:111261. doi: 10.1016/j.nut.2021.111261
32. Favaro-Moreira NC, Krausch-Hofmann S, Matthys C, Vereecken C, Vanhauwaert E, Declercq A, et al. Risk factors for malnutrition in older adults: a systematic review of the literature based on longitudinal data. *Adv Nutr.* (2016) 7:507–22. doi: 10.3945/an.115.011254
33. Cabrerizo S, Cuadras D, Gomez-Busto F, Artaza-Artabe I, Marín-Ciancas F, Malafarina V. Serum albumin and health in older people: review and meta analysis. *Maturitas.* (2015) 81:17–27. doi: 10.1016/j.maturitas.2015.02.009
34. Lavrador M, Cabral AC, Figueiredo IV, Veríssimo MT, Castel-Branco MM, Fernandez-Llimos F. Size of the associations between anticholinergic burden tool scores and adverse outcomes in older patients. *Int J Clin Pharm.* (2021) 43:128–36. doi: 10.1007/s11096-020-01117-x
35. Kouladjian L, Gnjidic D, Chen TF, Mangoni AA, Hilmer SN. Drug Burden Index in older adults: theoretical and practical issues. *Clin Interv Aging.* (2014) 9:1503–15. doi: 10.2147/CIA.S66660
36. Wilson NM, Hilmer SN, March LM, Cameron ID, Lord SR, Seibel MJ, et al. Associations between drug burden index and physical function in older people in residential aged care facilities. *Age Ageing.* (2010) 39:503–7. doi: 10.1093/ageing/afq053
37. Nishtala PS, Narayan SW, Wang T, Hilmer SN. Associations of drug burden index with falls, general practitioner visits, and mortality in older people. *Pharmacoepidemiol Drug Safety.* (2014) 23:753–8. doi: 10.1002/pds.3624
38. Nishtala PS, Allore H, Han L, Jamieson HA, Hilmer SN, Chyou TY. Impact of anticholinergic burden on cognitive performance: a cohort study of community-dwelling older adults. *J Am Med Directors Assoc.* (2020) 21:1357–8.e3. doi: 10.1016/j.jamda.2020.03.027
39. Gnjidic D, Le Couteur DG, Abernethy DR, Hilmer SN. Drug burden index and beers criteria: impact on functional outcomes in older people living in self-care retirement villages. *J Clin Pharmacol.* (2012) 52:258–65. doi: 10.1177/0091270010395591

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Dietary Patterns Are Associated With Multi-Dimensional Cognitive Functions Among Adults Aged 55 and Older in China

*Qiumin Huang[1], Hongru Jiang[1], Jiguo Zhang[1], Xiaofang Jia[1], Feifei Huang[1], Huijun Wang[1], Bing Zhang[1], Liusen Wang[1], Minxia Gu[2], Yuelong Huang[3], Wei Shi[4], Yuxia Ma[5], Xinjing Zhang[5] and Zhihong Wang[1]**

[1] National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, Beijing, China, [2] Ninghai Country Center for Disease Control and Prevention, Ningbo, China, [3] Hunan Provincial Center for Disease Control and Prevention, Changsha, China, [4] Shaanxi Provincial Center for Disease Control and Prevention, Xian, China, [5] School of Public Health, Hebei Medical University, Shijiazhuang, China

Background: The intake of certain food and nutrients may play a crucial role in cognitive health. However, research on the relationship between dietary patterns and cognitive function is limited. This study aims to investigate the associations between dietary patterns and multi-dimensional cognitive functions, such as global cognitive status and related domain profiles, mild cognitive impairment (MCI), and four major subtypes of Chinese adults.

Methods: Using the baseline data from the Community-based Cohort Study on Nervous System Diseases (2018–2019), we selected 4,309 Chinese adults aged 55 years and older as subjects with complete diet, cognition, and other related data. We collected food data for the past 12 months with a valid semi-quantitative food frequency questionnaire. Diving 49 food items into 13 subgroups, we used factor analysis to derive the main dietary patterns. We evaluated cognitive functions based on the scores of the Montreal Cognitive Assessment (MoCA) and used quantile regression and multivariable logistic regression to examine the relationship between dietary patterns and cognitive-related outcomes.

Results: We identified four dietary patterns, explaining 50.11% of the total variance: "meat-preferred" pattern, "plant-preferred" pattern, "eggs- and dairy-preferred" pattern, and "grain-preferred" pattern. After adjusting for all potential confounders, the "meat-preferred" pattern and the "plant-preferred" pattern were associated with higher scores of global cognition and several cognitive domains ($p < 0.05$), while the "grain-preferred" pattern was associated with lower scores of global cognition ($\beta = -0.36$, $p < 0.05$), execution ($\beta = -0.19$, $p < 0.05$), visuospatial ($\beta = -0.09$, $p < 0.05$), and language ($\beta = -0.05$, $p < 0.05$). Adults adhering to the "meat-preferred" pattern and the "plant-preferred" pattern had decreased odds of MCI and some MCI subtypes (*p-trend* < 0.05); in contrast, those in the top quartiles of the "grain-preferred" pattern had increased odds of MCI [adjusted odds ratio (*AOR*) = 1.34, 95% CI: 1.11–1.63, *p-trend* = 0.003].

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Gang Wang,
Shanghai Jiao Tong University, China

Reviewed by:
Yongmei Shi,
Shanghai Jiao Tong University, China
Yong Zhao,
Chongqing Medical University, China

***Correspondence:**
Zhihong Wang
wangzh@ninh.chinacdc.cn

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Conclusions: Adhering to the "plant-preferred" pattern and the "meat-preferred" pattern may help improve the multi-dimensional cognitive functions; on the contrary, adhering to the "grain-preferred" pattern may worse cognitive health. More prospective studies in this field are needed to strengthen the evidence.

Keywords: dietary patterns, cognition, cognitive domains, mild cognitive impairment, Chinese population

INTRODUCTION

There are 264.02 million people aged 60 years or older in China, accounting for 18.7% of the total population of 1.40 billion in 2020, an increase of about 5.4% over 2010. Due to the aging population, neurodegenerative disorders are expected to increase significantly. In 2020, it was reported that an estimated 9.83 million Chinese aged 60 years and older suffered from Alzheimer's disease (AD), whose cognition declines with age; and 38.77 million suffered from mild cognitive impairment (MCI), the most common outcome of pathological cognitive decline in the elderly (1). MCI, as an intermediate state, 8.1% of MCI may become AD (2) and 10–14% of MCI may return to normal cognition (3). To date, there are no effective measures to fight the progression of AD (4). Therefore, it is extremely important to fully investigate relevant risk factors associated with AD and provide sight into evidence-based intervention for early prevention among adults at early stages.

Diet may have an impact on the cognitive health. Studies in the United States, France, Brazil, and China found that regular consumption of fish, nuts, fruit, or vegetables is associated with improved cognitive function (5–7). Considering the complex interaction among various nutrients and food, research on the relationship between dietary patterns and cognition has received more and more attention. Observation studies showed that adherence to the Mediterranean diet can improve the global cognitive function (8) and decrease the risk of MCI in the elderly (9). Among the elderly in Japan, the "plant food and fish" pattern with high intake of vegetables, soy products, fruit, and fish determined by factor analysis is positively correlated with cognitive function score (10). Almost all of these studies have been carried out in Western countries, some of which have limitations in controlling cofounding factors. However, large-scale research in China is limited, and few studies have explored the associations between dietary patterns and cognitive outcomes in the subtypes of MCI. Accumulating more evidence in different regions of China is crucial to prevent the cognitive decline. Because of food items, nutrients in foods, and eating behaviors, especially the obvious nutrition transition in China. The beneficial dietary patterns identified in some regions may not be consistent with those in other regions (11).

This study aims to determine the main dietary patterns related to multi-dimensional cognitive functions, such as global cognitive status, six cognitive domain functions, prevalence of MCI, and four main MCI subtypes in Chinese residents aged 55 years and older, using the data from the Community-based Cohort Study on Nervous System Diseases (CCSNSD).

MATERIALS AND METHODS

Study Population

Community-based Cohort Study on Nervous System Diseases, a longitudinal study established by the National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention in 2018–2019, focuses on potential factors associated with risks of epilepsy, AD and Parkinson's disease (PD). We used a multistage stratified random sampling approach to draw participants without such diseases in Hebei, Zhejiang, Shaanxi, and Hunan provinces. Detailed survey design has been reported elsewhere (12). The Institutional Review Board of the National Institute for Nutrition and Health approved the study (No. 2017020, November 6, 2017). All participants provided their written informed consent.

This study recruited 6,143 eligible subjects aged 55 years and older without clinical diagnosis of AD or comorbidities. In the analysis, we used a subsample of 4,309 subjects (1,956 men and 2,353 women) aged between 55 and 86 years, excluding 1,769 subjects with incomplete data on sociodemographic characteristic, disease history, cognitive examination, food frequency questionnaire (FFQ), psychological evaluation, or basic abilities of daily living, and further excluding 65 subjects unable to perform basic activities of daily living, such as eating, dressing, bathing, toileting, grooming, moving to bed or chair, walking through the room, or incontinence.

Identification of Dietary Patterns

We assessed dietary intake using a validated semi-quantitative FFQ covering 65 food items. Participants reported frequency categories (daily, weekly, monthly, annually, or never) and the average amount for each food item consumed in the last 12 months. We calculated their intake of each item based on the average intake frequency and quantity. If the subject reported the "never" intake frequency, we set his/her intake to 0 g/day or week. According to the participant's habitual intake and the similarity of types, we categorized 49 major food items into 13 subgroups, such as rice, wheat, tubers, legumes, fresh vegetables, fresh fruit, pork, beef and mutton, poultry, fish, eggs, dairy, and nuts. **Supplementary Table S1** provides detailed information on the foods in each subgroup.

Abbreviations: AIS, Attention index score; EIS, Executive index score; LIS, Language index score; MCI, Mild cognitive impairment; MIS, Memory index score; OIS, Orientation index score; VIS, Visuospatial index score; aMCI-SD, Amnestic MCI single domain; aMCI-MD, Amnestic MCI multiple domains; naMCI-SD, Non-amnestic MCI single domain; naMCI-MD, Non-amnestic MCI multiple domains.

We used factor analysis with varimax rotation to analyze the 13 food subgroups and determined four factors, "meat-preferred" pattern, "plant-preferred" pattern, "eggs- and dairy-preferred" pattern, and "grain-preferred" pattern, based on eigenvalues >1.0, a scree plot, and the interpretability. Food items with an absolute factor load value >0.4 are the main contributors to the dietary pattern, representing the characteristics of each pattern. We calculated the factor scores of each participant for each pattern by summing the intake of food groups weighted by their factor loadings and grouped them into quartiles for further analysis. A higher quartile indicates more consistency with the pattern being calculated.

Assessment of Cognitive Function

We used the Montreal Cognitive Assessment (MoCA) to assess the cognitive function of participants. The sum of the scale items produces a total MoCA score ranging from 0 to 30, which is positively associated with the global cognitive function (13). The MCI criteria is based on the Chinese MoCA norms (14): total MoCA score ≤13 for illiterate individuals, ≤19 for individuals with 1–6 years of education, and ≤24 for individuals with 7 years of education or more.

We evaluated the cognitive domain functions of memory, execution, visuospatial, language, attention, and orientation by the memory index score (MIS), executive index score (EIS), visuospatial index score (VIS), language index score (LIS), attention index score (AIS), and orientation index score (OIS), respectively. We calculated these index scores based on the MoCA cognitive domain index score (13). We defined each impaired cognitive domain as participants who scored <1.5 SDs below the age- and education-adjusted mean value in that cognitive domain (15). We defined MCI subtypes as participants having MCI and characterized by different cognitive domain deficits (16, 17): amnestic MCI single domain (aMCI-SD): only memory impairment; non-amnestic MCI single domain (naMCI-SD): a deficit in one cognitive domain other than memory; amnestic MCI multiple domains (aMCI-MD): memory impairment plus one other impaired domain; and non-amnestic MCI multiple domains (naMCI-MD): deficits in at least 2 domains other than memory.

Assessment of Covariates

Interviewers with a degree in medicine or public health who have received two rounds of training by the national or provincial experts and passed the qualification examination collected all survey data, such as gender (male or female), age (years), education level (below primary school, primary school, or secondary school or above), residential area (rural or urban), current employment status (yes or no), and monthly household income per capita [<1,000, 1,000–3,999, or ≥4,000 (Chinese yuan RMB)], smoking (never or ever/current), drinking (never or ever/current), physical activity (occupations, household chores, leisure time, and transportation activities), sleep duration (hours), and medication usage (yes or no), and other. We converted total physical activity hours into metabolic equivalent of tasks (METs) hours per week based on the Compendium of Physical Activities recommended by the American College of Sports Medicine Association (18), and divided them into tertiles (low, medium, and high). We categorized sleep hours based on the cutoff points recommended by the National Sleep Foundation, 7–9 h for participants aged between 55 and 64 years, and 7–8 h for participants 65 years and older (19). We calculated the total energy intake based on the China Food Composition Table.

We defined history of diet-related chronic diseases as participant having hypertension, diabetes, stroke, or myocardial infarction diagnosed or treated by physicians. We defined obesity as body mass index (BMI) ≥ 28 kg/m^2, and central obesity as a waist circumference ≥90 cm for men and ≥85 cm for women. Detailed information on physical measurements has been reported elsewhere (12).

Statistical Analysis

For continuous variables, we used mean ± SD to describe the distribution and Wilcoxon signed rank test or Kruskal–Wallis *H*-test to examine the differences among groups as appropriate. For categorical variables, we used quantity (percentage) and the chi-square test. We used a series of quantile regression models to estimate the associations of each dietary pattern with the global cognitive score and cognitive domain subtypes. We built a set of modes, such as model without any adjustment (Model 1), model adjusted for age, gender, residential area, education level, current employment status, and household income level (Model 2), model further adjusted for physical activity, smoking, drinking, sleep duration, and total food energy (Model 3), and model further adjusted for diet-related chronic disease history, obesity, and central obesity (Model 4). We used multivariable logistic regression models to calculate the adjusted odds ratio (*AOR*) and 95% *CI* to estimate the relationship between each dietary pattern and the prevalence of MCI and its subtypes, controlling for covariates including age, gender, residential area, education level, current employment status, household income level, physical activity, smoking, drinking, sleep duration, total energy intake, diet-related chronic disease history, obesity, and central obesity. In addition, we tested the linear trends for the prevalence of MCI and its subtypes by assigning the median value to the quartiles of each dietary pattern score as a continuous variable. We used SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA) for all statistical analysis, and considered the statistical significance as a two-sided $p < 0.05$.

RESULTS

Characteristics of Subjects

As shown in **Table 1**, the average age of the participants was 68.4 years. Among the 4,309 participants, 45.4% were men, 50.6% lived in rural areas, 85.8% achieved a primary education level or above, and 76.4% had a per capita monthly income more than 1,000 yuan. The proportion of participants with a history of diet-related chronic diseases, obesity, or central obesity were 36.3, 13.2, and 46.5%, respectively. Compared with participants with the normal cognitive function, MCI participants were older and poorer, and had lower energy intake ($p < 0.05$).

TABLE 1 | Subject characteristics by cognitive status among Chinese adults aged 55 years and above in the Community-based Cohort Study on Nervous System Diseases (CCSNSD) 2018–2019.

Characteristics	*N* (%)	MCI		*P*-value
		Yes	No	
Total	4,309 (100.0)	42.6	57.4	
Age group (years)[a]				<0.001
55–64	1,586 (36.8)	38.1	61.9	
65–74	1,864 (43.3)	42.0	58.0	
≥75	859 (19.9)	51.9	48.1	
Gender[a]				0.476
Male	1956 (45.4)	42.0	58.0	
Female	2353 (54.6)	43.1	56.9	
Residential area[a]				<0.001
Urban	2,130 (49.4)	38.5	61.5	
Rural	2,179 (50.6)	46.5	53.5	
Education level[a]				<0.001
Illiteracy	613 (14.2)	39.0	61.0	
≤Primary school	1,784 (41.4)	38.6	61.4	
≥Secondary school	1,912 (44.4)	47.4	52.6	
Current employment[a]				0.011
Yes	772 (17.9)	38.5	61.5	
No	3,537 (82.1)	43.5	56.5	
Monthly household income per capital (RMB)[a]				<0.001
<1,000	1,017 (23.6)	54.2	45.8	
1,000–3,999	2,653 (61.6)	42.2	57.8	
≥4,000	639 (14.8)	25.5	74.5	
Physical activity level[a]				<0.001
Low	1,431 (33.2)	38.1	61.9	
Medium	1,442 (33.5)	43.7	56.3	
High	1,436 (33.3)	45.9	54.1	
Smoking[a]				0.006
Ever/current	1,036 (24.0)	46.2	53.8	
Never	3,273 (76.0)	41.4	58.6	
Drinking[a]				0.214
Ever/current	743 (17.2)	40.5	59.5	
Never	3,566 (82.8)	43.0	57.0	
Meeting sleep duration recommendation[a]				0.187
Yes	1,505 (34.9)	43.9	56.1	
No	2,804 (65.1)	41.8	58.2	
Medical history[a]				0.004
Yes	1,562 (36.3)	45.5	54.5	
No	2,747 (63.7)	40.9	59.1	
Obesity[a]				0.165
Yes	568 (13.2)	45.2	54.8	
No	3,741 (86.8)	42.2	57.8	
Central obesity[a]				0.969
Yes	2,005 (46.5)	42.6	57.4	
No	2,304 (53.5)	42.5	57.5	
Energy[b]	1,522.0 ± 620.7	1,484.3 ± 592.9	1,549.8 ± 639.3	0.003

[a]*Values are expressed as N (%) or % and examined using chi-square test.*
[b]*Values are expressed as mean ± SD (kcal) and examined using Wilcoxon signed rank test.*

Dietary Patterns

We identified four dietary patterns using factor analysis. **Table 2** presents the factor load in each food group. These four patterns explained 50.1% of the variance in dietary intake (i.e., 23.0% by factor 1, 10.9% by factor 2, 8.9% by factor 3, and 7.3% by factor 4). The first factor "meat-preferred" pattern, was characterized by a large intake of pork, fish, poultry, and beef or lamb. The second pattern, "plant-preferred" pattern, included a large intake of tubers, legumes, fresh vegetables, fresh fruit, and nuts. The third pattern, "eggs- and dairy-preferred" pattern, referred to a large intake of eggs and dairy products. The fourth pattern, "grain-preferred" pattern, was characterized by a large intake of rice- and wheat-based foods. The intake of each food group was significantly different among the quartiles of each dietary pattern ($p < 0.05$, **Supplementary Table S2**).

Supplementary Table S3 presents sociodemographic status, lifestyle variables, and health-related factors for each quartile of dietary pattern scores. The distributions of residential area, education level, employment status, household income level, drinking, and energy intake were significantly different among the quartiles of the four dietary patterns ($p < 0.05$). Physical activity and smoking status were significantly different among the quartiles of the "meat-preferred" pattern, the "plant-preferred" pattern, and the "grain-preferred" pattern ($p < 0.05$). The history of diet-related chronic diseases, obesity, and central obesity were significantly different among the quartiles of the "meat-preferred" pattern and the "eggs- and dairy-preferred" pattern ($p < 0.05$).

Dietary Patterns and Global Cognition and Cognitive Domains

Table 3 presents the global cognitive score and cognitive domain subscores of the quartiles of each dietary pattern. Participants in the highest quartile of the "meat-preferred" pattern had higher global cognitive function scores and higher scores in most cognitive domains ($p < 0.05$) compared with those in other quartiles. The global cognitive function scores and cognitive domains were significantly increased as the "plant-preferred" pattern score increased from the first quartile to the fourth quartile ($p < 0.05$), while they were decreased as the "grain-preferred" pattern score from the lowest to the highest quartile ($p < 0.05$). The lowest and the highest scores of global cognitive function, execution, visuospatial, language, and attention were distributed in the third quartile and the fourth quartile of the "eggs- and dairy-preferred" pattern ($p < 0.05$).

Table 4 presents the association of the four dietary patterns with the global cognitive score and cognitive domain subscores. In the final multivariate models, the average scores of the global cognition, memory, execution, and attention were increased significantly as the quartiles of the "meat-preferred" pattern score increased ($p < 0.05$); similarly, participants with high intake of the "plant-preferred" pattern may have higher scores of 0.45, 0.25, 0.18, 0.13, 0.07, and 0.30 in the global cognition, memory, execution, visuospatial, language, and attention function ($p < 0.05$), respectively. In contrast, the average scores of global cognition, execution, visuospatial, and language function were

TABLE 2 | Factor load in food groups of dietary patterns[a].

Food groups	Meat-preferred pattern (factor 1)	Plant-preferred pattern (factor 2)	Eggs- and dairy-preferred pattern (factor 3)	Grain-preferred pattern (factor 4)
Pork	**0.773**	−0.029	0.040	0.020
Fish	**0.701**	0.153	−0.119	−0.020
Poultry	**0.448**	0.330	0.245	0.002
Beef or mutton	**0.445**	0.212	0.342	−0.041
Tubers	−0.034	**0.716**	−0.029	0.148
Legumes	0.090	**0.605**	0.317	−0.101
Fresh vegetables	0.520	**0.573**	0.116	0.071
Fresh fruit	0.183	**0.539**	0.353	0.055
Nuts	0.112	**0.441**	−0.052	−0.078
Eggs	0.218	−0.053	**0.719**	0.186
Dairy	−0.160	0.191	**0.615**	−0.174
Wheat	−0.294	0.034	0.243	**0.742**
Rice	0.217	0.001	−0.192	**0.703**

[a]*The absolute value of factor load was >0.4 is shown in bold. For food group load with more than one dietary pattern, only the highest absolute value of the load is shown in bold.*

TABLE 3 | Differences in global cognitive score and cognitive domain subscores by quartiles of each dietary pattern score[a].

Dietary patterns	Global cognitive function	Cognitive domain scores					
		MIS	EIS	VIS	LIS	AIS	OIS
Meat-preferred pattern							
Q1	20.22 ± 6.06	10.48 ± 4.46	8.00 ± 3.26	5.06 ± 1.73	4.38 ± 1.40	12.52 ± 4.18	5.31 ± 1.17
Q2	20.82 ± 6.27	10.42 ± 4.43	8.47 ± 3.31	5.20 ± 1.70	4.38 ± 1.47	12.54 ± 4.11	5.46 ± 1.04
Q3	22.28 ± 6.36	11.38 ± 4.10	9.09 ± 3.37	5.46 ± 1.76	4.58 ± 1.43	13.58 ± 3.99	5.57 ± 0.92
Q4	22.80 ± 6.00	12.00 ± 3.67	9.22 ± 3.35	5.33 ± 1.89	4.65 ± 1.36	14.00 ± 3.60	5.63 ± 0.78
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Plant-preferred pattern							
Q1	20.26 ± 6.67	10.51 ± 4.41	8.09 ± 3.60	4.92 ± 1.83	4.25 ± 1.49	12.34 ± 4.25	5.32 ± 1.11
Q2	20.86 ± 6.27	10.70 ± 4.50	8.38 ± 3.31	5.09 ± 1.84	4.41 ± 1.42	12.89 ± 3.94	5.41 ± 1.08
Q3	21.83 ± 6.00	11.35 ± 3.96	8.76 ± 3.25	5.41 ± 1.73	4.56 ± 1.39	13.26 ± 4.01	5.56 ± 0.92
Q4	23.17 ± 5.66	11.72 ± 3.91	9.55 ± 3.06	5.64 ± 1.62	4.78 ± 1.32	14.15 ± 3.69	5.68 ± 0.81
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Eggs- and dairy-preferred pattern							
Q1	21.83 ± 6.60	11.25 ± 4.25	8.93 ± 3.44	5.09 ± 1.98	4.44 ± 1.51	13.46 ± 3.96	5.54 ± 0.95
Q2	21.26 ± 5.98	11.04 ± 4.14	8.46 ± 3.27	5.28 ± 1.71	4.47 ± 1.37	12.97 ± 3.92	5.50 ± 0.95
Q3	20.67 ± 6.23	10.74 ± 4.24	8.16 ± 3.35	5.12 ± 1.76	4.38 ± 1.44	12.65 ± 4.18	5.43 ± 1.07
Q4	22.36 ± 6.09	11.25 ± 4.27	9.23 ± 3.26	5.57 ± 1.61	4.70 ± 1.34	13.56 ± 3.98	5.51 ± 1.00
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.160
Grain-preferred pattern							
Q1	22.96 ± 5.89	11.63 ± 3.79	9.44 ± 3.17	5.69 ± 1.59	4.79 ± 1.33	13.71 ± 3.87	5.66 ± 0.82
Q2	21.77 ± 6.31	11.26 ± 4.15	8.78 ± 3.38	5.21 ± 1.84	4.46 ± 1.44	13.36 ± 3.91	5.58 ± 0.88
Q3	21.35 ± 6.23	10.92 ± 4.32	8.61 ± 3.34	5.16 ± 1.86	4.40 ± 1.46	13.16 ± 4.04	5.55 ± 0.95
Q4	20.05 ± 6.26	10.45 ± 4.53	7.94 ± 3.36	5.00 ± 1.73	4.35 ± 1.40	12.41 ± 4.18	5.19 ± 1.22
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a]*MIS, memory index score; EIS, executive index score; VIS, visuospatial index score; LIS, language index score; AIS, attention index score; OIS, orientation index score. Q1–Q4 are quartiles of each dietary pattern score. Global cognitive function score and cognitive domain subscores are expressed as mean ± SD, evaluated by Montreal Cognitive Assessment (MoCA, Beijing Version) and examined using Kruskal–Wallis H-test.*

decreased significantly as the quartiles of the "grain-preferred" pattern score increased ($p < 0.05$). After adjusting for all potential factors, only a positively significant relationship was observed between the visuospatial function score and the "eggs- and

TABLE 4 | Quantile regression analysis of associations of four dietary pattern scores with global cognitive score and cognitive domain subscores[a].

Dietary patterns	Global cognitive function		Cognitive domain scores											
	β	*p*-value	MIS		EIS		VIS		LIS		AIS		OIS	
			β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
Meat-preferred pattern														
Model 1	1.00	<0.001	0.50	<0.001	0.50	<0.001	0.00	1.000	0.00	1.000	0.67	<0.001	0.00	1.000
Model 2	0.43	<0.001	0.25	<0.001	0.15	0.001	0.00	1.000	0.00	0.814	0.25	<0.001	0.00	1.000
Model 3	0.42	<0.001	0.23	<0.001	0.12	0.034	−0.01	0.576	0.00	0.999	0.26	<0.001	0.00	0.986
Model 4	0.42	<0.001	0.20	<0.001	0.11	0.021	−0.01	0.669	0.01	0.653	0.22	<0.001	0.00	0.994
Plant-preferred pattern														
Model 1	1.00	<0.001	0.33	<0.001	0.50	<0.001	0.00	1.000	0.00	1.000	0.50	<0.001	0.00	1.000
Model 2	0.40	<0.001	0.17	0.012	0.17	<0.001	0.14	<0.001	0.00	0.957	0.32	<0.001	0.00	1.000
Model 3	0.47	<0.001	0.22	<0.001	0.18	0.001	0.15	<0.001	0.06	0.035	0.30	<0.001	0.00	1.000
Model 4	0.45	<0.001	0.25	<0.001	0.18	<0.001	0.13	<0.001	0.07	0.008	0.30	<0.001	0.00	1.000
Eggs- and dairy-preferred pattern														
Model 1	0.00	1.000	0.00	1.000	0.00	1.000	0.00	1.000	0.00	1.000	0.00	1.000	0.00	1.000
Model 2	0.00	1.000	0.00	0.976	−0.04	0.389	0.08	<0.001	0.00	0.817	0.00	0.968	0.00	1.000
Model 3	0.00	0.983	0.00	0.981	−0.04	0.403	0.07	0.003	0.03	0.228	0.00	0.991	0.00	1.000
Model 4	0.05	0.568	0.04	0.412	−0.04	0.395	0.08	0.002	0.04	0.056	−0.02	0.758	0.00	1.000
Grain-preferred pattern														
Model 1	−1.00	<0.001	−0.33	<0.001	−0.50	<0.001	0.00	1.000	0.00	1.000	−0.50	<0.001	0.00	1.000
Model 2	−0.50	<0.001	0.00	0.977	−0.23	<0.001	−0.11	<0.001	0.00	0.970	0.00	0.997	0.00	1.000
Model 3	−0.35	<0.001	0.00	0.998	−0.22	<0.001	−0.10	<0.001	−0.03	0.222	−0.12	0.048	0.00	0.986
Model 4	−0.36	<0.001	0.00	0.911	−0.19	0.001	−0.09	<0.001	−0.05	0.039	−0.11	0.071	0.00	0.894

[a]*MIS, memory index score; EIS, executive index score; VIS, visuospatial index score; LIS, language index score; AIS, attention index score; OIS, orientation index score. Global cognitive function score and cognitive domain subscores are evaluated by MoCA (Beijing Version). Model 1 was unadjusted; Model 2 adjusted for age, gender, residential area, education level, current employment status, and household income level; Model 3 further adjusted for physical activity, smoking, drinking, sleep duration, and total food energy; and Model 4 further adjusted for diet-related chronic disease history, obesity, and central obesity.*

dairy-preferred" pattern ($p < 0.05$). No significant association was observed between the orientation function score and any of the four dietary patterns ($p > 0.05$).

Dietary Patterns and MCI and Its Subtypes

As shown in **Figure 1**, the prevalence distributions of MCI and some of its subtypes differed among the quartiles of each dietary pattern. Participants in the highest quartile of the "meat-preferred" pattern had lower prevalence of MCI, aMCI-SD, naMCI-SD, and aMCI-MD ($p < 0.05$); and similarly, those in the highest quartile of the "plant-preferred" pattern had lower prevalence of MCI, aMCI-MD, and naMCI-MD ($p < 0.05$), compared with those in other quartiles of the corresponding pattern. In contrast, participants in the bottom quartile of the "eggs- and dairy-preferred" pattern had lower prevalence of MCI, aMCI-SD, and naMCI-SD ($p < 0.05$), and those in the bottom quartile of the "grain-preferred" pattern had lower prevalence of MCI, naMCI-SD, aMCI-MD, and naMCI-MD ($p < 0.05$).

Figure 2 presents *AOR* and the 95% *CI* of MCI and its subtypes in the quartiles of each dietary pattern score. After controlling for multi-covariates, participants in the highest quartile of the "meat-preferred" pattern had 24 and 56% lower odds of MCI (*AOR* = 0.76, 95% *CI* 0.63–0.92) and aMCI-SD (*AOR* = 0.44, 95% *CI* 0.28–0.71), respectively, compared with those in the lowest quartile; similarly, those in the highest quartile of the "plant-preferred" pattern were less likely to have MCI, aMCI-MD, and naMCI-MD. The *AOR* was 0.72 (95% *CI* 0.60–0.80), 0.49 (95% *CI* 0.34–0.72), and 0.41 (95% *CI* 0.27–0.63), respectively. In addition, the increase in each quartile level of these pattern scores significantly reduced the odds of MCI and the corresponding subtypes mentioned above (*p-trend* < 0.05). On the contrary, participants in the highest quartile of the "grain-preferred" pattern score tended to have 34% (*AOR* = 1.34, 95% *CI* 1.11–1.63, *p-trend* = 0.003) and 65% (*AOR* = 1.65, 95% *CI* 1.13–2.40, *p-trend* = 0.007) higher odds of MCI and naMCI-SD compared with those in the lowest quartile, and those in the second quartile of the "eggs- and dairy-preferred" pattern score had an AOR of 1.22 (95% *CI* 1.02–1.47) for the prevalence of MCI.

DISCUSSION

In this cross-sectional study, we identified four different dietary patterns: "meat-preferred" pattern (pork, fish, poultry, and beef or mutton), "plant-preferred" pattern (tubers, legumes, fresh vegetables, fresh fruit, and nuts), "eggs- and dairy-preferred" pattern (eggs and dairy products), and "grain-preferred" pattern (rice- and wheat-based foods). We found that adherence to the "meat-preferred" pattern and the "plant-preferred" pattern

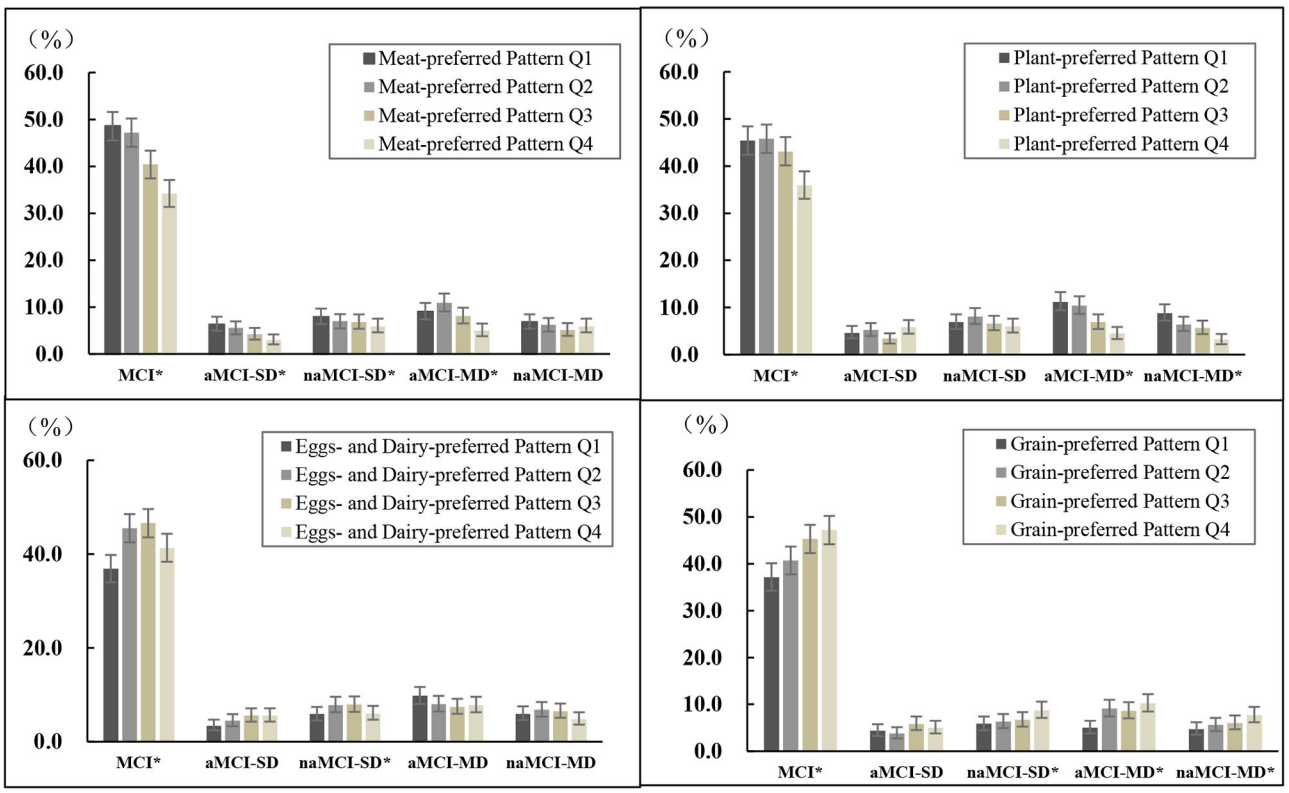

FIGURE 1 | Prevalence of MCI and its subtypes by quartiles of each dietary pattern score[a]. [a]Q1–Q4 are quartiles of each dietary pattern score. MCI, mild cognitive impairment; aMCI-SD, amnestic MCI single domain; naMCI-SD, non-amnestic MCI single domain; aMCI-MD, amnestic MCI multiple domains; naMCI-MD, non-amnestic MCI multiple domains. *$p < 0.05$ within dietary pattern score category, examined by chi-square test.

increased the scores of the global cognitive function and several cognitive domain functions and decreased the odds of MCI and some subtypes after adjustments for all potential confounding variables. Conversely, better adherence to the "grain-preferred" pattern reduced cognitive function scores and increased the odds of MCI and naMCI-SD in adjusted models. In addition, we observed that the "eggs- and dairy-preferred" pattern was associated with higher cognitive domain scores of visuospatial function. Participants in the second quartile of this pattern score had a 22% (95% *CI*: 1.02–1.47) higher odds of MCI compared with those in the bottom quartile.

In this study, the "meat-preferred" pattern is conducive in improving the cognitive function and reducing the odds of MCI, aMCI-MD, and naMCI-MD, supported by its food intake profile that is characterized by high intake of pork, fish, poultry, beef, and lamb. Previous studies have showed that these foods play a potentially beneficial role in cognition (12, 20). Specifically, we found that participants in the third or top quartile intake of pork, beef or mutton, poultry, and fish had about 20–30% lower *OR*s of MCI and its some subtypes, respectively, compared with those in the bottom intake of the corresponding food group (12). Moreover, the results of a large longitudinal study of 5,934 French people 65 years and older with an average follow-up of 9.8 years showed that the low-meat intake is associated with an increased risk of cognitive impairment compared with regular intake frequency (7). In fact, some researchers thought that the high-meat intake with high saturated fat content increases inflammation and circulating cytokines, and have a negative impact on cognition (21), but this may be offset by the protein source itself which is beneficial to cognitive function by promoting the production and release of catecholamines, when maintaining a reasonable balance of this pattern. In addition, increasing the intake of fish rich in polyunsaturated fatty acids could significantly combat the pathologic cognitive impairment of the elderly shown by mechanism studies and epidemiologic analyses (22, 23), which supports that this pattern is related to superior cognitive function.

Our findings show that the "plant-preferred" pattern is positively associated with a variety of cognitive functions ranging from the global mental status to specific domains. Food groups that are good for to brain are fruit, vegetables, legumes, and nuts, which are rich in fiber, beta carotene, vitamins K and C, folate, and especially magnesium (24, 25). These antioxidants, which are considered to have anti-inflammatory properties, have shown to be significantly associated with the prevention of cognitive impairment (26), because oxidative stress and inflammation are the triggers of the cognitive decline process (27). Some epidemiological studies are consistent with the above findings, despite the dietary pattern analysis is different (28, 29). For

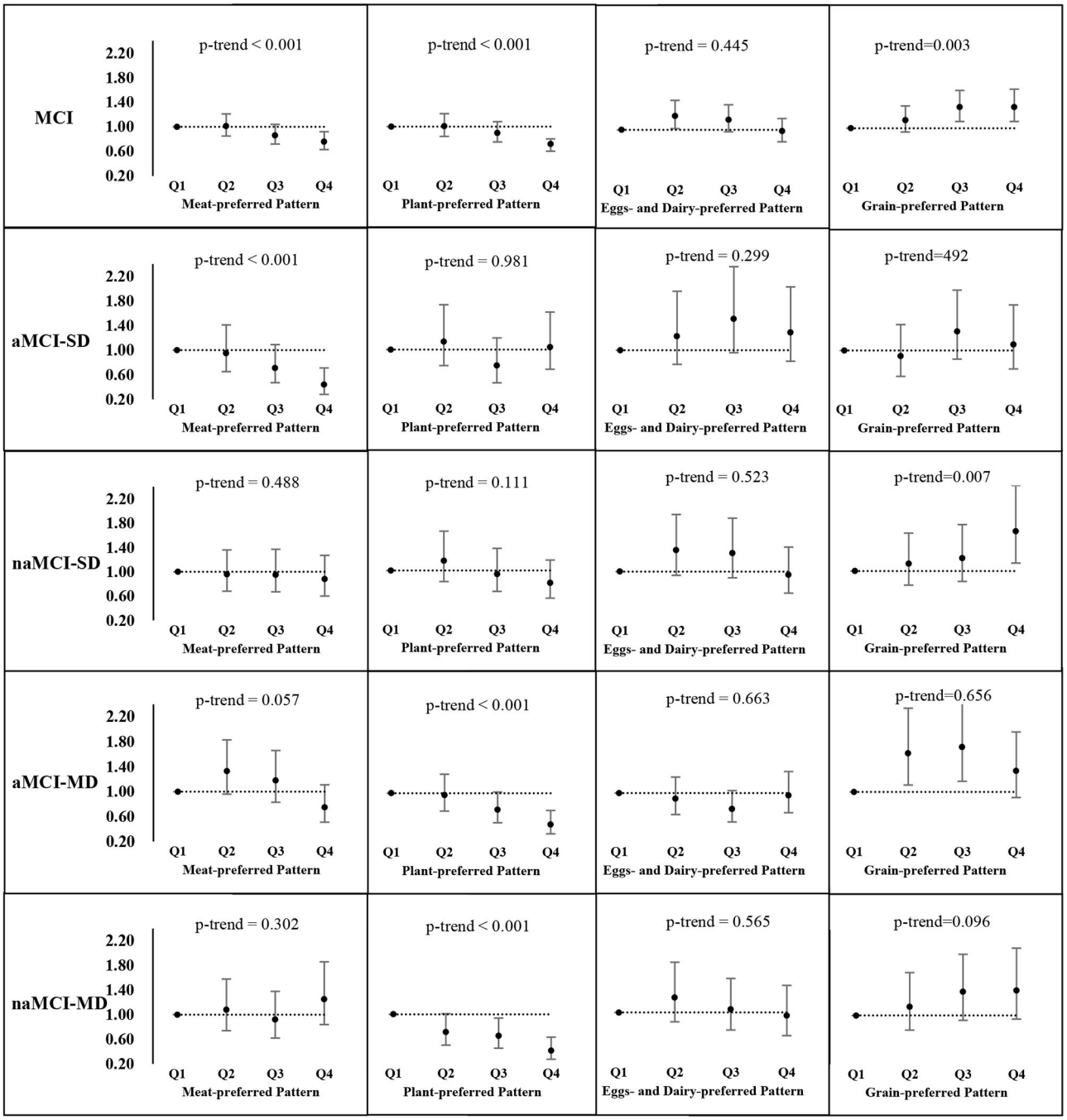

FIGURE 2 | Adjusted odds ratios (*AOR*) and the corresponding 95% *CI*s for MCI and its subtypes across quartiles of each dietary pattern score[a]. [a]MCI, mild cognitive impairment; aMCI-SD, amnestic MCI single domain; naMCI-SD, non-amnestic MCI single domain; aMCI-MD, amnestic MCI multiple domains; naMCI-MD, non-amnestic MCI multiple domains. Adjusted for age, gender, residential area, education level, current employment status, household income level, physical activity, smoking, drinking, sleep duration, total food energy, diet-related chronic disease history, obesity, and central obesity.

example, a food pattern related to high intake of vegetables, fruit, legumes, and nuts, derived through a reduced rank regression analysis of 2,311 Chinese elderly adults aged 60–88 years, is associated with better memory and language function and a lower likelihood of MCI (29). Although the main criticism of plant-based diets is the risk of vitamin B_{12} deficiency (30, 31), which is correlated to cognitive dysfunction (e.g., fatigue, memory, and movement problems) (32). However, moderate intake of poultry, beef or lamb can provide efficient vitamin B_{12} intake. This is another important feature of the "plant-preferred" pattern in this study, providing a plausible explanation to our findings.

Eggs, milk, and dairy products are nutritious foods that contain a variety nutrients associated with improving cognitive function, such as folate, vitamin B_{12} (33), choline (34), and protein (35). In 2019, a systematic review of six studies summarized the effects of milk and dairy products intake on cognitive function, indicating that dairy products may help prevent cognitive decline (36). However, a study of 3,835 American adults 65 years conducted in the same year did not find a similar relationship between egg intake and cognitive health (37). Our study found that eggs and dairy foods intake is positively correlated with higher scores of the "eggs- and dairy-preferred" pattern, which may improve visuospatial ability. In addition, we observed that the intake of eggs in the third quartile of this pattern score reached the recommended amount (40–50 g/day) of the Chinese Dietary Guidelines, but even in the top quartile the dairy intake did not reach its recommended level (300 g/day). In this regard, the difference in the intake of eggs and dairy foods intakes in the first two quartiles of the pattern is not significant. Therefore, the observation that subjects in the second quartile tended to have higher odds of MCI than subjects in the bottom quartile may be caused by data variances.

In addition, in our study, adults who adhere to the "grain-preferred" pattern consume more rice- and wheat-based foods, and their cognitive functions are often incomplete, which is consisting with the findings in the literature. There is evidence that high glycemic index foods may cause cognitive impairment through adverse effects on neuronal integrity and glucose metabolism in the nervous system (38, 39). It is worth noting that there is evidence that moderate intake of rice is negatively associated with cognitive functions (12, 40). Given that wheat intake increased as the quartiles of this pattern score increased, but rice intake did not increase, although both intakes were dominant. Therefore, we speculate that the detrimental effects of this pattern on cognition may be mainly due to the large intake of wheat-based foods. Of course, large-scale prospective cohort studies are needed to provide more clues to the underlying mechanism.

This study has several limitations. First, the cross-sectional nature of our study cannot provide evidence of any causal conclusions between dietary patterns and multidimensional cognition. It must consider the possibility of reverse causality that changes in dietary patterns lead to cognitive decline that cannot be captured. Second, the intake of food groups comes from a valid, semi-quantitative FFQ covering 1 year. Ranking subjects based on the food intake and presenting the profiles information of dietary nutrients intake in patterns is not completely accurate and may also lead to recall bias. However, the census concluded that recall errors due to cognitive impairment are believed to bias the results toward the null hypothesis. Third, the factor analysis method is highly data-driven, and thus dominant food groups are still interacted with other second or minor food groups. Fourth, although we carefully adjusted some covariates during the data analysis process, we may not consider residual confounding factors (e.g., hyperlipidemia, social engagement, living conditions, etc.). Finally, because this study covers adults in four provinces of China only, any generalization of the results we obtained to other regions should be carried out with caution. However, the CCSNSD is specifically designed to investigate the risk factors of neurological diseases in China. The population-based design reduces the selection bias, and a comprehensive evaluation of participant's cognitive function by trained evaluators with a degree in medicine or public health increases the internal validity of the findings.

This study showed the associations of four distinct dietary patterns with cognitive functions among Chinese adults, and thus findings can contribute to the establishment of dietary guidelines targeting older Chinese adults to reduce the cognitive impairment. It is of great significance to provide upgraded scientific evidence and strategies for early nutrition intervention and have important theoretical and social significance for promoting healthy aging.

CONCLUSION

To conclude, the study is the first to examine the relationship between dietary patterns and multidimensional cognitive function, global cognitive status, six cognitive domain functions, and cognitive-related outcomes of MCI and its four subtypes in Chinese adults. The study showed that the "plant-preferred" pattern and the "meat-preferred" pattern are associated with higher cognitive function scores and lower odds of MCI and some of its subtypes, which further provide evidence that higher intake of diet rich in vegetables, fruit, legumes, nuts, and meat may help prevent cognitive decline, and people who prefer grain foods, especially wheat-based foods, are negatively associated with cognitive health. However, the exact role of the "eggs- and dairy-preferred" pattern in cognition remains to be determined. Future prospective cohort studies are needed to examine the effects of these patterns on brain health to prove the causal relationship between dietary patterns and cognitive function.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of the National Institute for Nutrition and Health (No. 2017020, November 6, 2017). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

QH, XJ, JZ, and ZW contributed to the conceptualization of the manuscript. QH and JZ performed the methodology. QH is responsible for the formal analysis and writing—original draft preparation. FH performed data curation. XJ, JZ, and ZW contributed to writing—review. ZW and BZ contributed to project administration. HW is responsible for supervision. FH, LW, HJ, MG, YH, WS, YM, and XZ contributed

to the investigation. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.806871/full#supplementary-material

REFERENCES

1. Jia L, Du Y, Chu L, Zhang Z, Li F, Lyu D, et al. Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. *Lancet Public Health.* (2020) 5:e661–71. doi: 10.1016/S2468-2667(20)30185-7
2. Davis M, O Connell T, Johnson S, Cline S, Merikle E, Martenyi F, et al. Estimating Alzheimer's disease progression rates from normal cognition through mild cognitive impairment and stages of dementia. *Curr Alzheimer Res.* (2018) 15:777–88. doi: 10.2174/1567205015666180119092427
3. Xue H, Hou P, Li Y, Mao X, Wu L, Liu Y. Factors for predicting reversion from mild cognitive impairment to normal cognition: a meta-analysis. *Int J Geriatr Psychiatry.* (2019) 34:1361–8. doi: 10.1002/gps.5159
4. Liu-Seifert H, Schumi J, Miao X, Tian Y, Rabbia M, Andersen SW, et al. Disease modification in Alzheimer's disease: current thinking. *Ther Innov Regul Sci.* (2020) 54:396–403. doi: 10.1007/s43441-019-00068-4
5. Solfrizzi V, Custodero C, Lozupone M, Imbimbo BP, Valiani V, Agosti P, et al. Shits with Alzheimer's disease and late-life cognitive disorders: a systematic review. *J Alzheimers Dis.* (2017) 59:815–49. doi: 10.3233/JAD-170248
6. Li M, Shi Z. A prospective association of nut consumption with cognitive function in chinese adults aged 55+ China health and nutrition survey. *J Nutr Health Aging.* (2019) 23:211–6. doi: 10.1007/s12603-018-1122-5
7. Ngabirano L, Samieri C, Feart C, Gabelle A, Artero S, Duflos C, et al. Intake of meat, fish, fruits, and vegetables and long-term risk of dementia and Alzheimer's disease. *J Alzheimers Dis.* (2019) 68:711–22. doi: 10.3233/JAD-180919
8. Mantzorou M, Vadikolias K, Pavlidou E, Tryfonos C, Vasios G, Serdari A, et al. Mediterranean diet adherence is associated with better cognitive status and less depressive symptoms in a Greek elderly population. *Aging Clin Exp Res.* (2021) 33:1033–40. doi: 10.1007/s40520-020-01608-x
9. Trichopoulou A, Kyrozis A, Rossi M, Katsoulis M, Trichopoulos D, La Vecchia C, et al. Mediterranean diet and cognitive decline over time in an elderly Mediterranean population. *Eur J Nutr.* (2015) 54:1311–21. doi: 10.1007/s00394-014-0811-z
10. Okubo H, Inagaki H, Gondo Y, Kamide K, Ikebe K, Masui Y, et al. Association between dietary patterns and cognitive function among 70-year-old Japanese elderly: a cross-sectional analysis of the SONIC study. *Nutr J.* (2017) 16:56. doi: 10.1186/s12937-017-0273-2
11. Zhang J, Wang Z, Du W, Huang F, Jiang H, Bai J, et al. Twenty-five-year trends in dietary patterns among Chinese adults from 1991 to 2015. *Nutrients.* (2021) 13:e1327. doi: 10.3390/nu13041327
12. Huang Q, Jia X, Zhang J, Huang F, Wang H, Zhang B, et al. Diet-cognition associations differ in mild cognitive impairment subtypes. *Nutrients.* (2021) 13:e1341. doi: 10.3390/nu13041341
13. Julayanont P, Brousseau M, Chertkow H, Phillips N, Nasreddine ZS. Montreal cognitive assessment memory index score (MoCA-MIS) as a predictor of conversion from mild cognitive impairment to Alzheimer's disease. *J Am Geriatr Soc.* (2014) 62:679–84. doi: 10.1111/jgs.12742
14. Lu J, Li D, Li F, Zhou A, Wang F, Zuo X, et al. Montreal cognitive assessment in detecting cognitive impairment in Chinese elderly individuals: a population-based study. *J Geriatr Psychiatry Neurol.* (2011) 24:184–90. doi: 10.1177/0891988711422528
15. Ding D, Zhao Q, Guo Q, Meng H, Wang B, Luo J, et al. Prevalence of mild cognitive impairment in an urban community in China: a cross-sectional analysis of the Shanghai Aging Study. *Alzheimers Dement.* (2015) 11:300–9.e302. doi: 10.1016/j.jalz.2013.11.002
16. Winblad B, Palmer K, Kivipelto M, Jelic V, Fratiglioni L, Wahlund LO, et al. Mild cognitive impairment–beyond controversies, towards a consensus: report of the International Working Group on Mild Cognitive Impairment. *J Intern Med.* (2004) 256:240–6. doi: 10.1111/j.1365-2796.2004.01380.x
17. Petersen RC. Mild cognitive impairment. *Continuum (Minneap Minn).* (2016) 22:404–18. doi: 10.1212/CON.0000000000000313
18. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc.* (2000) 32:S498–504. doi: 10.1097/00005768-200009001-00009
19. Hirshkowitz M, Whiton K, Albert SM, Alessi C, Bruni O, Doncarlos L, et al. National Sleep Foundation's sleep time duration recommendations: methodology and results summary. *Sleep Health.* (2015) 1:40–3. doi: 10.1016/j.sleh.2014.12.010
20. Zhu J, Xiang YB, Cai H, Li H, Gao YT, Zheng W, et al. A prospective investigation of dietary intake and functional impairments among the elderly. *Am J Epidemiol.* (2018) 187:2372–86. doi: 10.1093/aje/kwy156
21. Tan B, Norhaizan M. Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. *Nutrients.* (2019) 11:2579. doi: 10.3390/nu11112579
22. Qin B, Plassman BL, Edwards LJ, Popkin BM, Adair LS, Mendez MA. Fish intake is associated with slower cognitive decline in Chinese older adults. *J Nutr.* (2014) 144:1579–85. doi: 10.3945/jn.114.193854
23. Zhang Y, Chen J, Qiu J, Li Y, Wang J, Jiao J. Intakes of fish and polyunsaturated fatty acids and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies. *Am J Clin Nutr.* (2016) 103:330–40. doi: 10.3945/ajcn.115.124081
24. Chan R, Chan D, Woo J. A cross sectional study to examine the association between dietary patterns and cognitive impairment in older Chinese people in Hong Kong. *J Nutr Health Aging.* (2013) 17:757–65. doi: 10.1007/s12603-013-0348-5
25. Jiang X, Huang J, Song D, Deng R, Wei J, Zhang Z. Increased consumption of fruit and vegetables is related to a reduced risk of cognitive impairment and dementia: meta-analysis. *Front Aging Neurosci.* (2017) 9:18. doi: 10.3389/fnagi.2017.00018
26. Mcgrattan AM, Mcguinness B, Mckinley MC, Kee F, Passmore P, Woodside JV, et al. Diet and inflammation in cognitive ageing and Alzheimer's disease. *Curr Nutr Rep.* (2019) 8:53–65. doi: 10.1007/s13668-019-0271-4
27. Den H, Dong X, Chen M, Zou Z. Efficacy of probiotics on cognition, and biomarkers of inflammation and oxidative stress in adults with Alzheimer's disease or mild cognitive impairment - a meta-analysis of randomized controlled trials. *Aging.* (2020) 12:4010–39. doi: 10.18632/aging.102810
28. Rajaram S, Jones J, Lee GJ. Plant-based dietary patterns, plant foods, and age-related cognitive decline. *Adv Nutr.* (2019) 10:S422–s436. doi: 10.1093/advances/nmz081

29. Su X, Zhang J, Wang W, Ni C, Hu S, Shao P, et al. Dietary patterns and risk of mild cognitive impairment among Chinese elderly: a cross-sectional study. *PLoS ONE.* (2020) 15:e0235974. doi: 10.1371/journal.pone.0235974
30. Gilsing AM, Crowe FL, Lloyd-Wright Z, Sanders TA, Appleby PN, Allen NE, et al. Serum concentrations of vitamin B12 and folate in British male omnivores, vegetarians and vegans: results from a cross-sectional analysis of the EPIC-Oxford cohort study. *Eur J Clin Nutr.* (2010) 64:933–9. doi: 10.1038/ejcn.2010.142
31. Medawar E, Huhn S, Villringer A, Veronica Witte A. The effects of plant-based diets on the body and the brain: a systematic review. *Transl Psychiatry.* (2019) 9:226. doi: 10.1038/s41398-019-0552-0
32. Moore E, Mander A, Ames D, Carne R, Sanders K, Watters D. Cognitive impairment and vitamin B12: a review. *Int Psychogeriatr.* (2012) 24:541–56. doi: 10.1017/S1041610211002511
33. Morris MC. Nutritional determinants of cognitive aging and dementia. *Proc Nutr Soc.* (2012) 71:1–13. doi: 10.1017/S0029665111003296
34. Blusztajn JK, Slack BE, Mellott TJ. Neuroprotective actions of dietary choline. *Nutrients.* (2017) 9:e815. doi: 10.3390/nu9080815
35. La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PJ. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. *Am J Clin Nutr.* (1997) 65:20–9. doi: 10.1093/ajcn/65.1.20
36. Cuesta-Triana F, Verdejo-Bravo C, Fernandez-Perez C, Martin-Sanchez FJ. Effect of milk and other dairy products on the risk of frailty, sarcopenia, and cognitive performance decline in the elderly: a systematic review. *Adv Nutr.* (2019) 10:S105–s119. doi: 10.1093/advances/nmy105
37. Bishop NJ, Zuniga KE. Egg consumption. Multi-domain cognitive performance, and short-term cognitive change in a representative sample of older US adults. *J Am Coll Nutr.* (2019) 38:537–46. doi: 10.1080/07315724.2019.1566035
38. Sunram-Lea SI, Owen L. The impact of diet-based glycaemic response and glucose regulation on cognition: evidence across the lifespan. *Proc Nutr Soc.* (2017) 76:466–77. doi: 10.1017/S0029665117000829
39. Taylor MK, Sullivan DK, Swerdlow RH, Vidoni ED, Morris JK, Mahnken JD, et al. A high-glycemic diet is associated with cerebral amyloid burden in cognitively normal older adults. *Am J Clin Nutr.* (2017) 106:1463–70. doi: 10.3945/ajcn.117.162263
40. Kim KY, Yun JM. Association between diets and mild cognitive impairment in adults aged 50 years or older. *Nutr Res Pract.* (2018) 12:415–25. doi: 10.4162/nrp.2018.12.5.415

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Nutrition and Congruent Care Improve Wellbeing of Residents With Dementia in Slovenian Care Homes

*Anamarija KejŽar[1], Liljana Rihter[1], Jakob Sajovic[2] and Gorazd Drevenšek[3]**

[1] Faculty of Social Work, University of Ljubljana, Ljubljana, Slovenia, [2] Department of Stomatology, University Medical Centre Ljubljana, Ljubljana, Slovenia, [3] Laboratory for Cardiovascular Pharmacology, Institute for Pharmacology and Experimental Toxicology, Medical Faculty, University of Ljubljana, Ljubljana, Slovenia

Introduction: Current nutritional strategies for people with dementia focus on nutritional diets and regimens, although in recent years congruent care for people with dementia has been increasingly recognized to improve their wellbeing. This includes consistency of care, respecting the variability of psycho-sociological factors, emphasizing the importance of participation in activities, and congruence with the individual's needs and capabilities. When applied to the nutritional aspects of care, it aims to empower people with dementia to have an active role in their care and during meals. Congruent care has previously shown promising results in improving the quality of life of residents, reducing the incidence of negative social interactions and daily intake of medicines.

Methods: A mixed methods qualitative-quantitative study was carried out. Out of 102 residential care homes for the elderly in Slovenia, a non-random sample of homes was selected. Seven homes that have implemented congruent care and five who have not implemented it agreed to participate. Content analysis of the transcripts of focus group interviews was carried out, to establish how the congruent care model was included into their everyday practice of care for people with dementia. Qualitative comparative analysis was used to describe the differences in the practice of care between the two groups of homes, in the fields of nutritional and general care. Frequencies and assigned importance of statements relating to different aspects of nutritional care were statistically compared.

Results: The introduction of congruent care improved the wellbeing of the people with dementia, as observed by caregivers. The homes that had implemented congruent care gave more attention to the food choice aspects of nutritional care ($p = 0.0474$, $95\%CI_{Congruent} = 50.77–72.35\%$, $95\%CI_{Non-congruent} = 27.65–49.23\%$), while the homes that had not were more attentive to the dietary intake aspects ($p = 0.0067$, $95\%CI_{Congruent} = 22.79–44.74\%$, $95\%CI_{Non-congruent} = 55.26–77.21\%$). In the homes for the elderly that had implemented congruent care, both caregivers and management reported that the frequency of use of *pro re nata* medication decreased, which is supported by the results of the linear regression ($R^2_{adjusted} = 78.4$, $p = 0.005$), although the data available is limited.

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***Correspondence:**
Gorazd Drevenšek
gorazd.drevensek@mf.uni-lj.si

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Conclusion: First, the people with dementia in the care homes that had implemented congruent care were observed to have improved in mood, attitudes toward eating and wellbeing, as reported by caregivers. Second, the implementation of congruent care was well received by the management and caregivers of the care homes. A model of implementation of congruent nutritional care for people with dementia is presented.

Keywords: nutrition, wellbeing, dementia, elderly, person-centered approach, congruent care

INTRODUCTION

Due to increasing life expectancy, the number of people with dementia is rising sharply, making these disorders a global public health priority. The most common types of dementia are Alzheimer's disease, frontotemporal dementia, and dementia due to vascular disease. Worldwide, ∼50 million people over 65 years are living with dementia, with 70% of them affected by Alzheimer's disease (1), a figure that is predicted to increase to 78 million in 2030 and 152 million by 2050 (2).

The common characteristics of dementia are impairment of memory and at least one domain of cognitive functioning, such as language, visuospatial skills, judgment or personality (3). Complex nutritional problems arise in dementia over the course of the disease, with the progressive decline in cognitive and behavioral functions ultimately leading to an inability to independently function in all aspects of life (4). During the onset of the disorder, impairments usually include difficulties in purchasing and preparing products, the preparation of simple dishes, forgetting to eat and drink, or eating multiple times a day. A decline in the sense of smell is also characteristic of the early stages of dementia (5). People with dementia may also experience increased appetite and rapid eating, and may repeatedly ask for food or exhibit compulsive eating (4).

As dementia progresses, so do the problems with eating—the inability to hold a spoon, to guide food to the mouth, impaired recognition of utensils or food, difficulty chewing or swallowing, loss of appetite or overeating, and the inability to drink from a glass. Especially in the later stages of the disease, malnutrition often occurs due to the refusal of food and fluids (6). Combined with the behavioral and psychological symptoms of dementia, such as depression, apathy and aggression, the capabilities of a person to continue eating autonomously are progressively impaired. It is therefore important to consider factors that positively affect the wellbeing of people with dementia at mealtimes and to consider their wishes and habits (7). One way to prolong the capabilities of people with dementia to eat on their own is by making meals as stress-free and enjoyable as possible. The general guidelines for the nutritional care of people with dementia are attractive and inviting meals, a daily routine and encouragement during mealtimes, variety in the menu, and respect for choice. The timing of meals and a calm and comfortable dining environment, as well as the food quality and suitability, are all important factors to consider (5, 8, 9).

In Slovenia, 59 public institutions and 43 concession providers offer long-term care (institutional care, day care and home care) with the capacity to care for 21,150 elderly people. The approaches with which care homes increase the wellbeing of their residents vary, but all adapt their processes of feeding to accommodate people with dementia. Some common adjustments that are made are in the consistency of the food, e.g. pureed and chopped food, and assistance with eating, such as spoon-feeding, in addition to the adherence to nutritional guidelines for the elderly. Aside from the nutritional aspect, many Slovenian care homes are implementing programmes of modern, integrated care for people with dementia to ensure the best possible quality of life. Since 2008, the household groups model has increasingly been adopted, featuring household units that provide individual care for 10–15 people with dementia (10).

Some public homes decided to take a further step, in addition to adopting the household groups model, and have introduced the congruent care programme, which includes a person-centered approach in all areas of life in a home for the elderly. Residents are treated as individuals and are equal partners in the process of care. It is personalized, coordinated and enabling, focusing on extending the autonomy of the residents and providing a supportive environment for when it becomes compromised (11). The implementation of congruent care, however, requires a large investment of both funds and time, as it is necessary to train all employees, from the management to the janitorial staff, in the methods of work according to the model. The process of implementation thus usually takes 5 years.

As one of the last areas of functioning that remain under the control of people with dementia, dedicating attention to the area of nutrition is of paramount importance in congruent care. In the spirit of congruent care, the individual's habits, desires, and possible dietary guidelines or restrictions need to be recognized and respected (12). Nutrition and the intake of food and drink can thus represent a key point of contact between the residents and caregivers, where the attunement of the caregiver to the person's wellbeing, wishes and habits places the resident at the center of care. To achieve this, caregivers strive to provide each individual with a meal in an environment where they feel safe and comfortable, and a meal they enjoy eating and can eat autonomously as long as they are able—with or without cutlery, mealtime extensions, snack corners and other adjustments (13, 14).

To more easily identify and evaluate the different areas of nutritional care that must be monitored and adjusted in everyday congruent care, Fostinelli et al. (5) suggest a taxonomy of three components: food choice (food preference and preparation); eating behavior (outcomes related to consumption, eating habits, eating disorders); and dietary intake (what is consumed, overall intake, specific nutrients). The authors further

provide the theoretical framework that the three factors depend on (or are related to), i.e. the physiological (hunger, satiety, innate preference for sweet food) and psychological processes (learned food preferences, knowledge, motivation, attitudes, values, personality traits, cognitive processes, self-regulation). In addition to these, social factors also influence food consumption and must be considered, for example, a person eats more when eating in the company of others (15).

In addition to improving the quality of life of the residents, other health benefits were observed after the implementation of congruent care. Of specific interest is the observation by Galiana and Haseltine (16), who report a significant reduction in the daily intake of medicines and a significantly reduced weight loss in residents. Moreover, the authors also reported a significant reduction in adverse resident-to-resident interactions, less agitation of the residents at sundown, and a deeper connectedness of the caregivers to the residents. The less frequent agitation in particular was tied to the freedom of choice in eating habits, food items and other nutrition-related aspects of congruent care.

These results inspired us to design the present qualitative-quantitative study, where we aimed to examine how nutritional guidelines are included in the everyday care of the care homes that have adopted the congruent care model and those who have not. We also hypothesized that the use of PRN (lat. *pro re nata*, "as needed") medication could be reduced in the care homes after the implementation of the congruent care model.

During our research, however, we found that there was little emphasis on dietary guidelines for people with dementia in either group of homes. Instead, the care homes organize their nutritional care not around guidelines, but according to their mode of operation, experience and capabilities. We therefore focused on the comparison and insight into how the two groups of homes approach nutritional care for people with dementia in general and in what ways they differ. The data on PRN medication use was very sparse, as we have discovered that these data are not routinely recorded and kept by the homes for the elderly. Thus only the data for PRN medication use in one home for the elderly was available, providing limited insight into the frequency of PRN medication use after the implementation of congruent care. Furthermore, we examined how care homes that adopted the congruent care model adapted their nutritional programmes for people with dementia. Based on these findings, we present a model for implementing congruent care in the field of nutrition of people with dementia.

METHODS

The study was a combined qualitative and quantitative study. The units of analysis were care homes, sampled from the population of 102 homes in Slovenia. The sampling in our study was targeted, meaning we chose 16 care homes, of which 8 had implemented the congruent care model for supporting people with dementia. We contacted the management of the homes and inquired whether they would be interested in participating in the current study, which would include focus group interviews being carried out with the management and the caregivers. From here on, the care homes are treated as statistical units, except for the analysis of medication consumption data, which was available only for one home, where the residents were treated as statistical units. From the group of care homes that implemented the congruent care model 7 responded, and from the second group 5 homes participated in our research. The frequencies of the response did not differ between the two groups, as tested by the Chi-square test of independence ($\chi^2_{\text{Pearson}} = 1.333$, df $= 1$, $p = 0.248$). The two groups equally did not statistically differ in the number of participating homes ($\chi^2 = 0.333$, $df = 1$, $p = 0.564$). We can thus conclude that the sampling is most likely unbiased by group characteristics. Additionally, all of the involved homes follow the same procedures for the procurement of food and are subject to the same cultural norms and values.

Qualitative data was obtained with the use of four focus groups based on semi-structured interviews with management and caregivers in the 12 care homes in May and June 2021. All the focus groups were recorded with the agreement of the participants. They lasted between an hour and a half to two hours. The focus groups were transcribed and analyzed by qualitative analysis in a process where units (parts of sentences, sentences or whole paragraphs) of analysis were identified first and then open codes were defined for each part. Next, axial coding was performed (codes with similar meanings were grouped into categories and themes). Finally, relations were found between categories and/or themes that were relevant for our research questions (17).

We adopted the three-component taxonomy of dietary behavior of Fostinelli et al. (5), as it is designed to form a consensus in the levels of nutritional care needed for people with dementia. The three components (food choice, eating behavior and dietary intake) were thus used as the basis for our results, according to which the statements of the interviewees were grouped, and statistical analyses were performed.

Qualitative comparative analysis (18) was used to assess the attitudes of the caregivers and management of care homes toward the three main aspects of nutritional care; food choice, eating behavior and dietary intake. The analysis was carried out by first identifying all the statements made by the caregivers and management that at least somewhat related to any of the three categories ($N_{\text{statements}} = 336$). This pool of statements was then further narrowed down by the consensus of three researchers on whether the statement did in fact relate to any one of the three aspects ($N_{\text{statements}} = 242$). The statements were then divided into three groups, according to the aspect of nutritional care they pertained to, with some statements being included in multiple groups, as their contents related to two or all of the aspects ($N_{\text{foodchoice}} = 74$, $N_{\text{eatingbehavior}} = 109$, $N_{\text{dietaryintake}} = 67$, $N_{\text{statmentsinmultiplegroups}} = 6$). Next, three researchers independently evaluated the statements with regard to the importance of the aspect of nutritional care to the home they conveyed, in a way blind to whether the statement originated from a care home with congruent care or without. The scores attributed were:

- 0 (We do not concern ourselves with this)

- 1 (We recognize this as a factor in the care for people with dementia)
- 2 (We are actively striving to pay attention to this factor in our everyday care and plan accordingly)
- 3 (This factor is exceptionally important to us, so we have gone to great lengths to ensure that needs associated with it are fulfilled)

Scoring was then averaged across evaluators, with the statements that had a minimum to maximum score difference of more than one (e.g. lowest score 1 and highest 3) removed from further evaluation. This was done in order to ensure that only statements where independent evaluators sufficiently agreed were further analyzed. For the food choice group of statements 11 statements were thus removed (14.9%), for the eating behavior group 17 (15.6%), and 7 statements (10.5%) for the dietary intake group.

Statistical testing was performed on the final scores, where the score of a statement was the mean of the three evaluators' scores. Testing of group differences was carried out with three separate Mann-Whitney *U* tests, with an alpha of 0.05 considered to be statistically significant. All tests were two-tailed. Mann-Whitney tests were used due to the non-normal distributions of all data, as assessed by one-sample Kolmogorov-Smirnov tests of normality (all $p < 0.05$), except for the congruent care homes dietary intake data, which also approached significance in the one-sample Kolmogorov-Smirnov test ($p = 0.066$). Two-sample Kolmogorov-Smirnov tests were used to corroborate the results of the Mann-Whitney U tests and to verify that the data came from the same distributions ($p_{foodchoice} = 0.180$, $p_{eatingbehavior} = 0.972$, $p_{dietaryintake} = 0.99$). The testing of the final scores was performed in order to determine if the two groups of homes differed in the expressed importance of the three aspects of nutritional care, where the null hypothesis was that they did not differ.

The group frequencies of statements, meaning whether a statement was given by the caregivers of a care home with congruent or non-congruent care, were tested by three separate binomial tests. These were performed in order to determine whether the two groups of homes differed in the number of statements given, with the null hypothesis being that they did not differ.

The amount of sedative or anxiolytic medication used as required (PRN) in people with dementia was available for one care home for 6 years after the implementation of congruent care. A linear regression model was used to assess the relationship between time (by year) and the number of events requiring this medication, in the years before and after the implementation of congruent care. The data fulfilled the criteria for linear regression (i.e. linearity).

SPSS v.25 (IBM, Armonk, New York, USA) and PRISM v.9 (GraphPad, San Diego, California, USA) were used to analyse and visualize the statistically tested data.

RESULTS

The response rates were 87.5% (seven homes) for the congruent care group and 62.5% (five homes) for the non-congruent group. Our sample encompassed 11.76% (12 out of 102) of all the care homes in Slovenia, meaning our sample is representative of the state of care for people with dementia in Slovenia at the institutional level. The care homes are located in most of Slovenia's major regions, giving a geographically sufficiently representative sample.

During the course of carrying out the research and after the analysis of the focus group results, it was discovered that there is not much focus on dietary guidelines for the care of people with dementia; instead, the caregivers and management of care homes attend more to the concept of nutritional care, regardless of whether the homes implemented congruent care or not. While implementation of specific nutritional guidelines for people with dementia, which would include nutrient ratios, amounts, etc., are lacking, the homes approach the problem of nutrition from a more comprehensive perspective, incorporating psychosocial aspects of human functioning into their care. This does not mean however, that no nutritional guidelines are adhered to, as care homes employ dieticians who construct the menus and ensure the nutritional quality of the food. The results merely point to the fact that no specific dementia-adjusted guidelines are observed.

We divided the results into two main sections: the first aims to describe how congruent care is implemented in practice, as well as the possible positive effects of such an approach to care. The second presents the differences between the homes that have implemented congruent care and those that have not. Both sections of the results first present representative statements obtained from the focus group interviews which illuminate the results of the qualitative analysis, which are then followed by limited quantitative analysis which helps to further the depth of analysis.

The Results of Implementing Congruent Care

The results of the focus group interview data pertaining to implementation of congruent care, the specific areas of dietary behavior that are focused upon, and the perceived effects of the implementation are presented in **Figure 1**.

How Was Congruent Care Implemented?

The results[1] showed that the implementation of congruent care was based on three main factors. The first was the education of both caregivers and management in the model of congruent care, and their acquiring the communication and cooperation skills needed.

Some statements outlining this are quoted below:

- "… [We] *first enrolled management in these courses, and finally included autonomous work teams in them.*"

[1] As the interviews were carried out in Slovene, only representative statements were translated into English and were kept as literal as possible to maintain the meaning and provide the closest approximation of the originals. For a full translation, please contact the corresponding author.

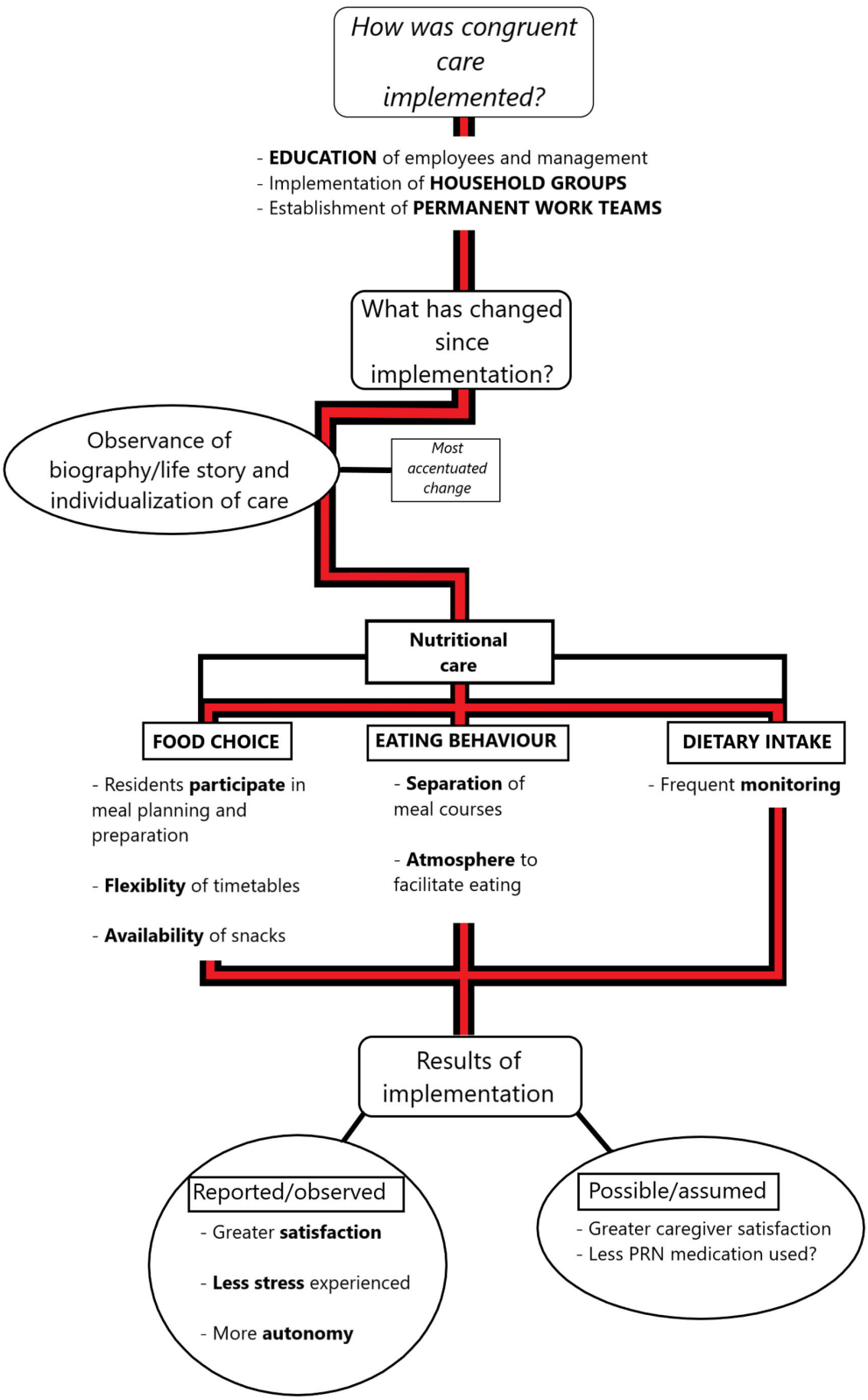

FIGURE 1 | A summary of the results of implementing congruent care in caretaking of people with dementia. The red-and-black line shows the course of themes covered by the questions asked. The square boxes with the rounded edges show the three main areas of response, the ovals show important, highlighted results, and the emboldened text highlights keywords pertinent to answers that relate to specific topics.

- "*The growth in the process of education, it is really important to ensure that what was learned becomes the new normal*."

These statements show the trend observed, where the education of both management and caregivers is seen as crucial. Next, we see a desire to improve, and to base the improvements on expert opinion and established models. Finally, we see that the self-improvement of all employees is seen as important in ensuring that the changes implemented are successful.

The second factor was the establishment of household groups, an organizational approach, where the residents live in groups of up to 20 people, with each group having their own assigned permanent support by caregivers (such as housekeepers, nurses etc.). This was seen as another crucial step in implementing congruent care. The representative statements which show this include:

- "*Our 200 residents live in household groups, divided into 10 groups of 20 residents*."
- "*The dynamics of life, of residence, are different in household groups*."
- "*You just have to think of this as living with family. And we were able to implement this. Not only the household group, the whole unit can be as a family. We began this way of working some time before the implementation of congruent care in our residential units, where the residents and caregivers are permanent*."

As these statements show, the household groups enable a more individualized approach to care, with deepening relationships between caregiver and resident.

The third factor was the establishment of permanent work teams, the importance of which is evident from the following statements:

- "*It's really important to have permanent teams of caregivers in household units*."
- " *…the key to effective care is really having staff permanently assigned to the resident's household groups."*
- "*...the employees know each other, their own life stories, the life stories and residents themselves, as the residents know them in return*."

The knowing of people and their personal characteristics, and the mutual familiarity of caregivers is important for creating a stable working and living environment that enables a focus on individual aspects of care. It also means that deeper relationships can be established. Having permanent staff in the household units is additionally viewed as a key component in effective care.

What Changes Have Been Made Since the Implementation of Congruent Care?

Life Stories and Individualization of Care

The most common change observed was the focus on life stories or biographies of people, where nutritional history and preference is recognized as one of the key aspects of care. This means that the caregivers in the care homes collected information on the preferred dishes and food of people before their admission into the home. These were further tied to life events (such as strong memories in childhood, or other important events, such as weddings) and used to construct individual plans of care for the residents. The importance of these life stories for the working process after the implementation of congruent care is evident from the following statements:

- *"The focus is very much on the individual, on their life story, what they like and what makes them feel good."*
- *"We have many residents that walk around at night. For example, a female resident who used to be a nurse often comes to us at night when we are on call in the section of the care home for people with dementia and says: "Well, what now, who is going to sleep tonight? Me or you?," to which I reply: "You go ahead and lie down, I'll be on call today. If the doctor calls, I'll call you, don't worry." "Can I really go to sleep?" "Yes, go, sleep, I'll take care of it today." Truly, this shows how important it is to know what lives our residents have lived and to know their life story."*

Particularly from the second quote, the importance of using information from a person's life story to calm them down and take care of their concerns is evident. Without knowing that the person was previously a nurse, the caregiver might have been clueless as to what the female resident meant by who is going to sleep tonight and might have reacted differently, perhaps by attempting to return her to her own room or administering a sedative medicine.

The individual approach in the field of nutrition, specifically of food choice, is described in the following statement:

- "*… we make an effort to obtain information from the children of the residents about what their parents liked to eat before. Which dishes do they like, what don't they like? We then, of course, take this into account, enter it into their life story document and make that available to all the employees who work with that resident.*"

In turn, the knowledge of the persons' history and preferences allows for the formation of detailed, individualized nutritional and caretaking plans that provide the person with as much freedom, familiarity and support as they require. The importance of information transfer between different employees is also seen in the previous quote.

Nutritional Care

Food Choice

On the specific changes in the areas of food choice, eating behavior and dietary intake, changes to accommodate the specifics of people with dementia were made.

Pertaining to food choice, participating in meal preparation and menu planning was seen as very important. The way in which this is organized is seen in the following two statements:

- "*We organize a meeting with the residents once a week, so they can tell us what they want to eat*."
- "*We include residents that are able enough in setting the tables before meals, placing the chairs around the tables, arranging the eating sets and giving out the food. After they have finished eating, they also help with putting the plates and cutlery into trolleys to be taken away*."

Flexibility of timetables, meaning the residents are free to choose the time when they eat breakfast, for example, were identified as important, as well as the availability of snacks and finger foods. The snacks are used as an alternative method of food supplementation, ensuring the people eat enough calories and maintain their weight. The organization of this is illustrated by the following responses of caregivers:

- "*We leave them be; we do call out to them and invite them to join the meals, when the main mealtimes come round, but we see who comes and who doesn't. A resident might choose to first take a walk before a meal, as he prefers to eat alone, slowly, sometimes for more than an hour, while another might be punctual as a Swiss clock. We adapt to their wants and needs.*"
- "*…the introduction of this corner helped a lot, the residents liked it very much as well, the "Serve yourself" corner. There we always have fruit, in a form that is easy for them to consume, yogurts, warm drinks… For example, we have some whole and some sliced apples, as some residents are more likely to notice sliced apples and some more likely to see whole ones and help themselves. We also have grapes in bunches, cut into portions, so they are not too large. We also always have warm drinks, yogurt and cakes. The residents who normally eat less during meals often take advantage of this corner and eat something there.*"

We can see that in homes with congruent care, changes were made to enable people to make their own decisions with regard to food, the time of eating, and to provide them with alternative opportunities to take in enough calories and maintain weight. We can also see that the interventions are thought out with attention to the sensory and cognitive specifics of people with dementia (i.e. fruit that is in sliced and whole form). The maintenance of as much autonomy as possible is also seen as a priority, allowing extended independent functioning of people, where possible.

Eating Behavior

The two main changes that were made to accommodate eating behavior in people with dementia were made after adopting the congruent care model. The first was the separation of individual meal courses (e.g. soup, salad, main course, dessert), which allows people with dementia to focus on one dish at a time and prevents confusion due to too many different foods being available at once. It also prevents another issue with presenting all the meal courses at once, that is, people with dementia eating only the dessert and rejecting the other foods. The second change was the creation of an atmosphere appropriate for the consumption of food, removing distracting stimuli and encouraging the people to eat. A multisensory approach was taken in this case, including the use of smells (by cooking in a kitchen that is spatially connected by corridors to the rooms of the residents, allowing them to smell when meals are being cooked), verbal encouragement, help with cutting up or consuming the dishes, and controlling the noise levels (by turning down or turning off music). Sometimes preparation for the meal, such as assistance with dressing up and putting on makeup helps the people to partake in the meals with more motivation and encourages them to consume the food. The following statements outline the measures described above:

- [Separation of meal courses] " *…we serve the food to every resident, while they are sitting at the table. This means we do not use trays for anyone, not that this is possible for people with dementia, but neither do we use trays with our other residents. The soup and main course, dessert or salad, whatever is on the menu that day, we place the next course in front of them after they have eaten the previous dish; we approach them so they can see us, then we give them some encouragement, so the eating is a bit easier. We want them to feel as much at home as possible. It's the same as at home, where we do not put everything we have for that meal on the table at once.*"
- [Creation of atmosphere] "*They come out of their rooms and start smelling what's cooking. It's a very positive feature. When food is prepared within the residential unit, it is very different to normal; everything smells of cooking in the unit."*
- " *… music has to be relaxing and quiet.*"
- " *… it helps when they are all tidy and have their hair done in the morning.*"

Dietary Intake

Concerning dietary intake, more evaluation was implemented, while observing the individual life stories and data gathered by the staff. This allows for more rapid adjustments in the treatment of individuals. For example:

- "*… we make regular notes to use in the evaluation; that helps make sure nothing is missed when we evaluate our care.*"

Observed Effects of the Implementation of Congruent Care

Both caregivers and the management of care homes report that the people and their relatives are more satisfied with the food and treatment after implementation of congruent care. Caregivers also report higher levels of job satisfaction. Additionally, they report that they have had fewer negative interactions with the residents, in the sense that agitation of the residents is usually addressed in a different manner, with conversation, relaxing activities or gentle encouragement. This, coupled with reduced stress levels for both caregivers and the people with dementia, likely contributes to the observed reduction in the need for PRN medication, as reported by caregivers and management of the care homes. The greater autonomy of the people with dementia is achieved by the methods described in the results section pertaining to food choice. Some statements outlining these reported effects are presented below:

- [Satisfaction and stress reduction] "*We have seen a reduction in, I'll say, unwanted interactions with residents, stressful situations, and issues with residents, and this also benefits the caregivers."*
- "*…Using congruent care shows in the levels of satisfaction that we survey every year, in residents, their relatives and our employees—all report over 90% of possible scores denoting satisfaction with care and work done.*"
- "*… not one of our caregivers would willingly revert to the old way of doing things.*"
- [Reduction of negative interactions and PRN medication use] "*…we have implemented the concept of personal monitoring,*

where we set out to teach our caregivers the techniques they need to calm the residents down, other than with medication. We have… the therapeutic discussion is at the forefront, touching them gently and sitting down with them, embracing them, calming them down with conversation. As was said, if they are hungry, we offer them something to eat, and they are immediately happier. We also use music therapy, adjusted for every individual. They often accept the headphones that we put over their ears, they listen to the music and calm down. The next measure is, of course, a safe space. They know this environment, they are free to come and lie down in the living room—if they feel safer with us, they don't have to stay in their rooms."

- "*PRN medication use has been reduced due to us being constantly in their company—we know them well-enough to adjust any medication needed as the situation changes, so we can reduce the medication when needed, not only increase it.*"

It is evident from these statements that the implementation of congruent care significantly improved the experience of caregivers, residents and their relatives.

Reduction in the Use of Pro Re Nata (PRN) Medication

We also hypothesized that a reduction in PRN medication use would be seen in the homes that implemented congruent care, but as the care homes mostly did not keep records of the PRN medication used, we were only able to obtain the data for one home that had implemented congruent care. We were unable to acquire any quantitative data from the homes that had not implemented congruent care on this topic, so between-group comparisons are impossible. The results of this analysis are a potential indicator of a relationship between the implementation of congruent care and PRN medication use, but are not grounds for any strong conclusions on the topic. Regardless, we present the results of a linear regression of PRN medication use since the implementation of congruent care, with **Figure 2** presenting the results.

The linear regression model of time since the implementation of congruent care and PRN medication use proved to be significantly better than the null model ($F = 22.75$, $p = 0.005$). The time since implementation explained 78.4% of the

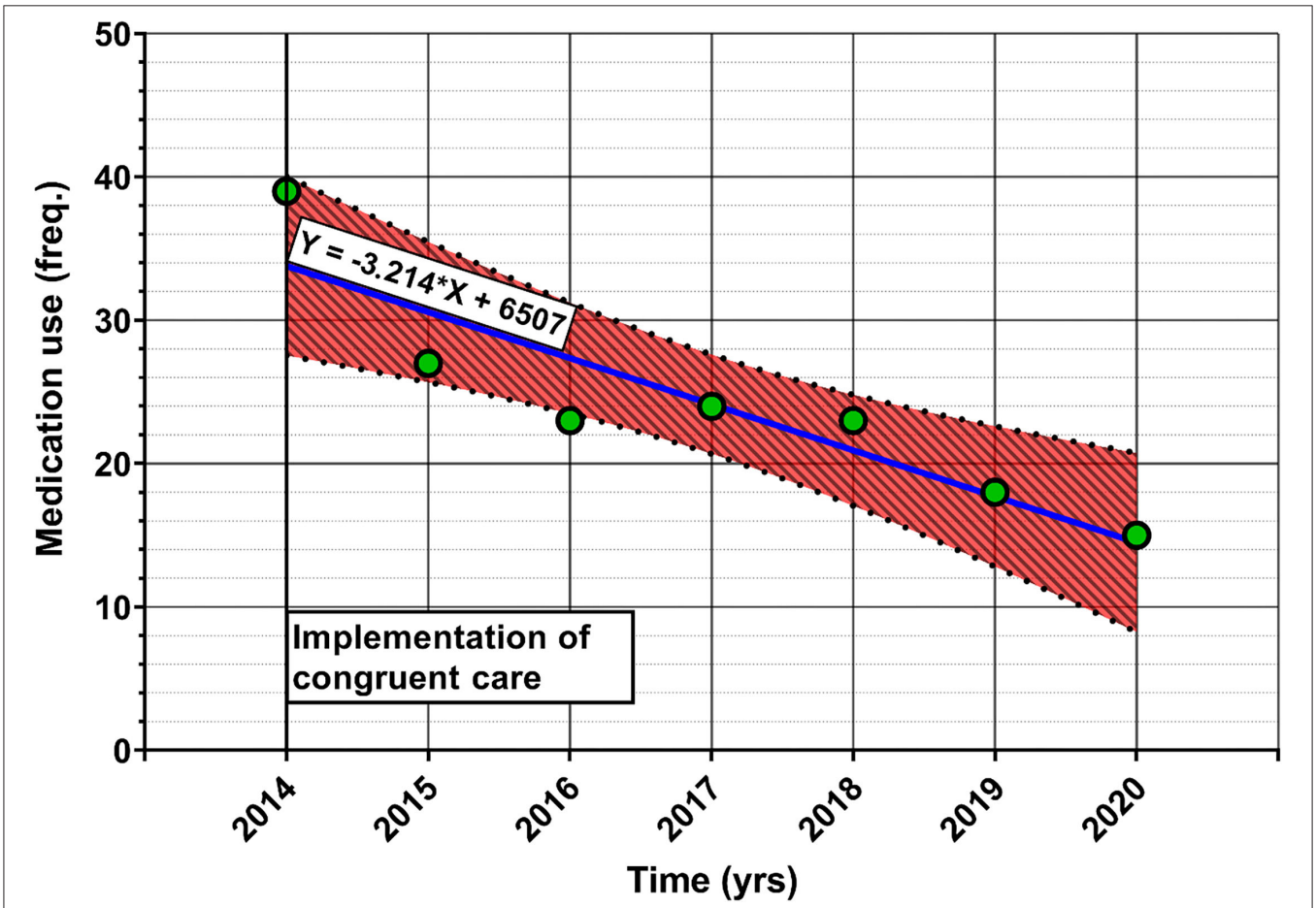

FIGURE 2 | Linear regression of the frequency of PRN medication use (Y-axis) and time (in years, X-axis) since the implementation of congruent care in one home for the elderly. The red shaded area represents 95% CI for the regression line, with the blue line representing the regression line itself. The green dots represent individual data points. The equation on the regression line represents the equation of the line. The data is obtained from a congruent care home that decided to closely monitor the use of PRN medication after they began the process of implementation of congruent nutritional care.

variance (adjusted R^2) in PRN medication use. These results show a significant association of the use of PRN medication and time since congruent care implementation, but warrants further research, with more variables and care homes included in the model to explain in more detail how the two variables are associated. From parameter B (or the slope parameter in the equation) of −3.214 [95% CI; −4.946–(−1.482)], however, we can assume, barring any confounding factors that are discovered in the future, that the implementation of congruent care could reduce the frequency of PRN medication administration by 3 (0.214) times per year, for at least 6 years after implementation.

Differences Between the Homes That Implemented Congruent Care and Those Which Did Not

Figure 3 presents the results of a qualitative and quantitative analysis of data gathered from the focus groups that pertains to differences in care for people with dementia in Slovenian care homes.

During the analysis of the transcripts of the focus group recordings it became evident that the care homes that had not implemented congruent care took a more general approach to nutritional care than the homes with congruent care. In summary, both caregivers and management reported that less attention is paid to the individualization of meals and preferred foodstuffs, which can be seen in the following statements (**Table 1**).

On the other hand, more attention is given to the medical and deficiency aspects, even when individualization of nutritional care is present, meaning that in homes with congruent care there is a focus on the individual so they can quickly observe if a resident does not want to eat. Yet in non-congruent homes (more) focus of care is evident when people already face malnutrition or other medical complications. These differences are shown in **Table 2**.

The care homes with congruent care also gave more detailed descriptions of evaluation and information transfer between caregivers in different roles, with such descriptions being absent from the answers of homes without congruent care.

These results are corroborated by the analysis of the number of statements given by each group of homes on the specific topic of nutritional care for people with dementia (**Figure 4**).

The results of the comparisons with binomial tests are: for food choice $p = 0.0474$, $95\%CI_{Congruent} = 50.77–72.35\%$, $95\%CI_{Non-congruent} = 27.65–49.23\%$; for eating behavior $p = 0.4437$, $95\%CI_{Congruent} = 44.79–63.18\%$, $95\%\ CI_{Non-congruent} = 36.82–55.21\%$; and for dietary intake $p = 0.0067$, $95\%CI_{Congruent} = 22.79–44.74\%$, $95\%CI_{Non-congruent} = 55.26–77.21\%$. All confidence intervals are reported as % of all statements.

As the frequency of mentioning a specific topic can be construed as that topic being more in the forefront of a person's

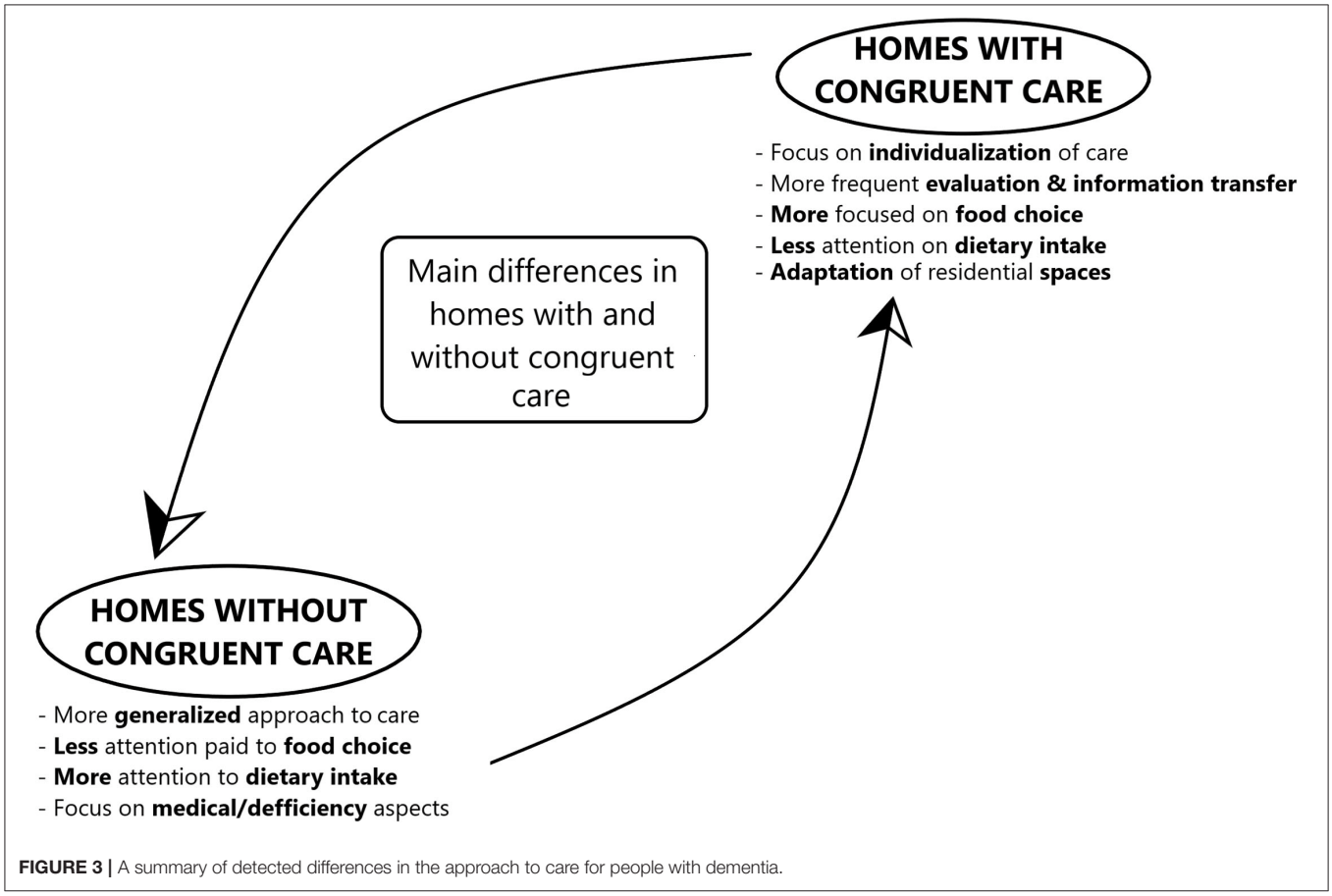

FIGURE 3 | A summary of detected differences in the approach to care for people with dementia.

TABLE 1 | Selected statements on the approach to nutritional care.

	Care homes with congruent care	Care homes without congruent care
Approach to nutritional care	*"My residents would like to eat beans, potatoes and cabbage all the time. That's the fact of our location. So we have a joint group of caregivers and residents, with the head nurse and cook, to make a menu for each month."*	*"Once every three months all the cooks and dieticians meet and make the menu and so on."*
	"Absolutely, we take mealtimes into account. We also offer a choice, as my colleague said. Everyone can choose what they eat for breakfast—will they eat a spread of one sort or another, maybe that one, will they eat salami, an egg, maybe three eggs?"	*"…we adjust, so the food that the people with dementia find difficult to eat, we offer something else, as with the other residents"*
	"We honor their wishes, so they can sleep in and join the meal later."	*"During the day the activities are structured in the same way as for the other residents, but they have a timetable, because we find it very important to provide a routine to the residents with dementia. This means when there is breakfast, it is breakfast, and after that we have activities. When it's time for coffee it's time for coffee, maybe we offer some biscuits. Later we also have lunch, afternoon and morning snacks, as well as dinner."*

TABLE 2 | Selected statements on the focus on individualization in care homes, by group.

	Care homes with congruent care	Care homes without congruent care
Focus on individualization	*"Absolutely, if a person doesn't want to eat anything that is available, we always find something they like or we make something especially for them, so they can eat it that day. If they want something else, we always try to fulfill that wish."*	*"…in people where these difficulties occur, when they can no longer swallow normally, they don't know how to swallow the food. Then we consult with the dietician, or use our good practices from the past, which allow us to ensure they get the calorific intake they need."*
	"We try to fulfill their wishes, so they can sleep in or come for a meal later, not at a pre-set time, as we used to do."	*"We do individualize care, for example, in people with dementia, they often start to choke on their food."*
	"We try to be really focused on the wishes of the residents in the area of nutrition. But, of course, we cannot ignore the doctors' prescriptions."	*"…individually, we then, one way or another, individualize care, if we find that someone does or doesn't want something, or when we find that a person is malnourished or in need."*

mind, we can conclude that the caregivers and management of the care homes that have implemented congruent care pay more attention to the food choice aspects of care and less to dietary intake, while the reverse is true for the caregivers and management of homes with non-congruent care.

The results of the analysis of the statements, evaluated on the importance attributed to specific topics of nutritional care, were non-significant. From this, it follows that we cannot say that the two groups of homes differed in the assigned importance to each of the three aspects of congruent care: food choice, eating behavior and dietary intake.

DISCUSSION

The present study was designed and carried out to investigate how the implementation of congruent care affected adherence to dietary guidelines for people with dementia. In the process of the research, it was found that neither group of care homes adhered to any specific guidelines. Instead, a psychosocial approach to nutrition was favored, with both groups of homes assigning equal importance to the food choice, eating behavior and dietary intake aspects of nutritional care. The differences between the groups of homes that have implemented congruent care and those that have not are as follows (**Figure 4**; **Tables 1**, **2**):

1. The care homes with congruent care are focused more on the food choice aspects of care, while more attention is given to dietary intake by the homes with non-congruent care (**Figure 4**).
2. The focus of the individualization of care, while present in both groups of homes, is different. The group without congruent care focuses more on the medical aspects, and attention to dietary intake and care is evident when people

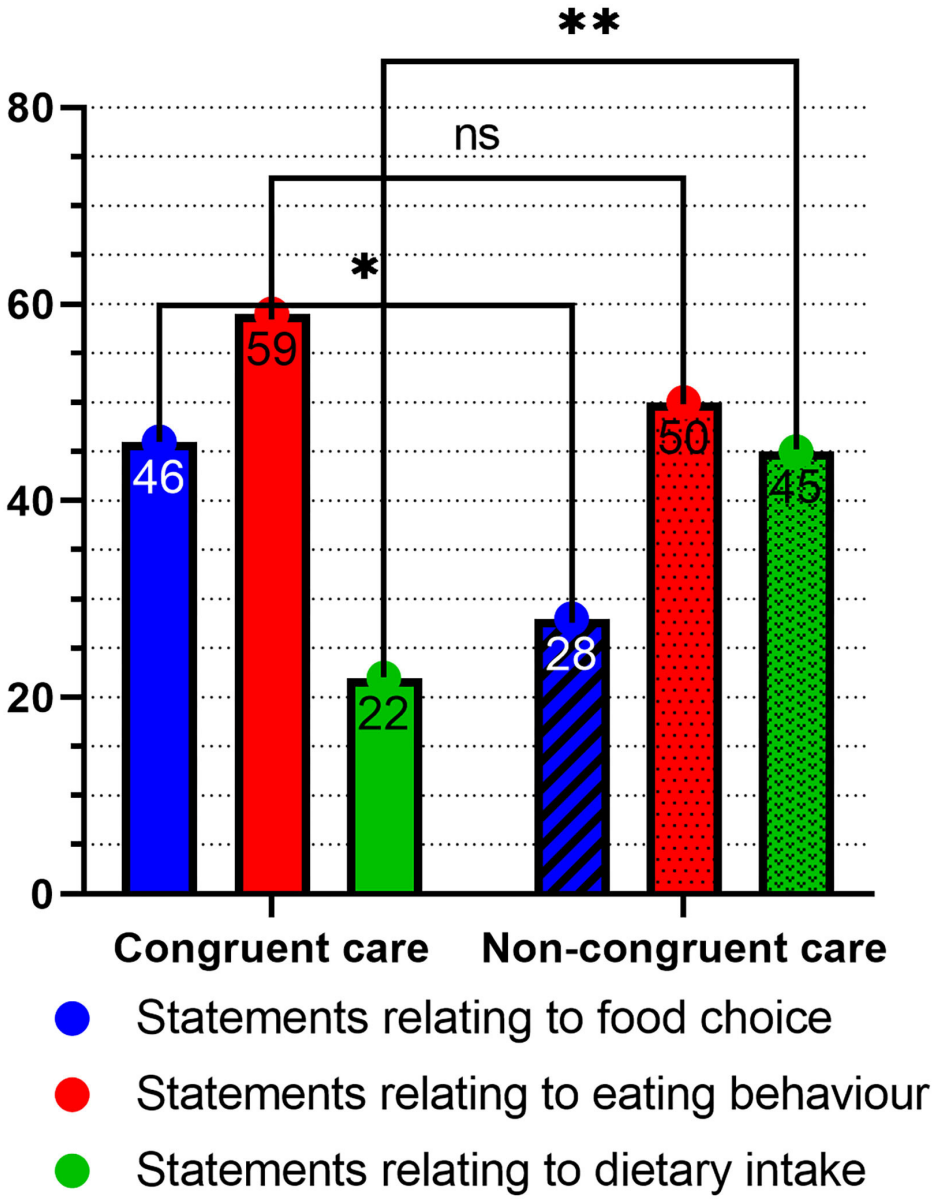

FIGURE 4 | Differences in frequency of statements relating to a specific area of nutritional care for people with dementia. Three separate binomial tests were run to determine the between-group differences. The * symbol denotes a statistical significance of <0.05, while the ** symbol denotes a statistical significance of <0.01.

already face malnutrition or other medical complications, while homes with congruent care favor aspects which improve quality of life (**Table 2**).

3. The approach to nutritional care is more generalized in homes with non-congruent care, while more accommodation of individual preferences and needs is made in homes with congruent care (**Table 1**).

The finding from the data on PRN medication use of one care home with congruent care warrants further investigation by future studies. Although the management and caregivers of the homes with congruent care reported that a reduction in the use of PRN medication is present, which concurs with the results of the data analysis of the one home, the limited amount of available records bars us from making strong conclusions on the topic.

Example statements on the reduction in medication use:

- "*PRN medication use was reduced, because we, the caregivers, know the residents, which means we are able to recognize a deterioration in the mental state of a resident, which enables us to attempt to steer them away from a difficult situation by talking to them.*"
- "*Additionally, daily medication use is reduced because we are there, with them all the time, and we know them well-enough*

to be able to reduce, not only increase, the dosage of medication when any changes are detected."

The unfortunate fact that most homes do not keep detailed records of PRN medication use should be overcome in the future, as this is an important variable to follow. This is recognized by the caregivers and employees of care homes, as it was stated during the focus group interviews that:

- "…[This] *has to be worked out, how to follow this* [the PRN medication use] *effectively*."
- "*As for PRN medication use in care homes, a resident pharmacist post should be created and filled in homes in Slovenia, … as someone who has knowledge of drug interactions and would closely follow medication use could surely reduce its use by 20–30%*."

Following on from these results, we recommend that the effects of the implementation of congruent care on PRN medication use is confirmed by future studies, and that care homes keep detailed records of their PRN medication use, where the data is not only gathered but also collated into useful information that can be analyzed and interpreted. This will enable more transparency in PRN medication use and easier adjustment when the data of an individual person is considered.

Some benefits of the implementation of congruent (nutritional) care are obvious. For example, a satisfaction level of over 90% in residents, relatives and caregivers was seen in care homes with congruent care, while no such reports were given by non-congruent homes. This is in line with previous results on this topic, by Galiana and Haseltine (16), who reported a similar level of satisfaction with care in the care homes they analyzed. Moreover, the fact that several caregivers of homes with congruent care stated that they would not willingly revert to the previous mode of work is salient to this discussion and supports the effectiveness of congruent care.

The nutritional aspects are recognized as crucial in the care for people with dementia, both in the extant literature (4, 16) and in the opinion of the caregivers interviewed in our study;

- "*As we know, food is one of the primary human needs, which remains important until the day we die, and as such contributes to quality of life*."

Regardless of the importance of food and nutrition in everyday care, the management and caregivers of the care homes that participated in our study expressed a concern that there is a lack of training courses or other options for education in the field of nutrition that are focused on these aspects of care for people with dementia.

To address this issue, we propose a four-part model for the implementation of congruent nutritional care for people with dementia, which synthesizes the experience of the staff and management of the care homes that adopted congruent care, and the current theoretical framework for nutritional care for people with dementia (4, 5, 16), presented in **Figure 5**.

Beginning with the requirements (**Figure 5**, red panel) for implementation, education for both caregivers and management is crucial when congruent nutritional care is to be implemented in care homes. Some care homes that have implemented congruent care have solved the issue of the shortage of available educational programmes by hiring professional chefs or by organizing internal education courses led by their resident dieticians in order to provide information for the caregivers on food preparation and dietary specifics.

Another important factor is the availability of space to reorganize the care and living arrangements into household groups. The residents ideally live in groups of 10 to 15 people in these household groups, with the care required being provided by the caregivers within the household. Each household has a permanent "housewife" assigned, who is in direct everyday interaction with the residents and acts as a "point of information" about the household group. Most of the daily life in these household groups revolves around a central kitchen and living room, where people can mingle and socialize. The food is also prepared there, which enables the residents to more easily help in its preparation, and allows for the smells of cooking to spread around the household, improving their interest in food. This way of organizing the care is important, as it allows the elderly people to retain the greatest possible resemblance of normal daily life, and builds the feeling of homeliness and familiarity, which has a calming effect on the residents (16). Moreover, it provides a constant point of contact between the residents and food preparation that fosters the retention of autonomy in the area of nutrition.

To support people with dementia in maintaining their autonomy as much as possible, permanent work teams are crucial (also see the Results section How Was Congruent Care Implemented?). Permanent work teams allow the formation of deeper personal connections, trust and mutual understanding between the residents and caregivers that enables easier, quicker and smoother adjustment of care to the needs of people with dementia.

A system of information gathering is the final requirement for the implementation of congruent nutritional care for people with dementia. To construct the life stories of their residents, caregivers should obtain information from multiple sources, ranging from the residents themselves and information from medical records to interviews with family members and their own observations. The caregivers and other staff of the care homes must have access to the life stories, which allows them to attune to the resident based on their past, and for a deeper understanding between the caregivers and the resident to develop. This is beautifully illustrated by the statement of a caregiver in a care home that had implemented congruent care, which describes how a female resident who used to be a nurse wandered the halls of the home at night and the way in which this issue was resolved (see Results section Life Stories and Individualization of Care). From the standpoint of nutrition, by knowing the preferences of people with dementia, their life stories and ancillary information, the caregivers are once more able to more efficiently adjust to the needs and wants of the residents (19, 20).

There are some areas where the methods of providing nutrition must change from the regular modus operandi (**Figure 5**, green panel). The three aspects of nutritional care for people with dementia (5) should be considered when providing

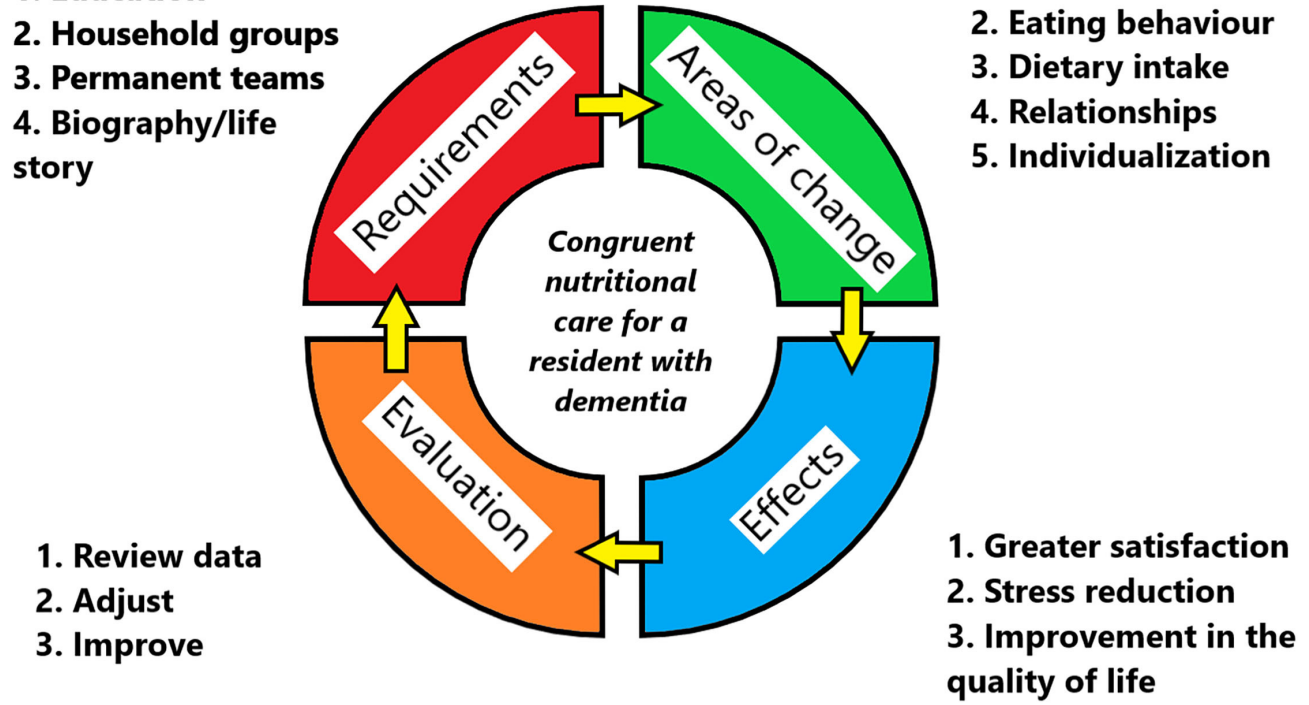

FIGURE 5 | The proposed model of the implementation of congruent nutritional care for people with dementia. Each quarter represents a crucial field of implementation, beginning with the requirements.

care, i.e. food choice, eating behavior and dietary intake. The purpose of congruent care, in nutrition or otherwise, to maintain autonomy, dignity and to provide compassionate treatment to the residents (11) should always be in the forefront of the mind when making a plan of care for people with dementia.

Thus the food choice aspect should be focused on, but not to the extent that the other two aspects are neglected. This is also seen in the results of the analysis of assigned importance (Results section Differences Between the Homes That Implemented Congruent Care and Those Which Did Not) and the frequency of statements (**Figure 4**), where no differences were found in the importance that the two groups of homes expressed with regard to the three aspects, but the homes with congruent care exhibited a greater amount of attention to the food choice aspect (**Figures 3**, **4**). With regard to the results of the differences in the number of statements made on the topic of dietary care, this is most likely due to the caregivers and management of the homes without congruent care operating differently, focusing more on the traditional medical modes of nutritional care and individualization (**Table 2**). The focus on food choice enables the residents to maintain their autonomy and is a key part of the individualization of care (8), and so should be given attention, allowing the person to participate in meal preparation and menu planning. Mealtimes should be flexible, to allow people with dementia to maintain their own rhythm of daily activities. To provide multiple opportunities to take in calories during the day, snacks should be made available, with a "serve yourself" corner being effective (**Figure 1**, Results section Food Choice).

With regard to eating behavior, it is important to recognize that meals are more than just an intake of food. While their primary function is to provide the body with the required nutrients, meals are important social events that are inextricably linked to the local culture (21, 22). To maintain the social function of meals, which can benefit the residents by reminding them of some of their core memories when congruent with their cultural background, the food-associated traditions of individuals should be understood. These are most often shaped by the individuals' upbringing and the social environment in which the food is served and eaten (23–26). The knowledge of this, linked to the initial data gathering, enables the creation of an appropriate atmosphere for the ingestion of food. Further important factors that influence the wellbeing of people with dementia at mealtimes are sensory: smell—preparing food together with a person with dementia or in front of them; sight—arrangement, food presentation, use of color contrasts, lighting; taste—preparing food that a person with dementia has known from an early age; and hearing—providing a calm environment during mealtimes. As no two people are alike in their preferences for food and its aesthetics, it is of great importance that the subject of nutrition is approached with individualized care and that appropriate strategies for preserving quality of life are adopted in the care of people with dementia (27–29). Considering the specifics of dementia, meal course separation, i.e. serving the soup, main

course and dessert consecutively, should always be applied (9) (**Figure 1**, Results section Food Choice). The dietary intake of residents should be regularly monitored and included in their biographical data, to enable easy overview and adjustment of nutrition, as required. The recognition of the importance of dietary intake in congruent care in the statements given by the caregivers and management of homes with congruent care can be found in the Results section What Changes Have Been Made Since the Implementation of Congruent Care?.

The combination of permanent work teams, knowledge of the people and a compassionate approach thus give rise to a person-centered approach to care, but additionally, one that is based on a genuine, affectionate and deep relationship between the caregivers and the residents, which enables the best quality of life and care for the elderly with dementia (12, 16).

The effects that can be expected (**Figure 5**, blue panel) after the implementation of congruent care and its application to nutrition are a reduction in stress and adverse social interactions, an increase in satisfaction with care of residents and their relatives, an increase in the job satisfaction of the caregivers (11, 16) (Results section What Changes Have Been Made Since the Implementation of Congruent Care?, **Figure 2**). A reduction in the need for PRN medication was observed on our data, as well as reported by the management and caregivers of the homes for the elderly. This association should be verified to clearly establish the relationship between the implementation of congruent care and the need for PRN medication. To ensure that the implementation is successful and has the desired effect, the satisfaction of the residents, their relatives and the caregivers should be monitored (**Figure 5**, orange panel). Additionally, detailed records of medication use should be kept, alongside dietary intake data and any events of note, such as data on the occurrences of adverse social interactions. The gathered data should be regularly evaluated and the care adjusted if the desired effects are not seen, in order to continuously improve and evolve the services provided.

To conclude, the implementation of congruent care has the potential to reduce stress and increase wellbeing, while improving both the living conditions of the residents and the working conditions for the caregivers and other employees of the care homes. A congruent approach to nutritional care can thus assist the caregivers in helping our elderly people with dementia retain the dignity and autonomy they deserve.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Figshare repository; https://doi.org/10.6084/m9.figshare.16817476.

AUTHOR CONTRIBUTIONS

GD, LR, and AK conceptualized and designed the study. LR and AK gathered the data. JS, LR, and AK analyzed the data. All authors participated in the writing of the manuscript. All authors have read and approved the final version.

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REFERENCES

1. Arco JE, Ramírez J, Górriz JM, Ruz M. Data fusion based on searchlight analysis for the prediction of Alzheimer's disease. *Expert Syst Appl*. (2021) 185:115549. doi: 10.1016/j.eswa.2021.115549
2. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, idr. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet*. (2020) 396:413–46. doi: 10.1016/S0140-6736(20)30367-6
3. Buffington ALH, Lipski DM, Westfall E. Dementia: an evidence-based review of common presentations and family-based interventions. *J Osteopath Med*. (2013) 113:768–75. doi: 10.7556/jaoa.2013.046
4. Murphy JL, Holmes J, Brooks C. Nutrition and dementia care: developing an evidence-based model for nutritional care in nursing homes. *BMC Geriatr*. (2017) 17:1–14. doi: 10.1186/s12877-017-0443-2
5. Fostinelli S, De Amicis R, Leone A, Giustizieri V, Binetti G, Bertoli S, idr. Eating behavior in aging and dementia: the need for a comprehensive assessment. *Front Nutr*. (2020) 16:604488. doi: 10.3389/fnut.2020.604488
6. Cloak N, Al Khalili Y. *Behavioral And Psychological Symptoms In Dementia*. Treasure Island, FL : StatPearls Publishing LLC (2019). Available oline at: http://europepmc.org/books/NBK551552 (accessed January 5, 2022).
7. Laver K, Cumming R, Dyer S, Agar M, Anstey K, Beattie E, et al. Clinical practice guidelines for dementia in Australia. *Med J Aust.* (2016) 204:191–3. doi: 10.5694/mja15.01339
8. Milte R, Shulver W, Killington M, Bradley C, Miller M, Crotty M. Struggling to maintain individuality – Describing the experience of food in nursing homes for people with dementia. *Arch Gerontol Geriatr*. (2017) 72:52–8. doi: 10.1016/j.archger.2017.05.002
9. Volkert D, Chourdakis M, Faxen-Irving G, Frühwald T, Landi F, Suominen MH, et al. ESPEN guidelines on nutrition in dementia. *Clin Nutr*. (2015) 34:1052–73. doi: 10.1016/j.clnu.2015.09.004
10. Mali J, KejŽar A. A model of integrated care of residents with dementia – a case of innovation in long-term care with accommodation in Slovenia. *Ljetop Soc Rada.* (2019) 26:447–69. doi: 10.3935/rsp.v26i2.1584
11. Coulter A, Oldham J. Person-centred care: what is it and how do we get there? *Futur Hosp J.* (2016) 3:114. doi: 10.7861/futurehosp.3-2-114

12. Nolan MR, Davies S, Brown J, Keady J, Nolan J. Beyond "person-centred" care: a new vision for gerontological nursing. *J Clin Nurs*. (2004) 13:45–53. doi: 10.1111/j.1365-2702.2004.00926.x
13. Cuellar NG. Providing culturally congruent health care to older adults. *J Transcult Nurs*. (2015) 26:109. doi: 10.1177/1043659615569540
14. Heikkilä K, Sarvimäki A, Ekman S-L. Culturally congruent care for older people: finnish care in Sweden. *Scand J Caring Sci*. (2007) 21:354–61. doi: 10.1111/j.1471-6712.2007.00480.x
15. Cohen-Mansfield J, Marx MS, Thein K, Dakheel-Ali M. The impact of stimuli on affect in persons with dementia. *J Clin Psychiatry*. (2011) 72:480–6. doi: 10.4088/JCP.09m05694oli
16. Galiana J, Haseltine WA. *Person-Centered Long-Term Care*. Singapore: Palgrave Macmillan (2019).
17. Corbin J, Strauss A. *Basics of Qualitative Research: Techniques and Procedures for Developing Grounded Theory*. Thousand Oaks, CA: SAGE Publications (2014).
18. Ragin CC. *The Comparative Method: Moving Beyond Qualitative and Quantitative Strategies*. Berkeley, CA: University of California Press (2014). doi: 10.1525/9780520957350
19. Isene T-A, Kjørven Haug SH, Stifoss-Hanssen H, Danbolt LJ, Ødbehr LS, Thygesen H. Meaning in life for patients with severe dementia: a qualitative study of healthcare professionals' interpretations. *Front Psychol.* (2021) 12:701353. doi: 10.3389/fpsyg.2021.701353
20. Kitwood T. *Dementia Reconsidered: The Person Comes First*. Maidenhead: Open Univeristy Press (1997).
21. Lam ITY, Keller HH. Honoring identity through mealtimes in Chinese Canadian immigrants. *Am J Alzheimers Dis Other Demen*. (2012) 30:662–71. doi: 10.1177/1533317512449727
22. Sidenvall B, Fjellström C, Ek A-C. Ritualized practices among caregivers at meals in geriatric care. *Scand J Caring Sci*. (1996) 10:53–61. doi: 10.1111/j.1471-6712.1996.tb00310.x
23. Chaudhury H, Hung L, Badger M. The role of physical environment in supporting person-centered dining in long-term care: a review of the literature. *Am J Alzheimers Dis Other Demen*. (2013) 28:491–500. doi: 10.1177/1533317513488923
24. Andersson EN. *Everyone Eats. Understanding Food and Culture*. New York, NY: New York University Press (2005).
25. Evans BC, Crogan NL, Shultz JA. The meaning of mealtimes: connection to the social world of the nursing home. *J Gerontol Nurs*. (2005) 31:11–7. doi: 10.3928/0098-9134-20050201-05
26. Meiselman HL. Dimensions of the meal. *J Foodserv*. (2008) 19:13–21. doi: 10.1111/j.1745-4506.2008.00076.x
27. Abdelhamid A, Bunn D, Copley M, Cowap V, Dickinson A, Gray L, et al. Effectiveness of interventions to directly support food and drink intake in people with dementia: systematic review and meta-analysis. *BMC Geriatr*. (2016) 16:1–18. doi: 10.1186/s12877-016-0196-3
28. Hsiao H-T, Chang C-C, Chen N-C, Chiu H-C, Huang C-W, Lee F-P, et al. Effects of a dementia dietary educational program on nutritional knowledge and healthy eating behavior of family caregivers. *Educ Gerontol*. (2020) 46:270–83. doi: 10.1080/03601277.2020.1744234
29. Barnes S, Wasielewska A, Raiswell C, Drummond B. Exploring the mealtime experience in residential care settings for older people: an observational study. *Health Soc Care Commun*. (2013) 21:442–50. doi: 10.1111/hsc.12033

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frontiers in Nutrition

REVIEW
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Potential Role of Ginger (*Zingiber officinale* Roscoe) in the Prevention of Neurodegenerative Diseases

Raúl Arcusa, Débora Villaño, Javier Marhuenda, Miguel Cano, Begoña Cerdà and Pilar Zafrilla*

Department of Pharmacy, Faculty of Health Sciences, Catholic University of San Antonio (UCAM), Murcia, Spain

Ginger is composed of multiple bioactive compounds, including 6-gingerol, 6-shogaol, 10-gingerol, gingerdiones, gingerdiols, paradols, 6-dehydrogingerols, 5-acetoxy-6-gingerol, 3,5-diacetoxy-6-gingerdiol, and 12-gingerol, that contribute to its recognized biological activities. Among them, the major active compounds are 6-shogaol and 6-gingerol. Scientific evidence supports the beneficial properties of ginger, including antioxidant and anti-inflammatory capacities and in contrast, a specific and less studied bioactivity is the possible neuroprotective effect. The increase in life expectancy has raised the incidence of neurodegenerative diseases (NDs), which present common neuropathological features as increased oxidative stress, neuroinflammation and protein misfolding. The structure-activity relationships of ginger phytochemicals show that ginger can be a candidate to treat NDs by targeting different ligand sites. Its bioactive compounds may improve neurological symptoms and pathological conditions by modulating cell death or cell survival signaling molecules. The cognitive enhancing effects of ginger might be partly explained *via* alteration of both the monoamine and the cholinergic systems in various brain areas. Moreover, ginger decreases the production of inflammatory related factors. The aim of the present review is to summarize the effects of ginger in the prevention of major neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and multiple sclerosis.

Keywords: ginger, neurodegenerative diseases, Alzheimer's disease, Parkinson's disease, multiple sclerosis, gingerol, antioxidants

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Shanghai University of Traditional Chinese Medicine, China

****Correspondence:***
Débora Villaño
dvillano@ucam.edu

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INTRODUCTION

The number of people over the age of 65 has progressively grown in Western countries, increasing the risk of age-related neurodegenerative diseases. The most common pathology is Alzheimer's disease, with more than 26 million people affected worldwide today. This number is expected to quadruple by 2050. No effective treatments are available for aging-related neurodegenerative diseases, which tend to progress in an irreversible manner and are associated with large personal and socioeconomic costs (1).

The prevention of these pathologies and the search for new nutraceuticals and drugs to combat them are the great challenges of scientific research. Plant-derived products are known to have protective effects including anti-inflammatory and antioxidant actions, related to improvements in cognitive impairment (2).

In recent years, several pharmacological activities of ginger and its bioactive compounds have been explored (3). *Zingiber officinale* is a perennial herb member of the *Zingiberaceae* family and

its thick tuberous rhizomes is very popular for medicinal uses and as a spice and additive agent for flavoring foods and drinks (4, 5). Its origin is little known but it is thought to be in South-East Asia or India (4). The composition in bioactive compounds of *Zingiber officinale* varies according to the place where it is grown and the drying techniques. In general terms, the rhizome of *Zingiber officinale* is mainly composed of essential oils in small quantities, oleoresins, mineral salts, sugars, mucilage, starch, gums and organic acids. Starch constitutes 40–60% of the dry weight of the rhizome of *Zingiber officinale*. Ginger contains a variety of bioactive compounds responsible of its biological activities (as 6-gingerol, 6-shogaol, 10-gingerol, gingerdiones, gingerdiols, paradols, 6-dehydrogingerols, 12-gingerol 3,5-diacetoxy-6-gingerdioal and 5-acetoxy-6-gingerol), among which 6-gingerol and 6-shogaol stand out (6).

In recent years, ginger has been found to possess biological activities, such as antimicrobial, anti-inflammatory, antioxidant, anticancer (by improvement in the expression level of markers for colorectal cancer risk) and anti-allergic activities (7). In this sense, numerous studies have demonstrated that ginger possesses the potential to prevent cardiovascular diseases and associated pathologies that act as risk factors (diabetes, obesity and metabolic syndrome), chemotherapy-induced emesis and nausea, arthritis, gastric dysfunction, pain, respiratory disorders and neurodegenerative diseases (8, 9). Ginger could modulate obesity through various potential mechanisms including increasing lipolysis and thermogenesis, inhibition of lipogenesis, decrease of fat absorption and appetite control (10). Ginger has been documented to ameliorate hyperglycemia and hyperlipidemia. These beneficial effects are mediated by modulation of transcription factors, such as nuclear factor κB, peroxisome proliferator–activated receptors and adenosine monophosphate–activated protein kinase (11). In this sense, Zhu et al. (12) showed that ginger improves insulin sensitivity, decreases the levels of glycosylated hemoglobin in type 2 diabetes mellitus and ameliorates plasma lipid profile.

Neurodegenerative diseases are generally characterized by neuroinflammation, oxidative stress and protein misfolding than leads to brain damage, synaptic dysfunction and neuronal apoptosis (13). In Alzheimer's disease, oxidative stress is mainly caused by mitochondrial dysfunction, the intracellular accumulation of hyperphosphorylated tau (τ) proteins in the form of neurofibrillary tangles, the excessive accumulation of extracellular plaques of beta-amyloid (Aβ), as well as environmental and genetic factors. Gingerols have shown antioxidant, anti-amyloidogenic, anti-inflammatory and anti-cholinesterase properties (14). The major component extracted from *Zingiber officinale*, 6-gingerol, showed antioxidant and anti-inflammatory activity and inhibition of astrocyte overactivation. Lipopolysaccharide stimulated microglia induced pro-inflammatory cytokines, such as IL-6, IL-1β, increments of intercellular nitric oxide concentrations, as well as iNOS enzyme activity, and all of them were suppressed by the treatment with 6-gingerol (15).

Parkinson disease is the second most common neurodegenerative pathology after Alzheimer's disease (16). Its prevalence increases with age and is characterized by the accumulation of α-synuclein protein within neurons, inside Lewy neurites and Lewy bodies (17). Parkinson disease can be caused by environmental and hereditary factors, including iron accumulation in the brain and oxidative stress. Medeiros et al. (18) showed than oxidative stress levels and inflammatory markers were significantly increased in Parkinson disease patients. Mohd et al. (19) suggests that the active compound in ginger may reduce the associated cognitive dysfunction by inhibiting the inflammatory response, increasing levels of nerve growth factor and stimulating synapse formation.

Multiple sclerosis is characterized by chronic inflammatory response-induced demyelination of the neurons and degeneration of the axons within the central nervous system. Factors as inflammatory, oxidative and immunopathological parameters are related in the development and progression of multiple sclerosis. Ginger and its bioactive compounds could be considered as potential agents to treat multiple sclerosis due to their anti-inflammatory, antioxidant and immunomodulatory properties (20).

As oxidative stress and inflammation play an important role in the pathogenesis of the above mentioned diseases, the introduction of anti-inflammatory and antioxidant agents, such as ginger and derived products, could be useful for the treatment and prevention of neurodegenerative conditions. Hence, the aim of the present review is to summarize the effects of ginger in the prevention of major neurodegenerative diseases, focusing on Alzheimer's disease, Parkinson's disease and multiple sclerosis. For this purpose, literature search has been carried out consulting the scientific publications related to ginger published in Web of Science, Scopus, Science Direct and Pubmed databases. Articles published in the last 10 years have been selected, with some exceptions on publication date in those previous works of major relevance.

BIOACTIVE COMPOUNDS PRESENT IN GINGER

Rhizome of *Zingiber officinale* is composed of 69 volatile compounds, which constitute 97 % of its total composition in essential oils. Those molecules present at higher concentrations are α-Zingiberene (28,62%), Camphene (9,32%), Ar-curcumene (9,09%), β-Phellandrene (7,97%), E-α-Farnesene (5,52%), β-Bisabolene (5,40%), α-Pinene (2,57%) (21). It has been documented their biological properties such as antimicrobial, antioxidant, cytotoxic, insecticidal and anti-inflammatory effects as well as their usefulness to preserve food characteristics (22).

Non-volatile compounds (oleoresins) are the main source of bioactive compounds in the rhizome of *Zingiber officinale*. At present, 34 oleoresins have been discovered, which constitute 88.6% of the total composition (21), among which Gingerols (1-(4-hydroxy-3-methoxyphenyl)-5-hydroxyalcan-3-one), Shogaols (1-(4-hydroxy-3-methoxy-phenyl)-4-decen-3-one) and Paradols are the most important groups. Shogaols are the more abundant components in the dried rhizome and gingerols are mainly found in the fresh rhizomes of ginger (23).

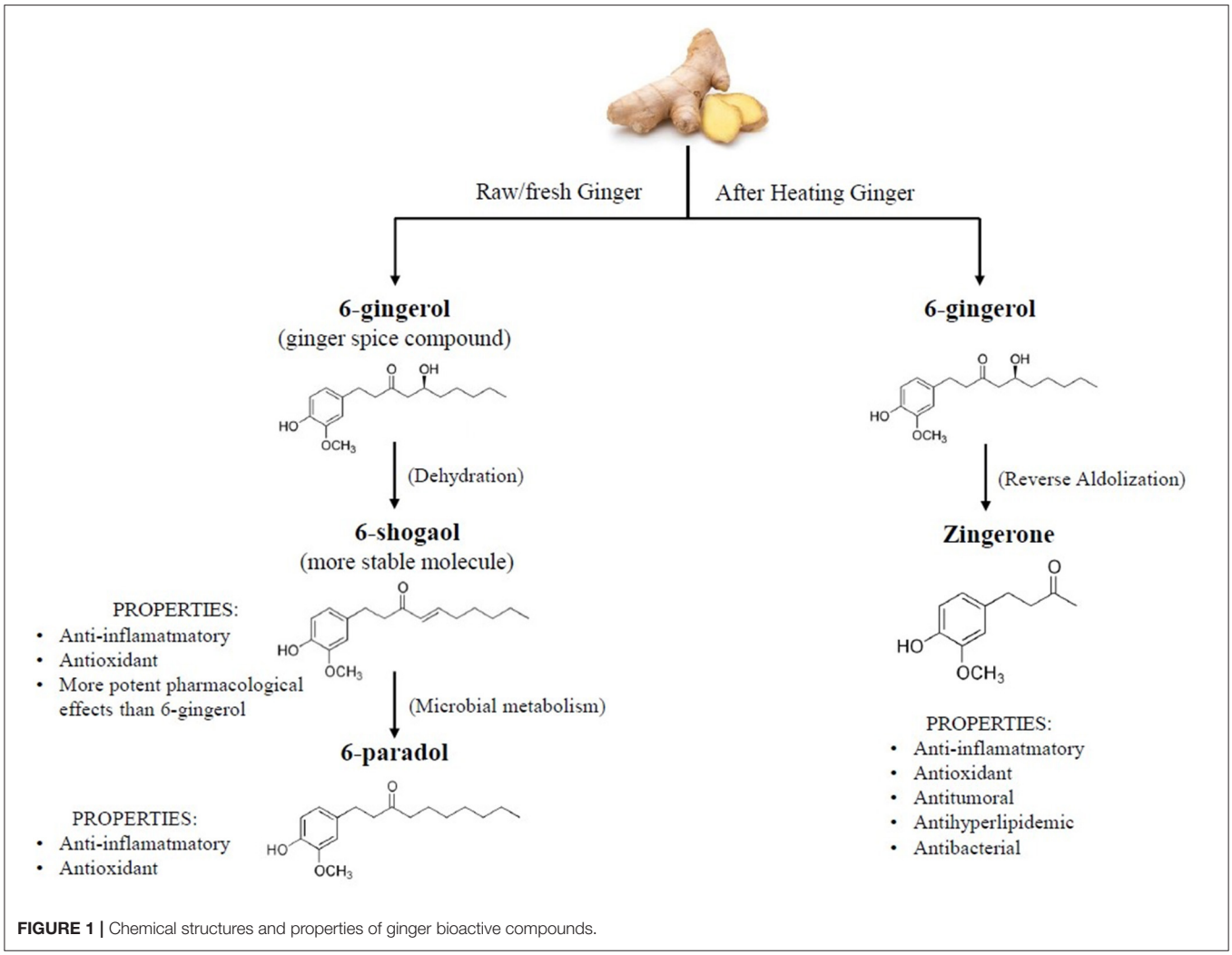

FIGURE 1 | Chemical structures and properties of ginger bioactive compounds.

Gingerol analogs are thermally labile and undergo dehydration reactions to form the corresponding shogaols, which are more stable and have greater pharmacological effects than their precursors and are responsible of the characteristic pungent taste of dried ginger. This chemical change occurs in the process of thermal drying of the rhizomes and long-term storage (24). 6-shogaol is converted to 6-paradol by bacterial metabolism (13, 25) (**Figure 1**). Other phenolic compounds are also present in ginger, as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione.

The maturation state, cultivar, environment, and processing steps are major factors that influence the biosynthesis and concentration of bioactive compounds in ginger. Besides, different composition of normal ginger and black ginger from different countries has been reported, evidencing that gingerol-related phenolic acids were present in normal ginger, while black ginger was characterized by the presence of methoxyflavones (26).

BIOAVAILABILITY AND PHARMACOKINETICS

Ingested dietary gingerols need to be available in the circulation and tissues to produce an effect in the organism. Multiple factors influence the amount of a compound distributed to the different tissues to exert its action, including the solubility in the gastrointestinal fluid and possible degradation in gastrointestinal tract, permeability of enterocytes membrane, protein-mediated intestinal efflux or pre-systemic gut and/or hepatic metabolism (27).

Nutritional and clinical use of ginger in nutraceuticals or enriched-food products is limited due to its poor bioavailability. Gingerols and derivatives are lipid soluble compounds and therefore it would be expected a good absorption by passive diffusion across intestinal epithelium. However, prior to absorption, they must reach brush border cells, what implies be solubilized in an aqueous media; due to their chemical structure, they present a low solubility in water. This phenomenon is related to the concept Bioaccesibility, the amount of ingested

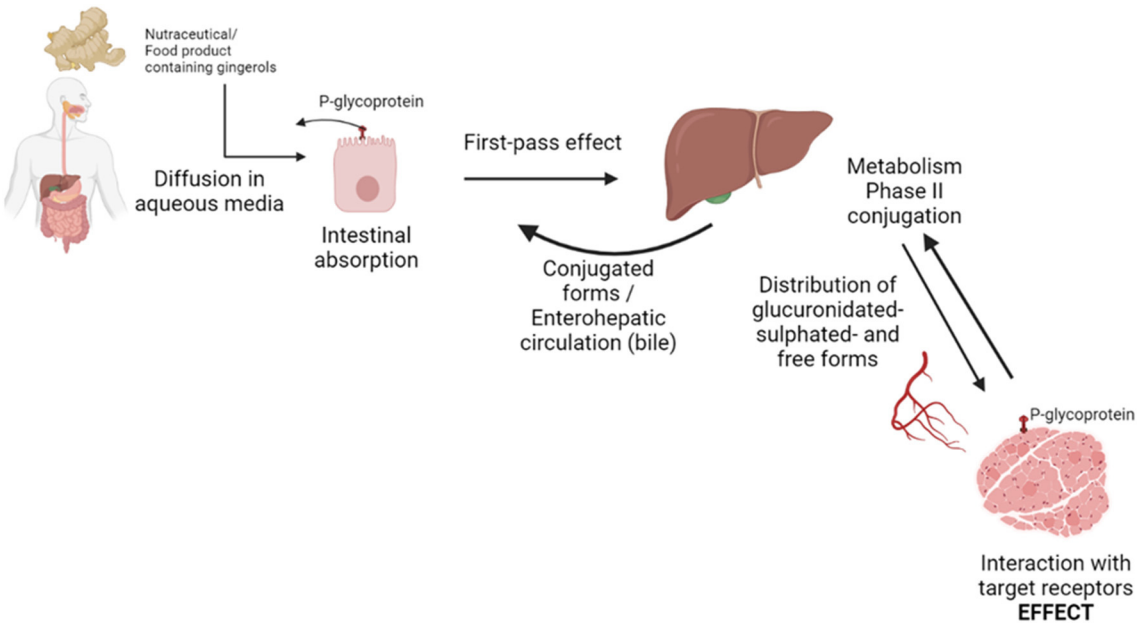

FIGURE 2 | Scheme of bioaccessibility and bioavailability routes of gingerols in the organism.

nutrient available for absorption, which is different to the concept Bioavailability, that represents a step forward, that is, the portion of the ingested dose of a compound that reaches the general circulation and specific sites where it can exert its action. Bioaccesibility is the first limiting step in whether or not a compound may exert an effect in the organism (28).

Unlike other types of compounds, such as flavonoids, gingerols are not naturally present in glycosylated form, so that they are not hydrolyzed by glycosidase enzymes of intestinal brush border. However, gingerols are substrates of P-glycoprotein. This protein is highly expressed in the outer membranes of the enterocytes in the small intestine, as well as in liver, brain and kidney. It behaves as a major barrier to the intestinal absorption of many drugs, as a defense mechanism against toxics (29).

Once absorbed, gingerols are carried by the hepatic portal vein to the liver and undergo hepatic metabolism or "first-pass effect." Half-life of these compounds is extremely low and they suffer from Phase II conjugative reactions, such as glucuronidation catalyzed by UDP-glucuronosyl-transferases (UGTs) and sulphation by sulphotransferases (SULTs), producing more polar molecules for biliary or renal excretion. Isoforms UGT1A1, 1A3 and 2B7 are responsible for gingerol conjugation (30).

Moreover, enterohepatic circulation occurs with these compounds, by biliary excretion and intestinal reabsorption. The hydrophilic metabolite 6-gingerol glucuronide diffuses out of the hepatic cells and is secreted in the bile into the small intestine. There it can be hydrolyzed by intestinal β-glucuronidases and re-enter into the blood stream through the enterocyte (31). All these phenomena are associated to extended half-life in plasma and prolonged pharmacological effect (**Figure 2**).

Human Studies on Bioavailability of Gingerols

Most studies on ginger activity and bioavailability have been performed in animal studies and human trials are scarce. Zick et al. (32) investigated the pharmacokinetics of 6-, 8- and 10-gingerol and 6-shogaol and related metabolites in healthy subjects, with doses ranging from 100 mg to 2 g. The compounds showed a rapid absorption, as glucuronide metabolites appeared within 1 h and the elimination half-lives ranged between 75 and 120 min, depending on the administered dose. All detected compounds were glucuronide conjugates, and no free forms were detected.

The authors used an HPLC method with LOQ ranging from 0.1 to 0.25 μg/mL. The determination of bioactive phytochemicals and their metabolites presents the difficulty of the low concentrations at which they are found in biological fluids. The development of highly sensitive techniques such as mass spectrometry coupled to liquid chromatography has made it possible to better detect and quantify metabolites in animal and human studies after ingestion of ginger or food products made with ginger. The same authors developed and validated a more sensitive, LC-MS/MS method to characterize the pharmacokinetics of 6-, 8-, and 10-gingerols and 6-shogaol in human plasma and colon tissues (33). After an oral dose of 2 g of ginger extract (GE), concentrations of free 10-gingerol and 6-shogaol were detected (peak concentrations of 9.5 and 13.6 ng/mL, respectively). Most compounds existed as glucuronide and sulfate metabolites, mainly 6-gingerol-glucuronide (0.47 μg/mL). LOQ was established in 5 ng/mL, with similar T_{max} between 45 and 60 min and half-lives of all compounds and their metabolites between 1 and 3 h.

Peak concentrations of sulfate metabolites were lower than glucuronide, being the higher value for 6-gingerol-sulfate (0.28 μg/mL). The multiple doses treatment consisted of 250 mg GE capsules daily for 28 days and no accumulation was observed for any of the quantified compounds, due to their short half-lives and fast clearance.

These studies have estimated the concentrations of gingerol glucuronides as the difference of gingerol concentrations prior to and after β-glucuronidase hydrolyzation, and not directly quantifying each compound. In this sense, Schoenknecht et al. (34) developed a direct liquid chromatography-tandem mass spectrometry method, using stable isotope synthesized standards of glucuronide forms, to detect and quantify gingerol glucuronides in human plasma. After SPE extraction and LC-MS analysis, the authors showed that the consumption of 1 liter of ginger tea led to a fast absorption and metabolization of gingerols, with maximum concentrations reached at 30 min post-ingestion. Plasma concentrations resembled the levels of each gingerol free form in the food product, with maximum plasma concentrations for 6-gingerol glucuronide (623.3 nmol/L), followed by 8-gingerol glucuronide (103.8 nmol/L) and 10-gingerol glucuronide (25.8 nmol/L). The authors collected pharmacokinetic parameters in plasma and urine, observing that the maximum concentrations and half-life in plasma were related to the carbon chain length and therefore to the hydrophobicity of the molecules. Pharmacokinetic parameters of urinary elimination indicated that the more lipid-soluble compounds remained longer in the body. 6-gingerol was still quantified in the interval 9–12 h. Recovery rates were between 45% of the administered dose for 6-gingerol and 10 % for 8-gingerol, expressed as glucuronide derivatives.

Some authors have hypothesized that glucuronide forms (inactive) interconvert to free (active) species in tissues by the presence of β-glucuronidase enzyme, establishing an equilibrium between both forms, what has been called "reverse pharmacokinetics." The free form would exert its effects on its multiple target receptors. This might explain the disconnect observed between the efficacy of free gingerols and their sub-therapeutic plasma concentrations (35). The authors demonstrated the accumulation of conjugated forms within various tissues, including brain, after repeated daily oral administration of ginger extract at 250 mg/kg for seven days.

New Technologies to Improve the Bioavailability of Ginger Bioactive Compounds

Low bioavailability of gingerols has been related to its poor water solubility and excessive phase II hepatic metabolism. Different strategies have been implemented to enhance the bioavailability of poorly water-soluble compounds. These technologies include nanoparticles, micelles, emulsions or solid dispersion (36, 37), liposomes (38) or self-microemulsifying drug delivery systems (39). Studies performed in animal models on these forms of encapsulation of single and combined ginger compounds revealed better pharmacokinetic profiles in all cases.

Xu et al. (39) conducted a bioavailability study with a 6-gingerol-loaded self-microemulsifying drug delivery system (SMEDDS) for oral administration in rats. It was formulated with 250 mg/kg dose of 6-gingerol and the system consisted of a mixture of oil phase and surfactants, creating an oil-in-water microemulsion. The 6-gingerol-SMEDDS exhibited prolonged plasma circulation, and significant higher absorption than free 6-gingerol ($t_{1/2}$ = 210 min and AUC = 2,987 min μg/mL, compared to free form $t_{1/2}$ = 82 min and AUC = 454 min μg/mL).

Similar results were observed by Wei et al. (40), who developed nanostructured lipid carriers (NLC) to improve oral solubility and bioavailability of 6-gingerol. After oral administration in rats, AUC was significantly higher compared to controls. Encapsulation of the drug in a lipid core coated with surfactants might help to first, increase the diffusion to epithelial space and improve the absorption and second, to avoid the first-pass effect. The small particle size contributes to a greater surface/volume ratio and major absorption.

Liposomes are new drug carriers prepared by the formation of vesicle enveloping drug molecules in the phospholipid bilayer membrane. Wang et al. (38) demonstrated that 6-gingerol encapsulated in proliposomes was retained in the blood stream much longer than the free form. The plasma concentration was significantly higher 30 min after oral administration of a dose of 250 mg/kg in rats.

Another approach conducted by Ogino et al. (31) was the solid dispersion of ginger extract (GE). Solid dispersions consist of a dispersion of a drug in a solid matrix made of either a small molecule or a polymer. The dispersed drug can exist in different isoforms or crystallization states. In this study, the solid dispersion was made using a hydrophilic polymer, hydroxypropyl cellulose, by a freeze-drying technique. Oral absorption (dose administered 100 mg GE/kg) was higher than that of GE alone, with enhanced AUC and C_{max} of each gingerol. 6-gingerol and 8-gingerol showed 5-fold higher bioavailability than their respective counterparts in free GE.

In an acute study with doses of 250 mg/kg of 6-gingerol administered in rats, polyethylene glycol-based polymeric micelles significantly improved (up to 3-fold) the bioavailability of 6-gingerol compared to 6-gingerol control group (37). Besides, there was a better brain distribution, what suggested that the micelle could overcome the brain-blood barrier. It has been hypothesized that the components of the micelles work as P-glycoprotein inhibitors, by suppressing its ATPase activity, hence improving the passage through biological barriers (41, 42).

In all studies, *in vitro* release assays were performed and an enhanced solubility of the compound was observed, compared to the free drug, which could be partly responsible of the improved oral bioavailability in circulation. Nevertheless, further research is required to confirm the usefulness of these preparations as nanocarriers, as well as thorough toxicity studies prior to human administration.

ANTIOXIDANT AND INFLAMMATORY ACTIVITY

The production of free radicals, such as reactive oxygen species (ROS) or nitrogen reactive species (NOS) leads to

the development of many oxidative-related disorders, such as the most common neurogenerative diseases (43). Hopefully, antioxidant bioactive compounds are widely spread over a large number of food matrices as fruits, vegetables, cereal grains, edible flowers or medicinal plants (44–50). Moreover, the latest scientific literature has found promising bioactive compounds contained in ginger that possess antioxidant and anti-inflammatory activities (20, 51, 52). Gingerols and shogaols have a plethora of biological activities such as antioxidant, antimicrobial, anticancer, anti-inflammatory, antiallergic and prevention of neurodegenerative diseases (7).

The antioxidant activity of ginger has been evaluated *in vitro*, showing better performance for dried ginger compared with fresh, stir-fried or carbonized ginger. This fact was principally related with the concentration of polyphenols, higher in dried ginger, as the temperatures applied for stir-fried or carbonized ginger could change gingerols into shogaols, leading to minor antioxidant capacity (53).

Moreover, the scientific literature has reported that ginger can be useful for the prevention of oxidative-related injury (51, 54). An extract from ginger showed antioxidant capacity related to interleukin-1β in human chondrocyte cell model, stimulating the expression of enzymes related to oxidative protection, reducing the generation of ROS leading to decreased lipid peroxidation (55). Ginger extract was also able to reduce ROS in human fibrosarcoma cells (56). Besides, another marker of lipid peroxidation as malondialdehyde was reduced in rat heart homogenates after the treatment with a ginger extract (54).

Particularly, ginger has shown antioxidant capacity *via* the nuclear factor erythroid 2-related factor 2 signaling pathway (Nrf2) (57, 58). In human colon cancer cells 6-shogaol is able to increase intracellular glutathione/glutathione disulfide ratio (GSH/GSSG), upregulating the expression of Nrf2, metallothionein 1 (MT1), heme oxygenase-1 (HO-1), ferritin light chain (FTL), aldo-keto reductase family 1 member B10 (AKR1B10), and γ-glutamyltransferase-like 4 activities (GGTLA4).

Despite the fact that doses, routes of administration and duration of treatment vary among studies, it has been reported that the effective anti-inflammatory and antioxidant doses of ginger extract *in vivo* studies range from 200 to 500 mg/kg/day, and the effective immunomodulatory doses range from 28 to 720 mg/kg/day. In human studies doses of 500 mg/day for 3 months, 1,000 mg/day for 2 months and 1,500 mg/day for 6 weeks were observed (20). Doses of up to 4 grams of ginger per day have been reported to be safe (59).

Both *in vitro* and *in vivo* studies have revealed that ginger and related bioactive secondary compounds, such as 6-shogaol, 6-gingerol, and oleoresin, exert potent antioxidant capacity by direct free radical scavenging. Additionally, the triggering of the Nrf2 signaling route is decisive to the underlying mechanisms of action. Importantly, the excess on the production of ROS and NOS is considered a cause of some diseases as neurodegenerative pathologies and antioxidants are crucial for their prevention (60, 61) (**Figure 3**).

Results on the anti-inflammatory capacity of ginger and its bioactive compounds have shown some variability (62, 63), which may be attributed to differences in the study design, length of interventions, individual characteristics, and doses administered. The anti-inflammatory mechanisms of ginger are probably associated with a decline in proinflammatory cytokines linked to the inhibition of Akt and NF-κB activation (8). NF-kβ pathway is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. NF-kβ is the key regulator of the inflammatory process, activating the expression of inflammatory target genes, including cytokines, chemokines, and COX2.This enzyme triggers the formation of some prostaglandins, responding to inflammation and enhancing the formation of proinflammatory cytokines. Ginger has being able to inhibit inflammatory response by suppressing NF-kβ, which lead to the reduction of cytokine gene expression (11). In 2016, a meta-analysis reported that C-reactive protein (CRP) and other acute-phase proteins were also suppressed after ginger supplementation (64). Naderi et al. (65) published that treatment for 12 weeks with ginger powder at a dose of 1 g/day was able to decrease the plasma concentration of CRP, in accordance to previous studies (66). Likewise, the anti-inflammatory capacity of ginger can be justified by its ability to inhibit COX-2 and 5-lipoxygenase enzymes, which results in the suppression of amino acid metabolism. In fact, it has demonstrated to reduce platelet aggregation, as well as the formation of pro-inflammatory thromboxanes and prostaglandins (67). Specifically, the anti-inflammatory effects of ginger are related to the inhibition of COX-2 without affecting COX-1, which seems to be an advantage over traditional NSAIDs due to the related side effects (68, 69). Van Breemen et al. through pulsed ultrafiltration mass spectrometry, showed that several compounds related to gingerol were COX-2 ligands. COX-2 inhibition would prevent the conversion of arachidonic acid into prostaglandin (PG) H2, preventing its subsequent conversion into proinflammatory prostaglandins such as PGD2 and PGE2 (68). It has also been reported the inhibition of the formation of nitric oxide, inflammatory cytokines, and the inhibition of the enzymatic activity of prostaglandin synthase, which could lead to a decrease in the inflammatory component (69–73) (**Figure 4**).

Health state, genetics, lifestyle habits and dietary factors of individuals, or the dosage and solubility aspects of ginger forms could affect the bioaccessibility and bioavailability and ultimately the bioactivity of ginger compounds, which may justify the contradictory or controversial results emerged from *in vitro* and *in vivo* studies.

ALZHEIMER'S DISEASE AND GINGER

Alzheimer's disease (AD) is a neurodegenerative condition linked to profound memory impairment and loss of cognitive function. Among others, cellular damage due to β-amyloid protein aggregation, tau protein hyperphosphorylation, neurotransmitter imbalances, oxidative stress, apoptosis and inflammatory responses is responsible for its occurrence (3, 74).

Due to the inadequate efficacy of the conventional drugs currently used, their adverse effects and pharmacokinetic

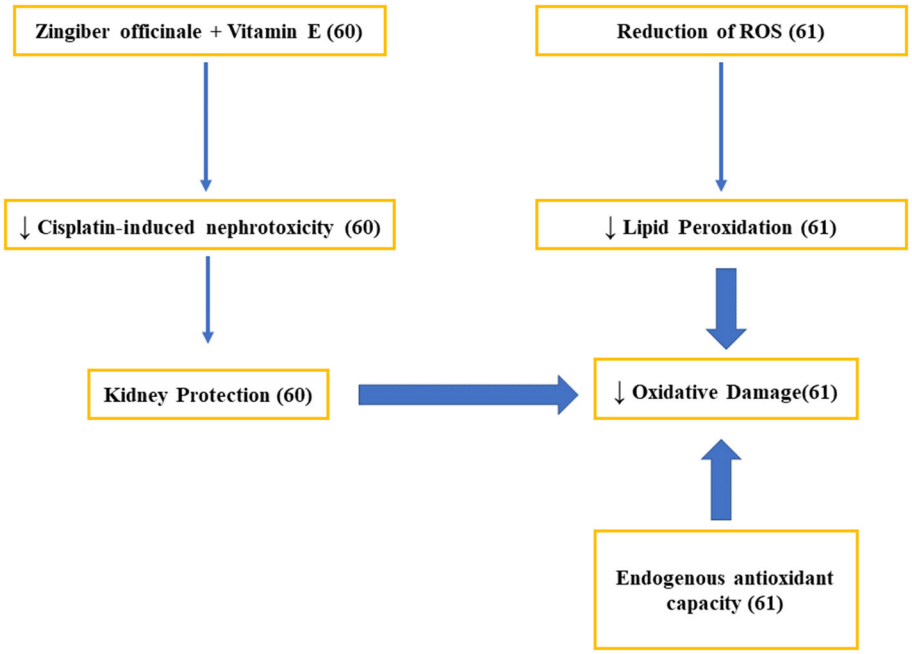

FIGURE 3 | Studies on the mechanisms related to the antioxidant activity of ginger.

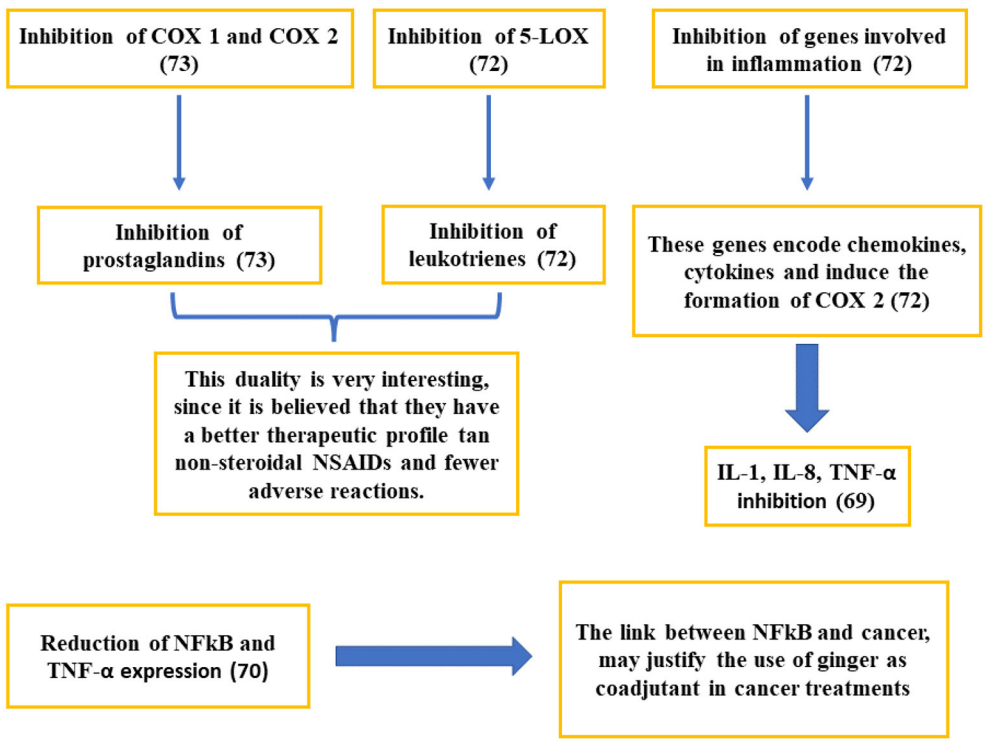

FIGURE 4 | Studies on the mechanisms of action related to the anti-inflammatory activity of ginger.

problems, together with the scientific evidence that in recent years suggests that traditional medicinal plants could be useful both in the prevention and treatment of a multitude of pathologies, a great opportunity has led for their evaluation in the treatment of memory disorders, as it is the case of *Zingiber officinale* (3, 75).

TABLE 1 | Preclinical studies of ginger and Alzheimer's disease.

Model Animal	Compound used	Conclusions	References
Mouse	Ginger	AChE activity, cytotoxic Effect, Lps-induced NO production, Supressed TNF-a, IL-6, IL-1B	(78)
Rat	6-gingerol	IL-6, TNF-α, NO, NOS_2 protein expresión in C6 cells.	(15)
Male Swiss albino mice	Gingerol	Reduction of the levels of AB42, B-secretase, APH1a and COX-2. Increased of a-secretase activity	(79)
Male Wistar rats	Extract of *Cyperus rotundus* and *Zingiber officinale*	Oxidate stress reduction in hippocampus improved cholinergic gunction, improved memory deficit	(80)
Male C57BL/6 mice	Ginger, 6-Gingerol	Upregulation of BDNF. Prevention of memory deficits	(81)

TABLE 2 | Human clinical studies on ginger and cognitive function.

Study design	Intervention	Outcomes	References
Elderly people with memory complaints. Open label study ($n = 30$)	"Cognitex" (12 weeks)	↑ memory abilities ↑ sustained attention ↑ visual learning ↑ activities of daily living	(84)
Double-blind randomized placebo-controlled. Middle-aged healthy Thai women ($n = 60$)	Placebo, 400, 800 mg/day standardized ginger extract (8 weeks)	↑ working memory ↓ latency to audit stimuli ↑ Word recognition ↑ Choice reaction time ↑ Numeric working memory ↑ Spatial working memory	(83)
Double-blind randomized placebo-controlled > 50 years, mild to moderate AD patients ($n = 50$)	Placebo, Davaie Loban capsules 500 mg, 3 times day (12 weeks)	Improvements in AD scores (ADAS-cog, CDR-SOB)	(85)
Open-label, crossover AD patients ($n = 10$)	Kihito herbs 2,5 g extract, 3times/day (16 weeks)	Improvements in AD scores (MMSE-J)	(86)

ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; CDR-SOB, Clinical Dementia Rating Scale Sum of Boxes; MMSE-J, Mini-Mental State Examination-Japanese.

The main characteristics of *Zingiber officinale* for its possible use in neurodegenerative diseases, specifically Alzheimer's, are its anti-inflammatory and antioxidant effects. In particular, clinical studies have shown that the use of ginger has increased the expression of nerve growth factor (NGF), playing a key role in improving memory function, simplifying long-term hippocampal enhancement and accelerating neurite outgrowth.

Preclinical trials in mice (**Table 1**) showed that increasing NGF levels in the hippocampus initiated the activation of extracellular signal regulatory kinases (ERK) and cAMP response element binding protein (CREB), leading to increased synaptogenesis (76). Furthermore, studies have shown that ginger blocks the expression of pro-inflammatory cytokines and chemokines in THP-1 cells. Animal studies concluded that the use of ginger significantly inhibited the expression of mRNA related to the expression of pro-inflammatory cytokines and endothelial adhesion activating factors such as LPS, TNF-α, IL-1 β, COX-2, MIP-1A, MCP-1 and IP-10, among others (77).

In vitro and animal studies conclude that various bioactive compounds of *Zingiber officinale* cross the blood-brain barrier, allowing us to think that the beneficial properties observed in diverse pathologies could have application against neurodegenerative diseases, specifically AD (82).

Zingiber officinale might also have therapeutic properties for other diseases affecting the nervous system, such as brain tumors, cardiovascular accidents, neurosis, depression, insomnia and psychiatric disorders. It is included on the US Food and Drug Administration's (FDA) "Generally Recognized as Safe" (GRAS) list and can be defined as a safe nutraceutical that could be used to combat neurodegenerative disorders (75).

However, clinical studies in humans are scarce and some of them refer to supplements consisting of a mixture of herbs, including ginger, used in traditional oriental medicine, as Davaie Loban or Kihito (**Table 2**). Other authors have reported improvements in cognitive abilities using Cognitex, a nutritional supplement containing sage, blueberry and Zingiber officinale. Saenhong et al. (83) evaluated the individual effect of ginger and findings are noteworthy. The researchers conducted a placebo-controlled study with standardized ginger extracts, observing an enhance in cognitive processing capabilities, with greater effects at higher doses of 800 mg/day (**Table 2**).

PARKINSON DISEASE AND GINGER

Parkinson's disease (PD) is a complex neurodegenerative process that appears in adulthood and is the second most common neurodegenerative disease behind Alzheimer's dementia. Its pathological basis is characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) of the midbrain, as well as the presence of intracellular inclusions called Lewy bodies, which are formed by insoluble aggregates of abnormally folded alpha-synuclein protein. The result of this neurodegeneration is the dopaminergic denervation

of the projections of the SNpc toward the striatum, which conditions an alteration in the normal physiology of the basal ganglia (87, 88). These phenomena leads to a deficit of dopamine (DA) and the subsequent appearance of the cardinal signs of the disease, that is, the tremor resting, bradykinesia, posture rigidity and instability. In addition to the motor symptoms, there is the manifestation of non-motor symptoms, the prevalence of which increases as the disease progresses (apathy or depression, sleep disturbances, autonomic dysfunction or sensory symptoms) (87, 89).

PD can be caused by hereditary and environmental factors, including oxidative stress and iron accumulation in the brain. It is clear that neuroinflammation plays an important role in the development and progression of PD and other neurodegenerative diseases (19, 90). In PD, oxidative stress is a result of mitochondrial deficiency, in addition to a chronic inflammatory process, in which both produce reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species meet the accumulated iron in the brain and harm structures, leading to the death of dopaminergic neurons in the substantia nigra. This process creates a cycle of cell damage, neuroinflammation, and ROS/RNS production, resulting in neuronal death (18, 91). The combination of oxidative stress and high levels of tissular iron cause harm to the brain structure, with the death of dopaminergic neurons in the substantia nigra. Consequently, the loss of these dopaminergic neurons in the substantia nigra lead to progressive motor impairment in PD (18). In fact, oxidative stress levels and inflammatory markers are significantly increased in PD patients.

Currently available treatments have a strictly symptomatic effect. The most effective drug for treating the motor manifestations of PD is levodopa. To date, there is no treatment that slows the progression of the disease, as current drugs improve the symptoms of PD but not the underlying neurodegeneration of PD. In recent years, interest has increased in the discover of the possible beneficial effect of natural products as ginger on the development and progression of PD. Park et al. (92) reported a neuroprotective effect of 6-shogaol in a PD model; 6-shogaol protected dopaminergic cells against MPP+ - and MPTP-induced neurotoxicity *via* the inhibition of neuroinflammatory responses of microglia. In this way, results of Moon et al. (76) suggest that 6-shogaol may play a role in inhibiting glial cell activation and reducing memory impairment in animal models of dementia (**Table 3**).

Kongsui et al. (90) suggested that ginger crude extract might be a potential neuroprotective agent for the treatment of lipopolysaccharide-induced neurodegenerative diseases. Other study carry out by Hussein et al. (93) clearly indicates a neuroprotective effect of ginger against MSG-induced neurodegenerative disorders and these beneficial effects could be attributed to the polyphenolic compounds present (**Table 3**).

TABLE 3 | Parkinson's disease and ginger.

Effect	References
Suppressing the overactivation of astrocytes Attenuated LPS-induced neuronal cell loss by reducing the expression of GFAP and IL-1ß in the hippocampus.	(90)
Low levels of antioxidants, incapable of controlling free radical and ROS/RNS production with subsequent inflammation, leading to neurodegeneration in PD.	(18)
Inhibited components of the inflammatory pathway such as TNF-α, NO, COX-2, and inducible nitric oxide synthase (iNOS)	(92)
6-shogaol may play a role in inhibiting glial cell activation and reducing memory impairment in animal models of dementia.	(76)

MULTIPLE SCLEROSIS AND GINGER

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS) characterized by inflammation, demyelination of neurons and axonal degeneration even in the early stages of the disease. MS is one of the most common causes of neurological disability in young people (94, 95) and usually appears in women with ages comprised between 25 and 30 years (95, 96).

Currently MS is considered as multifocal chronic inflammatory disease that associates neurodegeneration (97). Some individuals are genetically predisposed to such an abnormal autoimmune response, and the development and progression of the disease will be affected by various environmental factors. Genetic predisposition is mediated especially by the major histocompatibility complex. Among the risk factors with the best available evidence are the association with Epstein-Barr virus infection, high BMI during adolescence, low vitamin D levels and smoking (95, 96). The number of population affected by MS has increased in recent decades and it is estimated that 2.5 million people worldwide suffer from the disease, affecting some 700,000 population in Europe (96, 98).

As for the pathogenesis, despite decades of research, the exact etiology is still unknown to the scientific community and it is believed that the symptoms of MS result from damage to the myelin sheath and disruption of myelinated tracts in the CNS (99). In most patients, the characteristic clinical symptoms of the disease include cognitive, sensory, motor, and autonomic disturbances. These symptoms manifest as loss of coordination and balance, impaired vision, deficits in executive functioning, chronic pain and mood disturbance (94). There is currently no definitive cure for MS. However, different pharmaceutical and rehabilitation therapies are available to treat acute attacks, improve symptoms and modify the course of the disease (100). In recent years, complementary and alternative medicine methods such as the use of herbal therapy appear to have promising therapeutic approach to treat MS (101). Such therapies among which ginger is included, could be effective in the treatment of MS by reducing demyelination, enhancing remyelination and especially by suppressing/reducing inflammatory processes. Regarding the reduction of inflammatory processes, it occurs by inhibiting the infiltration of inflammatory cells in the CNS, reducing the proinflammatory cytokine production.

Demyelination and neurodegeneration are closely related to inflammation (a key feature in MS), being much more pronounced in acute and relapsing phases (101). Within the CNS there is an infiltration of leukocytes including neutrophils, DCs,

TABLE 4 | The anti-inflammatory, antioxidant activities and immunomodulatory effects of ginger and its components.

Down-regulation of the	Th1 cell-related immune responses
	Th17 cell-related immune responses
	B cell-related immune responses
	Antigen presenting cells
	Arachidonic acid-derived mediators
	Oxidative stress Expression of the chemokines and chemokines receptors
	Adhesion molecules
	Th2 cell-related immune responses
	Th9 cell-related immune responses
	Th22 cell-related immune responses
Modulation of the	Macrophage's responses
	Production of pro- and anti-inflammatory cytokines
	Toll-like receptor's-related signaling pathways
	Inflammasome-related responses
Up-regulate of the	Treg cell-related immune responses4

macrophages, CD4+ T cells, and CD8+ T cells), with $CD4^+$ T cells having the greatest impact on demyelination of neurons and axonal damage (101, 102). As for DCs they cross the damaged blood-brain barrier promoting a polarization of myelin-specific T-lymphocytes to different subsets of effector T-cells; Th1, Th2, Th9, Th17, Th22 and Treg cells. While Th1 and Th17 cells act pathogenically in the immunopathological process of MS, Treg and Th2 cells exert protective action against autoimmune diseases (103–105). Astrocytes and microglia cells also contribute to the pathogenesis of MS by releasing proinflammatory cytokines (20).

There are currently more than a dozen drugs on the market to treat MS. However, they are questioned both for their moderate efficacy and side effects. The possibility of using ginger to attenuate the symptoms of MS arises from the fact that there are certain components derived from plants with anti-inflammatory and immunomodulatory properties and with low side effects (20).

Among the possible therapeutic potentials of ginger and its components for the treatment of MS, its immunomodulatory, anti-inflammatory and antioxidant effects are depicted in **Table 4**, and their mechanisms are extensively detailed by Jafarzadeh et al. (20).

According to a recent systematic review on the concomitant consumption of ginger extract and other drugs it can be concluded that ginger consumption is safe and there is no potential risk of clinically relevant interactions in the treatment of MS (106). The only contraindications were observed in the coadministration together with anticoagulants, due to the anticoagulant properties of ginger.

Experimental autoimmune encephalomyelitis (EAE) is a model of inducible human MS in vulnerable animals. It is usually induced in mice due to the fact that it is a highly reliable model to study both the pathogenesis of MS to test drugs in development to treat MS (107, 108). In different studies performed in mice with EAE, it was observed that after the administration of ginger extract the clinical symptoms of EAE appeared later and the clinical scores of the disease were lower compared to placebo (109, 110). The main feature of MS and EAE is primary demyelination of axons, causing blocking of signal conduction or reduced conduction at the demyelinated site (111). Administration of ginger extract prior to EAE appears to reduce the clinical symptoms, through up-regulation of inflammatory cytokines and chemokines (IL-23, IL-33, IFN-γ, CCL20 and CCL22) (109, 112). Moreover, a recent investigation in mice with EAE showed that both 6-shogaol and 6-paradol appear to reduce clinical symptoms. In addition, they were also associated with attenuation of astrogliosis, microglial activation and TNF-α expression, suppressing neuroinflammatory responses. Therefore, it seems that 6-shogaol and 6-paradol could be the active ingredients responsible for the efficacy of ginger extract (111).

The research in mice with EAE seems to be effective to further our knowledge of all the possible mechanisms involved in the pathogenesis and treatment of MS, due to the similarities (113). Therefore, although more research is needed, we consider it a promising and necessary first step for the assessment of the efficacy prior to human studies.

CONCLUSIONS

Ginger contains diverse bioactive compounds, such as gingerols, shogaols, and paradols and possesses antioxidant and anti-inflammatory properties that might help reduce the levels of inflammation and oxidative stress in neurodegenerative diseases. In fact, several inflammatory, oxidative and immunopathological parameters are involved in their pathogenesis and drugs used for treatment are of limited efficacy and can also generate adverse side effects. It seems that ginger, given its antioxidant, immunomodulatory and anti-inflammatory capacity, has the ability to intercept all the main elements involved in the development of multiple sclerosis as well as to attenuate the symptoms of neurological diseases including Parkinson's, Alzheimer's, migraine, and epilepsy. Even though with the doses studied, no considerable adverse effects are observed, further research is needed to study whether higher doses and/or longer administration protocols are more effective without causing adverse side effects.

Inclusion of ginger or ginger extracts in nutraceutical formulations could provide valuable protection against neurodegenerative diseases. The low bioavailability and extensive phase II metabolism have limited the use of ginger in neurodegenerative pathologies and new pharmaceutical forms for delivering ginger's bioactive compounds that overcome these limitations are currently being developed. Further toxicological and pharmacokinetic studies of these new formulations will be necessary before their application in human trials, but the evidence is promising for the therapeutic potential of ginger in neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

PZ: conceptualization, supervision, and project administration. JM, BC, RA, MC, PZ, and DV: methodology, investigation, and writing—original draft preparation. JM, BC, RA, PZ, and DV: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Hou Y, Dan X, Babbar M, Wei Y, Hasselbalch SG, Croteau DL, et al. Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol.* (2019) 15:565–81. doi: 10.1038/s41582-019-0244-7
2. Reddy PH, Manczak M, Yin X, Grady MC, Mitchell A, Tonk S, et al. Protective effects of indian spice curcumin against amyloid-β in Alzheimer's disease. *J Alzheimers Dis.* (2018) 61:843–66. doi: 10.3233/JAD-170512
3. Talebi M, Ilgün S, Ebrahimi V, Talebi M, Farkhondeh T, Ebrahimi H, et al. Zingiber officinale ameliorates Alzheimer's disease and cognitive impairments: lessons from preclinical studies. *Biomed Pharmacother.* (2021) 133:111088. doi: 10.1016/j.biopha.2020.111088
4. Mann A. Biopotency role of culinary spices and herbs and their chemical constituents in health and commonly used spices in Nigerian dishes and snacks. *African J Food Sci.* (2011) 5:111–24. doi: 10.5897/AJFS.9000032
5. Banerjee S, Mullick HI, Banerjee J. Ghosh A. Zingiber officinale:'a natural gold'. *Int J Pharm Bio-Sci.* (2011) 2:283–94.
6. Tanaka K, Arita M, Sakurai H, Ono N, Tezuka Y. Analysis of chemical properties of edible and medicinal ginger by metabolomics approach. *Biomed Res Int.* (2015) 2015:671058. doi: 10.1155/2015/671058
7. Semwal RB, Semwal DK, Combrinck S, Viljoen AM. Gingerols and shogaols: Important nutraceutical principles from ginger. *Phytochemistry.* (2015) 117:554–68. doi: 10.1016/j.phytochem.2015.07.012
8. Mao Q-Q, Xu X-Y, Cao S-Y, Gan R-Y, Corke H, Beta T, Li H-B. Bioactive compounds and bioactivities of ginger (Zingiber officinale roscoe). *Foods.* (2019) 8:185. doi: 10.3390/foods8060185
9. Anh NH, Kim SJ, Long NP, Min JE, Yoon YC, Lee EG, et al. Ginger on human health: a comprehensive systematic review of 109 randomized controlled trials. *Nutrients.* (2020) 12:157. doi: 10.3390/nu12010157
10. Ebrahimzadeh Attari V, Malek Mahdavi A, Javadivala Z, Mahluji S, Zununi Vahed S, Ostadrahimi A, et al. Systematic review of the anti-obesity and weight lowering effect of ginger (Zingiber officinale Roscoe) and its mechanisms of action. *Phytother Res.* (2018) 32:577–85. doi: 10.1002/ptr.5986
11. Wang J, Ke W, Bao R, Hu X, Chen F. Beneficial effects of ginger zingiber officinale roscoe on obesity and metabolic syndrome: a review. *Ann N Y Acad Sci.* (2017) 1398:83–98. doi: 10.1111/nyas.13375
12. Zhu J, Chen H, Song Z, Wang X, Sun Z. Effects of ginger (Zingiber officinale Roscoe) on Type 2 diabetes mellitus and components of the metabolic syndrome: a systematic review and meta-analysis of randomized controlled trials. *Evid Based Complement Alternat Med.* (2018) 2018:5692962. doi: 10.1155/2018/5692962
13. Choi JG, Kim SY, Jeong M, Oh MS. Pharmacotherapeutic potential of ginger and its compounds in age-related neurological disorders. *Pharmacol Ther.* (2018) 182:56–69. doi: 10.1016/j.pharmthera.2017.08.010
14. Noori T, Dehpour AR, Sureda A, Sobarzo-Sanchez E, Shirooie S. Role of natural products for the treatment of Alzheimer's disease. *Eur J Pharmacol.* (2021) 898:173974. doi: 10.1016/j.ejphar.2021.173974
15. Zhang F, Zhang J-G, Yang W, Xu P, Xiao Y-L, Zhang H-T. 6-Gingerol attenuates LPS-induced neuroinflammation and cognitive impairment partially *via* suppressing astrocyte overactivation. *Biomed Pharmacother.* (2018) 107:1523–9. doi: 10.1016/j.biopha.2018.08.136
16. Çubukçu HC, Yurtdaş M, Durak ZE, Aytaç B, Güneş HN, Çokal BG, et al. Oxidative and nitrosative stress in serum of patients with Parkinson's disease. *Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol.* (2016) 37:1793–8. doi: 10.1007/s10072-016-2663-1
17. Pimentel C, Batista-Nascimento L, Rodrigues-Pousada C, Menezes RA. Oxidative stress in Alzheimer's and Parkinson's diseases: insights from the yeast saccharomyces cerevisiae. *Oxid Med Cell Longev.* (2012) 2012:132146. doi: 10.1155/2012/132146
18. Medeiros MS, Schumacher-Schuh A, Cardoso AM, Bochi GV, Baldissarelli J, Kegler A, et al. Iron and oxidative stress in parkinson's disease: an observational study of injury biomarkers. *PLoS ONE.* (2016) 11:e0146129. doi: 10.1371/journal.pone.0146129
19. Mohd Sahardi NFN, Makpol S. Ginger (Zingiber officinale Roscoe) in the prevention of ageing and degenerative diseases: review of current evidence. *Evid Based Complement Alternat Med.* (2019) 2019:5054395. doi: 10.1155/2019/5054395
20. Jafarzadeh A, Nemati M. Therapeutic potentials of ginger for treatment of Multiple sclerosis: a review with emphasis on its immunomodulatory, anti-inflammatory and anti-oxidative properties. *J Neuroimmunol.* (2018) 324:54–75. doi: 10.1016/j.jneuroim.2018.09.003
21. Asadi-Samani M, Rafieian-Kopaei M, Azimi N. Gundelia: a systematic review of medicinal and molecular perspective. *Pakistan J Biol Sci PJBS.* (2013) 16:1238–47. doi: 10.3923/pjbs.2013.1238.1247
22. An K, Zhao D, Wang Z, Wu J, Xu Y, Xiao G. Comparison of different drying methods on Chinese ginger (Zingiber officinale Roscoe): changes in volatiles, chemical profile, antioxidant properties, and microstructure. *Food Chem.* (2016) 197:1292–300. doi: 10.1016/j.foodchem.2015.11.033
23. Kiran CR, Chakka AK, Amma KPP, Menon AN, Kumar MMS, Venugopalan V V. Influence of cultivar and maturity at harvest on the essential oil composition, oleoresin and [6]-gingerol contents in fresh ginger from northeast India. *J Agric Food Chem.* (2013) 61:4145–54. doi: 10.1021/jf400095y
24. Balladin DA, Headley O, Chang-Yen I, McGaw DR. High pressure liquid chromatographic analysis of the main pungent principles of solar dried West Indian ginger (Zingiber officinale Roscoe). *Renew Energy.* (1998) 13:531–6. doi: 10.1016/S0960-1481(98)00029-9
25. Mohd Yusof YA. Gingerol and its role in chronic diseases. *Adv Exp Med Biol.* (2016) 929:177–207. doi: 10.1007/978-3-319-41342-6_8
26. Asamenew G, Kim H-W, Lee M-K, Lee S-H, Kim YJ, Cha Y-S, et al. Characterization of phenolic compounds from normal ginger (Zingiber officinale Rosc) and black ginger (kaempferia parviflora wall) using UPLC–DAD–QToF–MS. *Eur Food Res Technol.* (2019) 245:653–65. doi: 10.1007/s00217-018-3188-z
27. He S-M, Chan E, Zhou S-F, ADME. properties of herbal medicines in humans: evidence, challenges and strategies. *Curr Pharm Des.* (2011) 17:357–407. doi: 10.2174/138161211795164194
28. Bohn T, Carriere F, Day L, Deglaire A, Egger L, Freitas D, et al. Correlation between in vitro and in vivo data on food digestion. what can we predict with static in vitro digestion models? *Crit Rev Food Sci Nutr.* (2018) 58:2239–61. doi: 10.1080/10408398.2017.1315362
29. Gessner A, König J, Fromm MF. Clinical aspects of transporter-mediated drug-drug interactions. *Clin Pharmacol Ther.* (2019) 105:1386–94. doi: 10.1002/cpt.1360
30. Pfeiffer E, Heuschmid FF, Kranz S, Metzler M. Microsomal hydroxylation and glucuronidation of [6]-gingerol. *J Agric Food Chem.* (2006) 54:8769–74. doi: 10.1021/jf062235l
31. Ogino M, Yakushiji K, Suzuki H, Shiokawa K, Kikuchi H, Sato H, et al. Novel ginger extract formulation with enhanced pharmacokinetics and anti-hypothermic action. *Drug Metab Pharmacokinet.* (2017) 1:S30–1. doi: 10.1016/j.dmpk.2016.10.138
32. Zick SM, Djuric Z, Ruffin MT, Litzinger AJ, Normolle DP, Alrawi S, et al. Pharmacokinetics of 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol and conjugate metabolites in healthy human subjects. *Cancer Epidemiol biomarkers Prev..* (2008) 17:1930–6. doi: 10.1158/1055-9965.EPI-07-2934
33. Yu Y, Zick S, Li X, Zou P, Wright B, Sun D. Examination of the pharmacokinetics of active ingredients of ginger in humans. *AAPS J.* (2011) 13:417–26. doi: 10.1208/s12248-011-9286-5

34. Schoenknecht C, Andersen G, Schieberle P. A novel method for the quantitation of gingerol glucuronides in human plasma or urine based on stable isotope dilution assays. *J Chromatogr B Analyt Technol Biomed Life Sci.* (2016) 1036–7:1–9. doi: 10.1016/j.jchromb.2016.09.033
35. Mukkavilli R, Yang C, Tanwar RS, Saxena R, Gundala SR, Zhang Y, et al. Pharmacokinetic-pharmacodynamic correlations in the development of ginger extract as an anticancer agent. *Sci Rep.* (2018) 8:3056. doi: 10.1038/s41598-018-21125-2
36. Rafiee Z, Nejatian M, Daeihamed M, Jafari SM. Application of different nanocarriers for encapsulation of curcumin. *Crit Rev Food Sci Nutr.* (2019) 59:3468–97. doi: 10.1080/10408398.2018.1495174
37. Zhen L, Wei Q, Wang Q, Zhang H, Adu-Frimpong M, Kesse Firempong C, et al. Preparation and in vitro/in vivo evaluation of 6-Gingerol TPGS/PEG-PCL polymeric micelles. *Pharm Dev Technol.* (2020) 25:1–8. doi: 10.1080/10837450.2018.1558239
38. Wang Q, Wei Q, Yang Q, Cao X, Li Q, Shi F, et al. novel formulation of [6]-gingerol: Proliposomes with enhanced oral bioavailability and antitumor effect. *Int J Pharm.* (2018) 535:308–15. doi: 10.1016/j.ijpharm.2017.11.006
39. Xu Y, Wang Q, Feng Y, Firempong CK, Zhu Y, Omari-Siaw E, et al. Enhanced oral bioavailability of [6]-Gingerol-SMEDDS: Preparation, in vitro and in vivo evaluation. *J Funct Foods.* (2016) 27:703–10. doi: 10.1016/j.jff.2016.10.007
40. Wei Q, Yang Q, Wang Q, Sun C, Zhu Y, Niu Y, et al. Formulation, characterization, and pharmacokinetic studies of 6-gingerol-loaded nanostructured lipid carriers. *AAPS PharmSciTech.* (2018) 19:3661–9. doi: 10.1208/s12249-018-1165-2
41. Zhu H, Chen H, Zeng X, Wang Z, Zhang X, Wu Y, et al. Co-delivery of chemotherapeutic drugs with vitamin E TPGS by porous PLGA nanoparticles for enhanced chemotherapy against multi-drug resistance. *Biomaterials.* (2014) 35:2391–400. doi: 10.1016/j.biomaterials.2013.11.086
42. Cheng X, Yan H, Jia X, Zhang Z. Preparation and in vivo/in vitro evaluation of formononetin phospholipid/vitamin E TPGS micelles. *J Drug Target.* (2016) 24:161–8. doi: 10.3109/1061186X.2015.1064435
43. Poprac P, Jomova K, Simunkova M, Kollar V, Rhodes CJ, Valko M. Targeting free radicals in oxidative stress-related human diseases. *Trends Pharmacol Sci.* (2017) 38:592–607. doi: 10.1016/j.tips.2017.04.005
44. Li S, Li S-K, Gan R-Y, Song F-L, Kuang L, Li H-B. Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. *Ind Crops Prod.* (2013) 51:289–98. doi: 10.1016/j.indcrop.2013.09.017
45. Deng G-F, Lin X, Xu X-R, Gao L-L, Xie J-F, Li H-B. Antioxidant capacities and total phenolic contents of 56 vegetables. *J Funct Foods.* (2013) 5:260–6. doi: 10.1016/j.jff.2012.10.015
46. Deng G-F, Xu X-R, Guo Y-J, Xia E-Q, Li S, Wu S, et al. Determination of antioxidant property and their lipophilic and hydrophilic phenolic contents in cereal grains. *J Funct Foods.* (2012) 4:906–14. doi: 10.1016/j.jff.2012.06.008
47. Fu L, Xu B-T, Gan R-Y, Zhang Y, Xu X-R, Xia E-Q, et al. Total phenolic contents and antioxidant capacities of herbal and tea infusions. *Int J Mol Sci.* (2011) 12:2112–24. doi: 10.3390/ijms12042112
48. Fu L, Xu B-T, Xu X-R, Gan R-Y, Zhang Y, Xia E-Q, et al. Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chem.* (2011) 129:345–50. doi: 10.1016/j.foodchem.2011.04.079
49. Guo Y-J, Deng G-F, Xu X-R, Wu S, Li S, Xia E-Q, et al. Antioxidant capacities, phenolic compounds and polysaccharide contents of 49 edible macro-fungi. *Food Funct.* (2012) 3:1195–205. doi: 10.1039/c2fo30110e
50. Song F-L, Gan R-Y, Zhang Y, Xiao Q, Kuang L, Li H-B. Total phenolic contents and antioxidant capacities of selected chinese medicinal plants. *Int J Mol Sci.* (2010) 11:2362–72. doi: 10.3390/ijms11062362
51. Ji K, Fang L, Zhao H, Li Q, Shi Y, Xu C, et al. Ginger oleoresin alleviated γ-ray irradiation-induced reactive oxygen species *via* the Nrf2 protective response in human mesenchymal stem cells. *Oxid Med Cell Longev.* (2017) 2017:1480294. doi: 10.1155/2017/1480294
52. Abolaji AO, Ojo M, Afolabi TT, Arowoogun MD, Nwawolor D, Farombi EO. Protective properties of 6-gingerol-rich fraction from zingiber officinale (Ginger) on chlorpyrifos-induced oxidative damage and inflammation in the brain, ovary and uterus of rats. *Chem Biol Interact.* (2017) 270:15–23. doi: 10.1016/j.cbi.2017.03.017
53. Li Y, Hong Y, Han Y, Wang Y, Xia L. Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger. *J Chromatogr B, Anal Technol Biomed life Sci.* (2016) 1011:223–32. doi: 10.1016/j.jchromb.2016.01.009
54. Akinyemi AJ, Ademiluyi AO, Oboh G. Aqueous extracts of two varieties of ginger (Zingiber officinale) inhibit angiotensin I-converting enzyme, iron(II), and sodium nitroprusside-induced lipid peroxidation in the rat heart in vitro. *J Med Food.* (2013) 16:641–6. doi: 10.1089/jmf.2012.0022
55. Hosseinzadeh A, Bahrampour Juybari K, Fatemi MJ, Kamarul T, Bagheri A, Tekiyehmaroof N, et al. Protective effect of ginger (zingiber officinale roscoe) extract against oxidative stress and mitochondrial apoptosis induced by interleukin-1β in cultured chondrocytes. *Cells Tissues Organs.* (2017) 204:241–50. doi: 10.1159/000479789
56. Romero A, Forero M, Sequeda-Castañeda LG, Grismaldo A, Iglesias J, Celis-Zambrano CA, et al. Effect of ginger extract on membrane potential changes and AKT activation on a peroxide-induced oxidative stress cell model. *J King Saud Univ.* (2018) 30:263–9. doi: 10.1016/j.jksus.2017.09.015
57. Peng S, Yao J, Liu Y, Duan D, Zhang X, Fang J. Activation of Nrf2 target enzymes conferring protection against oxidative stress in PC12 cells by ginger principal constituent 6-shogaol. *Food Funct.* (2015) 6:2813–23. doi: 10.1039/C5FO00214A
58. Chen H, Fu J, Chen H, Hu Y, Soroka DN, Prigge JR, et al. Ginger compound [6]-shogaol and its cysteine-conjugated metabolite (M2) activate Nrf2 in colon epithelial cells in vitro and in vivo. *Chem Res Toxicol.* (2014) 27:1575–85. doi: 10.1021/tx500211x
59. Ryan JL, Morrow GR. *Ginger. Oncol nurse Ed.* (2010). p. 46–9.
60. Ajith TA, Nivitha V, Usha S. Zingiber officinale Roscoe alone and in combination with alpha-tocopherol protect the kidney against cisplatin-induced acute renal failure. *Food Chem Toxicol.* (2007) 45:921–7. doi: 10.1016/j.fct.2006.11.014
61. Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. *J Ethnopharmacol.* (2010) 127:515–20. doi: 10.1016/j.jep.2009.10.004
62. Arablou T, Aryaeian N, Valizadeh M, Sharifi F, Hosseini A, Djalali M. The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. *Int J Food Sci Nutr.* (2014) 65:515–20. doi: 10.3109/09637486.2014.880671
63. Kim SO, Chun K-S, Kundu JK, Surh Y-J. Inhibitory effects of [6]-gingerol on PMA-induced COX-2 expression and activation of NF-kappaB and p38 MAPK in mouse skin. *Biofactors.* (2004) 21:27–31. doi: 10.1002/biof.552210107
64. Mazidi M, Gao H-K, Rezaie P, Ferns GA. The effect of ginger supplementation on serum C-reactive protein, lipid profile and glycaemia: a systematic review and meta-analysis. *Food Nutr Res.* (2016) 60:32613. doi: 10.3402/fnr.v60.32613
65. Naderi Z, Mozaffari-Khosravi H, Dehghan A, Nadjarzadeh A, Huseini HF. Effect of ginger powder supplementation on nitric oxide and C-reactive protein in elderly knee osteoarthritis patients: a 12-week double-blind randomized placebo-controlled clinical trial. *J Tradit Complement Med.* (2016) 6:199–203. doi: 10.1016/j.jtcme.2014.12.007
66. Rahimlou M, Yari Z, Hekmatdoost A, Alavian SM, Keshavarz SA. Ginger Supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. *Hepat Mon.* (2016) 16:e34897. doi: 10.5812/hepatmon.34897
67. Azimi P, Ghiasvand R, Feizi A, Hariri M, Abbasi B. Effects of cinnamon, cardamom, saffron, and ginger consumption on markers of glycemic control, lipid profile, oxidative stress, and inflammation in type 2 diabetes patients. *Rev Diabet Stud.* (2014) 11:258–66. doi: 10.1900/RDS.2014.11.258
68. van Breemen RB, Tao Y, Li W. Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale). *Fitoterapia.* (2011) 82:38–43. doi: 10.1016/j.fitote.2010.09.004
69. Tjendraputra E, Tran VH, Liu-Brennan D, Roufogalis BD, Duke CC. Effect of ginger constituents and synthetic analogues on cyclooxygenase-2 enzyme in intact cells. *Bioorg Chem.* (2001) 29:156–63. doi: 10.1006/bioo.2001.1208
70. Phan PV, Sohrabi A, Polotsky A, Hungerford DS, Lindmark L, Frondoza CG. Ginger extract components suppress induction of chemokine expression in human synoviocytes. *J Altern Complement Med.* (2005) 11:149–54. doi: 10.1089/acm.2005.11.149

71. Habib SHM, Makpol S, Abdul Hamid NA, Das S, Ngah WZW, Yusof YAM. Ginger extract (Zingiber officinale) has anti-cancer and anti-inflammatory effects on ethionine-induced hepatoma rats. *Clinics.* (2008) 63:807–13. doi: 10.1590/S1807-59322008000600017
72. Srivastava KC, Mustafa T. Ginger (Zingiber officinale) in rheumatism and musculoskeletal disorders. *Med Hypotheses.* (1992) 39:342–8. doi: 10.1016/0306-9877(92)90059-L
73. Khan M, Ullah N, Azhar M, Komal W, Muhammad W. A mini-review on the therapeutic potential of Zingiber officinale (ginger). *Nat Prod An Indian J.* (2019) 15:125.
74. Melo A, Monteiro L, Lima RMF, Oliveira DMde, Cerqueira MDde, El-Bachá RS. Oxidative stress in neurodegenerative diseases: mechanisms and therapeutic perspectives. *Oxid Med Cell Longev.* (2011) 2011:467180. doi: 10.1155/2011/467180
75. Karam A, Nadia A, Abd EF, Nemat A, Siham M. Protective effect of ginger (Zingiber officinale) on Alzheimer's disease induced in rats. *J Neuroinfect Dis.* (2014) 5:2.
76. Moon M, Kim HG, Choi JG, Oh H, Lee PKJ, Ha SK, et al. 6-Shogaol, an active constituent of ginger, attenuates neuroinflammation and cognitive deficits in animal models of dementia. *Biochem Biophys Res Commun.* (2014) 449:8–13. doi: 10.1016/j.bbrc.2014.04.121
77. Grzanna R, Phan P, Polotsky A, Lindmark L, Frondoza CG. Ginger extract inhibits beta-amyloid peptide-induced cytokine and chemokine expression in cultured THP-1 monocytes. *J Altern Complement Med.* (2004) 10:1009–13. doi: 10.1089/acm.2004.10.1009
78. Kim JE, Shrestha AC, Kim HS, Ham HN, Kim JH, Kim YJ, et al. WS-5 extract of curcuma longa, chaenomeles sinensis, and zingiber officinale contains Anti-AChE compounds and improves β-amyloid-induced memory impairment in mice. *Evid Based Complement Alternat Med.* (2019) 2019:5160293. doi: 10.1155/2019/5160293
79. Halawany AME, Sayed NSE, Abdallah HM, Dine RSE. Protective effects of gingerol on streptozotocin-induced sporadic Alzheimer's disease: emphasis on inhibition of β-amyloid, COX-2, alpha-, beta - secretases and APH1a. *Sci Rep.* (2017) 7:2902. doi: 10.1038/s41598-017-02961-0
80. Sutalangka C, Wattanathorn J. Neuroprotective and cognitive-enhancing effects of the combined extract of cyperus rotundus and zingiber officinale. *BMC Complement Altern Med.* (2017) 17:135. doi: 10.1186/s12906-017-1632-4
81. Kim C-Y, Seo Y, Lee C, Park GH, Jang J-H. Neuroprotective effect and molecular mechanism of [6]-gingerol against scopolamine-induced amnesia in C57BL/6 mice. *Evid Based Complement Altern Med.* (2018) 2018:8941564. doi: 10.1155/2018/8941564
82. Simon A, Darcsi A, Kéry Á, Riethmüller E. Blood-brain barrier permeability study of ginger constituents. *J Pharm Biomed Anal.* (2020) 177:112820. doi: 10.1016/j.jpba.2019.112820
83. Saenghong N, Wattanathorn J, Muchimapura S, Tongun T, Piyavhatkul N, Banchonglikitkul C, et al. Zingiber officinale improves cognitive function of the middle-aged healthy women. *Evid Based Complement Alternat Med.* (2012) 2012:383062. doi: 10.1155/2012/383062
84. Richter Y, Herzog Y, Eyal I, Cohen T. Cognitex supplementation in elderly adults with memory complaints: an uncontrolled open label trial. *J Diet Suppl.* (2011) 8:158–68. doi: 10.3109/19390211.2011.569514
85. Tajadini H, Saifadini R, Choopani R, Mehrabani M, Kamalinejad M, Haghdoost AA. Herbal medicine davaie loban in mild to moderate Alzheimer's disease: A 12-week randomized double-blind placebo-controlled clinical trial. *Complement Ther Med.* (2015) 23:767–72. doi: 10.1016/j.ctim.2015.06.009
86. Watari H, Shimada Y, Matsui M, Tohda C. Kihito, a traditional Japanese Kampo Medicine, Improves Cognitive Function in Alzheimer's disease PATIENTS. *Evid Based Complement Alternat Med.* (2019) 2019:4086749. doi: 10.1155/2019/4086749
87. Obeso JA, Stamelou M, Goetz CG, Poewe W, Lang AE, Weintraub D, et al. Past, present, and future of Parkinson's disease: a special essay on the 200th anniversary of the shaking palsy. *Mov Disord.* (2017) 32:1264–310. doi: 10.1002/mds.27115
88. Rizek P, Kumar N, Jog MS. An update on the diagnosis and treatment of Parkinson disease. *CMAJ.* (2016) 188:1157–65. doi: 10.1503/cmaj.151179
89. Valadez-Barba V, Juárez-Navarro K, Padilla-Camberos E, Díaz NF, Guerra-Mora JR, Díaz-Martínez NE. Parkinson's disease: an update on preclinical studies of induced pluripotent stem cells. *Neurologia.* (2021) 11:S0213-4853(21)00020-7. doi: 10.1016/j.nrl.2021.01.005. [Epub ahead of print].
90. Kongsui R, Sriraksa N, Thongrong S. The neuroprotective effect of zingiber cassumunar roxb. extract on LPS-induced neuronal cell loss and astroglial activation within the hippocampus. *Biomed Res Int.* (2020) 2020:4259316. doi: 10.1155/2020/4259316
91. Perfeito R, Cunha-Oliveira T, Rego AC. Revisiting oxidative stress and mitochondrial dysfunction in the pathogenesis of Parkinson disease–resemblance to the effect of amphetamine drugs of abuse. *Free Radic Biol Med.* (2012) 53:1791–806. doi: 10.1016/j.freeradbiomed.2012.08.569
92. Park G, Kim HG, Ju MS, Ha SK, Park Y, Kim SY, et al. 6-Shogaol, an active compound of ginger, protects dopaminergic neurons in Parkinson's disease models *via* anti-neuroinflammation. *Acta Pharmacol Sin.* (2013) 34:1131–9. doi: 10.1038/aps.2013.57
93. Hussein UK, Hassan NE-HY, Elhalwagy MEA, Zaki AR, Abubakr HO, Nagulapalli Venkata KC, et al. Ginger and propolis exert neuroprotective effects against monosodium glutamate-induced neurotoxicity in rats. *Molecules.* (2017) 22:1928. doi: 10.3390/molecules22111928
94. Compston A, Coles A. Multiple sclerosis. *Lancet.* (2008) 372:1502–17. doi: 10.1016/S0140-6736(08)61620-7
95. Oh J, Vidal-Jordana A, Montalban X. Multiple sclerosis: clinical aspects. *Curr Opin Neurol.* (2018) 31:752–9. doi: 10.1097/WCO.0000000000000622
96. Perez-Carmona N, Fernandez-Jover E, Sempere AP. [Epidemiology of multiple sclerosis in Spain]. *Rev Neurol.* (2019) 69:32–8.
97. Río J, Montalbán X. [Current description of multiple sclerosis]. *Med Clin.* (2014) 143:3–6.
98. Browne P, Chandraratna D, Angood C, Tremlett H, Baker C, Taylor BV, et al. Atlas of multiple sclerosis 2013: a growing global problem with widespread inequity. *Neurology.* (2014) 83:1022–4. doi: 10.1212/WNL.0000000000000768
99. Dworsky-Fried Z, Chadwick CI, Kerr BJ, Taylor AMW. Multiple sclerosis and the endogenous opioid system. *Front Neurosci.* (2021) 15:741503. doi: 10.3389/fnins.2021.741503
100. Gilmour H, Ramage-Morin PL, Wong SL. Multiple sclerosis: PREVALENCE and impact. *Heal reports.* (2018) 29:3–8.
101. Yadav SK, Mindur JE, Ito K, Dhib-Jalbut S. Advances in the immunopathogenesis of multiple sclerosis. *Curr Opin Neurol.* (2015) 28:206–19. doi: 10.1097/WCO.0000000000000205
102. Glatigny S, Bettelli E. Experimental autoimmune encephalomyelitis (EAE) as Animal models of multiple sclerosis (MS). *Cold Spring Harb Perspect Med.* (2018) 8:28977. doi: 10.1101/cshperspect.a028977
103. Jafarzadeh A, Ebrahimi HA, Bagherzadeh S, Zarkesh F, Iranmanesh F, Najafzadeh A, et al. Lower serum levels of Th2-related chemokine CCL22 in women patients with multiple sclerosis: a comparison between patients and healthy women. *Inflammation.* (2014) 37:604–10. doi: 10.1007/s10753-013-9775-z
104. Buc M. Role of regulatory T cells in pathogenesis and biological therapy of multiple sclerosis. *Mediators Inflamm.* (2013) 2013:963748. doi: 10.1155/2013/963748
105. Jafarzadeh A, Bagherzadeh S, Ebrahimi HA, Hajghani H, Bazrafshani MR, Khosravimashizi A, et al. Higher circulating levels of chemokine CCL20 in patients with multiple sclerosis: evaluation of the influences of chemokine gene polymorphism, gender, treatment and disease pattern. *J Mol Neurosci.* (2014) 53:500–5. doi: 10.1007/s12031-013-0214-2
106. Petersen MJ, Bergien SO, Staerk D. A systematic review of possible interactions for herbal medicines and dietary supplements used concomitantly with disease-modifying or symptom-alleviating multiple sclerosis drugs. *Phytother Res.* (2021) 35:3610–31. doi: 10.1002/ptr.7050
107. Baker D, Amor S. Experimental autoimmune encephalomyelitis is a good model of multiple sclerosis if used wisely. *Mult Scler Relat Disord.* (2014) 3:555–64. doi: 10.1016/j.msard.2014.05.002
108. Lassmann H, Bradl M. Multiple sclerosis: experimental models and reality. *Acta Neuropathol.* (2017) 133:223–44. doi: 10.1007/s00401-016-1631-4
109. Jafarzadeh A, Mohammadi-Kordkhayli M, Ahangar-Parvin R, Azizi V, Khoramdel-Azad H, Shamsizadeh A, et al. Ginger extracts influence the expression of IL-27 and IL-33 in the central nervous system

in experimental autoimmune encephalomyelitis and ameliorates the clinical symptoms of disease. *J Neuroimmunol.* (2014) 276:80–8. doi: 10.1016/j.jneuroim.2014.08.614

110. Jafarzadeh A, Ahangar-Parvin R, Nemat M, Taghipour Z, Shamsizadeh A, Ayoobi F, et al. Ginger extract modulates the expression of IL-12 and TGF-β in the central nervous system and serum of mice with experimental autoimmune encephalomyelitis. *Avicenna J phytomedicine.* (2017) 7:54–65.
111. Sapkota A, Park SJ, Choi JW. Neuroprotective effects of 6-shogaol and its metabolite, 6-paradol, in a mouse model of multiple sclerosis. *Biomol Ther.* (2019) 27:152–9. doi: 10.4062/biomolther.2018.089
112. Jafarzadeh A, Arabi Z, Ahangar-Parvin R, Mohammadi-Kordkhayli M, Nemati M. Ginger extract modulates the expression of chemokines CCL20 and CCL22 and their receptors (CCR6 and CCR4) in the central nervous system of mice with experimental autoimmune encephalomyelitis. *Drug Res.* (2017) 67:632–9. doi: 10.1055/s-0043-113455
113. Mojaverrostami S, Bojnordi MN, Ghasemi-Kasman M, Ebrahimzadeh MA, Hamidabadi HG. A review of herbal therapy in multiple sclerosis. *Adv Pharm Bull.* (2018) 8:575–90. doi: 10.15171/apb.2018.066

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REVIEW
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Associations Between Dietary Patterns and Neuroimaging Markers: A Systematic Review

*Rebecca F. Townsend[1], Jayne V. Woodside[1,2], Federica Prinelli[3], Roisin F. O'Neill[1] and Claire T. McEvoy[1,2]**

[1] Centre for Public Health, Queen's University Belfast, Belfast, United Kingdom, [2] Institute for Global Food Security, Queen's University Belfast, Belfast, United Kingdom, [3] Institute of Biomedical Technologies, National Research Council, Milan, Italy

Dementia is a complex, growing challenge for population health worldwide. Dietary patterns (DPs) may offer an opportunity to beneficially influence cognitive ageing and potentially reduce an individuals' risk of dementia through diet-related mechanisms. However, previous studies within this area have shown mixed results, which may be partly explained by the lack of sensitivity and accuracy within cognitive testing methods. Novel neuroimaging techniques provide a sensitive method to analyse brain changes preceding cognitive impairment which may have previously remained undetected. The purpose of this systematic review was to elucidate the role of DPs in relation to brain ageing processes, by summarising current prospective and intervention studies. Nine prospective studies met the inclusion criteria for the review, seven evaluated the Mediterranean diet (MeDi), one evaluated the Alternative Healthy Eating Index-2010, and one evaluated *a posteriori* derived DPs. No intervention studies were eligible for inclusion in this review. There was some evidence of an association between healthy DPs and neuroimaging markers including changes within these markers over time. Consequently, it is plausible that better adherence to such DPs may positively influence brain ageing and neurodegeneration. Future studies may benefit from the use of multi-modal neuroimaging techniques, to further investigate how adherence to a DP influences brain health. The review also highlights the crucial need for further intervention studies within this research area.

Keywords: dietary patterns, neuroimaging, neurocognition, diet, brain health

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***Correspondence:**
Claire T. McEvoy
c.mcevoy@qub.ac.uk

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INTRODUCTION

Dementia is a global challenge within the twenty-first century. Over 50 million individuals worldwide are estimated to live with the condition, a number that is expected to treble by 2050 (1). Up to 40% of future cases could be prevented or delayed by targeting modifiable factors (2). Previous observational research suggests greater adherence towards a healthy dietary pattern (DP) is associated with slower cognitive decline (3–7) and reduced risk of Alzheimer's disease (AD) (the most common cause of dementia) (8–12) in later life. Yet, the limited data from randomised controlled trials (RCT) have not convincingly demonstrated a protective effect of diet on cognition. For example, two RCT's found intervention with an enhanced Mediterranean diet

(MeDi) enhanced with either nuts or extra-virgin olive oil (EVOO) improved Mini-Mental State Examination (MMSE) scores ranging from 4 to 6 years post-intervention, in older adults with high vascular risk (13, 14). However, a sub-study from the same cohort also found cognitive performance only improved significantly in those supplementing the MeDi with EVOO and not those that supplemented with nuts, when compared to controls (15). Similarly, another study also found intervention with a MeDi supplemented with dairy foods did not significantly improve cognition in middle-aged adults (16). Intervention studies investigating DPs other than the MeDi have also found inconsistent results (17–20).

A potential reason for the inconsistent findings may be the relatively short trial duration, making it difficult to discern effects on cognition and the narrow range of cognitive endpoints assessed. Subsequently, there is a need to determine which aspects of cognition are sensitive to dietary change, and the optimum timescale over which to observe these effects during trials. Furthermore, diet intervention is likely to have the most benefit during preclinical stages of AD, where it is more difficult to detect such minimal deterioration in cognition. However, due to advancements in neuroimaging techniques, there is now the ability to sensitively measure changes within brain health, which can help to elucidate potential diet-related mechanisms of neurodegeneration. For example, accumulating cross-sectional data suggest a protective association of healthy DPs on brain structures and functions. In particular, adherence to the MeDi has been associated with reduced brain atrophy, specifically in AD-vulnerable regions (21) including the hippocampus (22) and posterior cingulate cortex (23). Furthermore, better adherence to the MeDi has also been associated with less amyloid-β (Aβ) burden (24).

There have been limited comprehensive reviews of whole DPs and neuroimaging biomarkers (25–27). A previous systematic review (28) evaluated evidence published up to 2017. However, several prospective studies investigating DPs and neuroimaging outcomes have been published since 2017, which have not yet been synthesized. More recently, a systematic review (25) suggested evidence of associations between healthy DPs and brain imaging correlates, which preceded cognitive decline. However, only studies reporting both cognitive performance and neuroimaging biomarkers were reviewed, meaning that several studies reporting neuroimaging markers alone were not eligible for review. A further review (26) investigated DPs in relation to neuroimaging markers but only in middle-aged adults, meaning studies including both younger and older age adults were excluded. An additional review (27), investigated any aspect of diet or metabolism in relation to neuroimaging markers and included some, but not all available (29, 30) studies investigating DPs in relation to neuroimaging markers. It should be noted that these latter three reviews included evidence from cross-sectional studies, from which a causal relationship between diet and neurocognitive outcomes cannot be established. Therefore, we aimed to synthesise the available data from RCTs and prospective studies to evaluate effects of DPs on neuroimaging biomarkers. This review will address the following research questions; (1) What is the relationship between DPs and neuroimaging markers across the adult life course? and (2) What is the effect of DPs on changes in neuroimaging measures over time?

Abbreviations: AD, Alzheimer's Disease; AHEI-2010, Alternate Healthy Eating Index-2010; Aβ, Amyloid-β; Cmrglc, Cerebral Metabolic Rate Of Glucose; DP, Dietary pattern; DTI, Diffusion Tensor Imaging; EVOO, Extra Virgin Olive Oil; FDG-PET, (18)F-Fluorodeoxyglucose Positron Emission Tomography; FFQ, Food frequency questionnaire; GMV, Grey Matter Volume; HCV, Hippocampal Volume; MeDi, Mediterranean diet; MMSE, Mini-Mental State Examination; MRI, Magnetic Resonance Imaging; NOS, Newcastle Ottawa Scale; PCA, Principal Component Analysis; PET, Positron Emission Tomography; PiB, Pittsburgh compound B; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PROSPERO, International Prospective Register of Systematic Reviews; RCT, Randomised Controlled Trial; SUVR, Standard Uptake Volume Ratio; TBV, Total Brain Volume; WMI, White Matter Integrity; WMV, White Matter Volume.

METHODS

Study Design and Systematic Review Protocol

This systematic review was undertaken based on the Centre for Reviews and Dissemination (CRD) guidance for undertaking systematic reviews in health care. The protocol for this review was registered with PROSPERO database (PROSPERO 2020: CRD42020181423). The review presented here focuses on neuroimaging outcomes from part of a broader review on DPs and neurocognitive outcomes.

Search Strategy and Data Sources

A systematic literature search was conducted using two major databases; EMBASE and Ovid MEDLINE (a subset of PubMed), for relevant studies published up to 5 March 2020. A detailed search strategy (shown in **Supplementary Table 1**) was developed (28, 31), using key terms associated with DPs and neuroimaging markers. The primary search was limited to humans and English language publications. Articles were considered eligible for review if they were an RCT or prospective observational design and met the following criteria; (i) measured adherence/exposure to ≥1 whole DP, (ii) reported neuroimaging outcomes using any imaging measure/technique; (iii) evaluated the effect of diet in relation to any specified brain ageing outcome(s) or determined the association between diet and brain ageing outcomes. Intervention studies were eligible for inclusion if they were of RCT design and met the above criteria. We placed no restrictions on intervention duration or type. As studies were required to include ≥1 whole DP, those which focused on a single food, macronutrient group, were based on nutrients alone or focused on calorie restriction or weight loss, without overall consideration for dietary quality were excluded. Multi-domain lifestyle exposures/interventions were also excluded, due to the difficulty in disentangling potential effects from diet alone. Studies within the same cohort were excluded if the exposures and outcomes studied were analysed identically within each.

Data Extraction

The process of study selection and data extraction was managed using EndNote software version X9 and Rayyan online software.

Titles of all potentially eligible studies were screened by R.F.T. and those not including any keywords relating to the review were excluded. Two reviewers (R.F.T. and C.T.ME.) screened the abstracts of all remaining studies independently before assessing the remaining studies further for eligibility via retrieval and independent reading of full texts. Any discrepancies or queries regarding inclusion of studies were then resolved through discussion among the research team (R.F.T, C.T.ME., and J.V.W). For duplicated study cohorts reporting identical diet exposure and neuroimaging outcome(s) at different time points, the study with the longest follow-up period was included. Data was collected from each study identified as eligible for inclusion using a standardized form. For prospective studies included, the following information was collected; names of authors, year of publication and study cohort name (if applicable); country of study location; follow-up period (years); exclusion and inclusion criteria; method to derive DP (*a priori* or *a posteriori*); dietary assessment tools used (e.g., 24 h recall, food frequency questionnaire (FFQ), diet record, diet history); DP(s) examined; time points at which DP was examined; neuroimaging methods; primary outcomes effect size and/or summary of main findings; covariates included in adjusted models (where applicable).

Risk of Bias Assessment

Two reviewers (R.F.T and R.F.ON.) independently examined the quality of each study included in the review using the Newcastle-Ottawa Scale (NOS) (32) for prospective studies. Studies could be awarded a maximum of nine stars in total. Up to four stars could be awarded for selection of study (representation within sample, selection of sample, exposure measurement, and demonstration outcome not present at baseline). Up to two stars could be awarded within the comparability domain (controls for basic confounders or includes additional confounding factors) comparability and up to three stars for outcome measurement (including methodology of outcome assessment, follow-up length, and adequacy of follow-up cohort). Studies were considered high quality if they scored 9 stars, medium quality if 7–8 stars were scored, or low quality if $\leq$6 stars were scored.

Data Analysis

Due to the heterogeneity within the data collected in terms of neuroimaging markers, statistical analyses and reporting of data, a quantitative analysis was not possible. Consequently, a narrative synthesis was used to present results alongside detailed tables.

RESULTS

Study Selection

The preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram is shown in **Figure 1**. From the primary systematic search in March 2020, 10,194 articles were identified, 978 of which were removed due to duplication. Following title and abstract screening, 9,065 articles were excluded. From the remaining 158 full text articles, a further 149 were excluded (see **Figure 1**). A total of 79 articles [70 prospective studies and 9 randomised control trials (RCT)] were therefore included within the broader systematic review; 9 of which included neuroimaging markers and were included in this review.

Study Characteristics

All included articles were prospective studies published in the last 10 years (29, 30, 33–39). There were no RCTs identified. Sample sizes varied between studies ranging from 70 to 707, with a total of approximately 2,540 participants across all articles. Similarly, follow-up periods also differed greatly with the shortest period being 3 years to the longest period of 13 years. An overview of the key characteristics and findings of each study included within the review is provided in **Table 1**.

Populations Assessed

Most studies (6/9) included older aged adults (aged $\geq$ 60 years) (29, 30, 33, 35–38). Only two studies specifically included individuals from both young adulthood (age $\geq$ 30 years) and older adulthood (aged $\geq$ 60 years) (34, 39). Three studies were conducted in the United States of America (USA) (34, 37, 39), two from the United Kingdom (UK) (33, 36), two from Australia (30, 35), one from France (29), and one from Sweden (38).

All studies except for one (33) specified that at study baseline, participants must have been cognitively healthy and/or free from dementia. Although, one study did specifically select individuals categorised as Aβ accumulators (30). However, at the time of neuroimaging measurement, three studies reported the inclusion of individuals with cognitive impairment and/or a diagnosis of dementia (33, 37, 38). Two of these studies reported sensitivity analyses excluding those with cognitive impairment (33, 37).

The majority of studies were conducted in educated populations, with three specifically requiring $\geq$12 years of education to participate in the study (30, 34, 39).

Another demographic factor that may impact the relationship between DP and neuroimaging measures is ethnicity. Populations such as Black and Asian ethnic groups may experience a higher risk of developing AD (2). Yet, only four of nine included studies specifically reported participants' ethnic demographics. Three studies (33, 34, 39) reported a high prevalence ($\geq$70%) of white participants. In contrast, the proportion of white individuals within the Washington Heights-Hamilton Heights-Inwood Community Aging Project (WHICAP) study (37) (27%) was lower than African American individuals (35%) and Hispanic individuals (36%).

Measurement of Dietary Patterns

Assessment of Dietary Intake

Food frequency questionnaires were used in the majority of studies (8/9) to assess dietary intake. Of which, one study combined the FFQ used with another method, which was a 24-h dietary recall (29). The nature of FFQs means they lend themselves to self-reporting. However, from the studies within this review, three specifically reported that a trained individual (i.e., a dietitian or interviewer, respectively) administered the FFQ (29, 34, 37). In the one study that did not use an FFQ, a 7-day food diary was used (38).

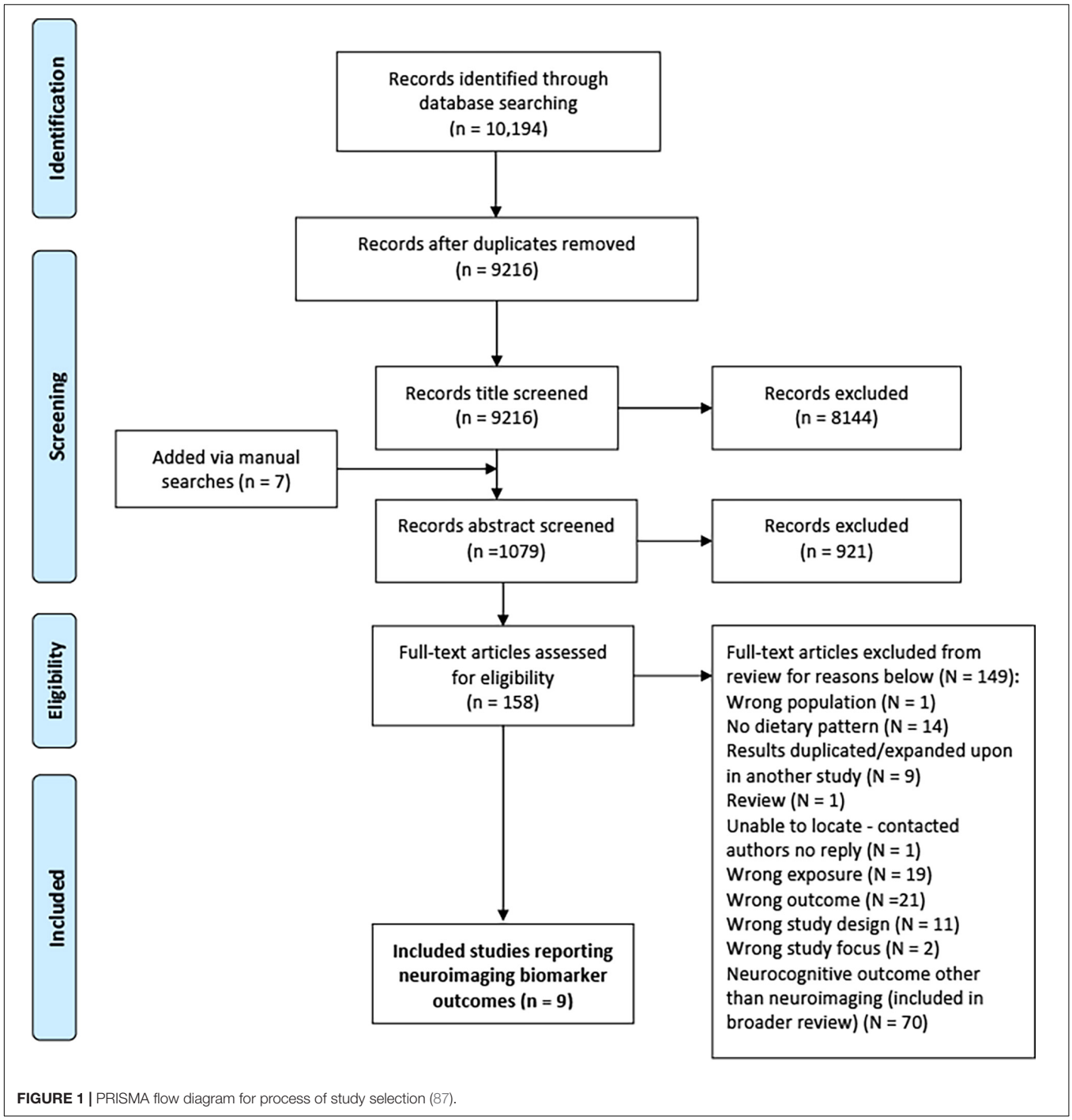

FIGURE 1 | PRISMA flow diagram for process of study selection (87).

Time Points of Diet Intake Assessment

Dietary intake data was captured at a single time point at baseline in nearly all studies (8/9) to measure adherence to the DPs studied in relation to neuroimaging markers (29, 30, 34–39). One study (33) evaluated diet exclusively retrospectively, to determine earlier life diet exposure. Diet measures were then repeated to evaluate a cumulative average and provide a value representative of longer-term adherence to the DP. It should be noted that one other study (29) also assessed diet stability through repeated diet measures within supplementary analyses, but not within main statistical analyses.

Dietary Pattern Indices

A priori scoring indices were used in eight studies to determine adherence towards a DP (29, 30, 33–39). One study utilised *a posteriori* methods (35). Details of the DPs studied within each article, including their respective scoring systems and food components are summarised in **Supplementary Table 2**.

TABLE 1 | Overview of included studies investigating DPs in relation to neuroimaging markers.

Authors, year, study name, country	Study characteristics			Diet measures			Neuroimaging measures	Results			
	Follow-up time (years)	N =	Population characteristics	Mean age at baseline, y	Food intake assessment; time point(s) assessed	DP(s) examined (scoring reference or *a posteriori*)	Specific methods/ techniques used:	Timepoint(s) of neuroimaging assessment(s)	Primary outcome(s) of interest	Main findings from primary outcome(s) of interest	Covariates
Pelletier et al. (29); *Three City Study;* France.	8.9	146	Adults ≥ 65 years who are non-institutionalised free from dementia at baseline.	73	40-item FFQ and 24-h diet recall, administered by trained dietitian; 1 time point used in analysis, but reassessments conducted to assess diet stability in supplementary analyses.	1. Mediterranean diet, Trichopoulou et al. (40)	3 T MRI and DTI.	1 time point; 8.9 years on average post-diet assessment.	GMV, WMV, WM microstructure via fractional anisotropy, axial diffusivity, radial diffusivity and mean diffusivity (global measure of diffusion).	Adherence to MeDi was not significantly associated with any structural MRI measures (GMV, WMV and TIV) but better MeDi adherence was associated with reduced mean diffusivity values and higher fractional anisotropy values assessed 8.9 years later.	Age, sex, education, APOE4 caloric intake, BMI, smoking, physical activity, vascular risk factors (including cardiovascular or cerebrovascular disease, hypertension, diabetes and hypercholesterolemia), cognitive performance on Isaac's set test.
Scarmeas et al. (37); WHICAP Study; USA.	5.8 ± 3.22	707	Adults ≥ 65 years who are non-institutionalised living in Manhattan from WHICAP 1992 and 1999. 66% female	80.3 ± 5.7	Interviewer administered 61-item Willett Semi-quantitative FFQ; 1 time point (baseline).	1. Mediterranean diet, Trichopoulou et al. (40)	1.5 T MRI	1 time point; 5.8 years after baseline.	Cerebral infarcts and WMH.	Adherence to MeDi was not significantly associated with WMH but was associated with reduced odds of cerebral infarcts assessed 5.8 years later. Significance was attenuated after excluding individuals with dementia and stroke.	Age, sex, ethnicity, education, APOE E4 status, caloric intake, BMI, duration between diet evaluation and MRI, smoking, diabetes, hypertension, and heart disease.
Titova et al. (38); *Prospective Investigation of the Vasculature in Uppsala Seniors Cohort;* Sweden.	5	194	Adults ≥ 70 years who are community-dwelling and mostly clinically and cognitively normal at baseline. 48% female	70.1 ± 0.01	7-day food diary; 1 time point (baseline).	1. Mediterranean diet, Trichopoulou et al. (40)	1.5 T MRI	1 time point; 5 years after baseline.	GMV, WMV, TBV (sum of GMV and WMV).	Adherence to MeDi was not significantly associated with any structural MRI measures (GMV, WMV or TBV) assessed 5 years later.	Age, gender, education, caloric intake, BMI, physical activity, systolic blood pressure, HOMA-IR, LDL cholesterol.

(Continued)

TABLE 1 | (Continued)

Authors, year, study name, country	Study characteristics			Diet measures			Neuroimaging measures	Results			
	Follow-up time (years)	N =	Population characteristics	Mean age at baseline, y	Food intake assessment; time point(s) assessed	DP(s) examined (scoring reference or *a posteriori*)	Specific methods/ techniques used:	Timepoint(s) of neuroimaging assessment(s)	Primary outcome(s) of interest	Main findings from primary outcome(s) of interest	Covariates
Berti et al. (34), USA.	3	70	Participants derived from multiple community sources. Must been aged 30-60 years at enrolment with ≥ 12 years of education. MeDi-group = 54% female MeDi + group = 64% female	MeDi- group = 50 ± 9; MeDi + group = 49 ± 9	Interviewer administered Willett Semi-quantitative FFQ; 1 time point.	1. Mediterranean diet, Trichopoulou et al. (40)	3 T MRI, PiB-PET and FDG-PET	2 time points; baseline and ≥ 2 years follow-up.	GMV, 11C Pittsburgh compound-B (PiB) uptake (a known marker of fibrillary amyloid-β) and 18-F fluorodeoxyglucose (FDG) (a known marker of glucose metabolism)-PET.	Adherence to MeDi was not significantly associated with GMV or change in GMV across 2 years. However lower adherence was significantly associated with increased CMRglc decline and increased Aβ load.	Age, sex, education, APOE E4 status. BMI, insulin resistance, hypertension.
Walters et al. (39), USA.	3	70	Cognitively and clinically normal middle-aged adults, age 30–60 years at baseline.	49 ± 8	Willett Semi-quantitative FFQ; 1 time point.	1. Mediterranean diet, Trichopoulou et al. (40)	3 T MRI, PiB-PET and FDG-PET	2 time points; baseline and ≥ 2 years follow-up.	Entorhinal and posterior cingulate cortices thickness, PiB uptake and FDG of posterior cingulate and frontal cortices.	Adherence to MeDi was not significantly associated with cortices thickness or PiB uptake at baseline or 2 year follow up. Lower adherence to MeDi was significantly associated with increased rate of FDG decline in posterior cingulate cortex but not in frontal cortex.	Age, sex, education, BMI, APOE E4 status, diet, physical activity, hypertension and intellectual scores, QUICKI scores, lab measures.
Luciano et al. (36), *Lothian Birth Cohort,* Scotland, UK.	3	562/401	Adults born in 1936 who are community dwelling and free from dementia at baseline. 47.9% female	72.65 ± 0.72	168-item FFQ; 1 time point (baseline).	1. Mediterranean diet, Trichopoulou et al. (40)	1.5 T MRI.	2 time points; wave 2 (2007–2010) and wave 3 (2011–2014).	GMV, and Total Brain Volume and (representing difference in volume of CSF, venous sinuses and meninges and ICV), cortical thickness.	Adherence to MeDi was not significantly associated with GMV or cortical thickness at baseline or change across 3 years. Increased adherence to MeDi was significantly associated with reduced 3 year change in TBV but not with TBV at baseline or follow-up.	Age, sex, education, BMI, APOE E4 status, diabetes, stroke, blood pressure, cardiovascular disease, cognitive ability and MMSE.

(Continued)

TABLE 1 | (Continued)

Authors, year, study name, country	Study characteristics			Diet measures			Neuroimaging measures	Results			
	Follow-up time (years)	N =	Population characteristics	Mean age at baseline, y	Food intake assessment; time point(s) assessed	DP(s) examined (scoring reference or *a posteriori*)	Specific methods/ techniques used:	Timepoint(s) of neuroimaging assessment(s)	Primary outcome(s) of interest	Main findings from primary outcome(s) of interest	Covariates
Rainey-Smith et al. (30), *Australian Imaging, Biomarkers and Lifestyle Study of Ageing*, Australia.	3	77	Healthy, cognitively normal individuals aged 60 or above residing within Australia (analysis only completed on individuals considered as "Aβ accumulators"). 49% female	71.1 ± 7.1	74-item FFQ; 1 time point (baseline).	1. Mediterranean diet, Trichopoulou et al. (40)	PiB-PET	3 time points; Baseline, 18 month follow-up and 36 month follow-up.	Cerebral Aβ load.	Increased adherence to MeDi was significantly associated with decreased Aβ accumulation over 3 years.	Age, gender, education, caloric intake, BMI, APOE E4 status.
Akbaraly et al. (33), *Whitehall II Imaging Sub-study;* UK.	13	459	Civil servants from London aged 35–55 at baseline of Whitehall II Study. Aged 60–85 at baseline of imaging sub-study. 19.2% female	59.6 ± 5.3	127-item semi-quantitative FFQ; 3 time point(s) across 11 year period.	1. AHEI-2010, Chiuve et al. (41).	3 T MRI	1 time point; 13 years following baseline diet assessment.	Total hippocampal volume (HCV); Left HCV and right HCV.	Better AHEI-2010 adherence was associated with larger total HCV, left HCV and right HCV. Compared to those who maintained a low AHEI-2010 score over 11 year follow up, those maintaining a high score or improving their score to high had larger hippocampal volumes.	Age, sex, caloric intake, ethnicity, occupational position, smoking, physical activity, cardio-metabolic health factors (CHD, dyslipidaemia, type 2 diabetes, BMI and hypertension), cognitive impairment, depressive symptoms.
Jacka et al. (35), *PATH Sub-study,* Australia.	4	255	Oldest cohort of the PATH study (60–64 years old at baseline). 46% female	62.6 ± 1.42	183-item FFQ; 1 time point (baseline).	1. Prudent diet, derived *a posteriori*. 2. Western diet, derived *a posteriori*.	1.5 T MRI.	2 time points; baseline and follow-up at 3–4 years.	Total HCV, left HCV, right HCV and amygdala volumes.	Increased adherence to prudent DP was significantly associated with larger left HCV but not right HCV. Whereas, increased adherence to western DP was significantly associated with smaller left HCV but not right HCV. No significant association was found between either DP and 4 year change HCV.	Age, sex, education, labour-force status, physical activity, smoking, hypertension, diabetes, depressive symptoms, medication, intracranial volume, time between MRI and change in intracranial volume over time.

Of the eight studies investigating *a priori* derived DPs, seven (29, 30, 34, 36–39) examined the MeDi and one (33) examined the Alternate Healthy Eating Index-2010 (AHEI-2010). Within the seven studies investigating the MeDi, all were based on the scoring index from Trichopoulou et al. (40), which is based on population median cut points for nine specific food components. Of which, five measured adherence to the MeDi as a continuous variable (29, 30, 36, 38, 39), one as a categorical variable (34), and one used both (37). The one study (33) which examined the AHEI-2010 used an adaptation of the index from Chiuve et al. (41). Adherence to the AHEI-2010 was measured as a continuous variable in main analyses and as a categorical variable in further analyses.

Only one study used *a posteriori* methodology in the form of principal component analysis (PCA). In contrast to *a priori* methodology, this adopts an exploratory approach and relies on dietary intake information provided to identify correlated items to enable the construction of common consumption patterns (DPs). The study included within this review defined the two DPs identified as prudent and western, which can be dichotomised as healthy and unhealthy, respectively (35). In regards to the management of energy misreporting from FFQs, some studies (30, 36, 38) reported using methods to address this.

Neuroimaging Measures

Magnetic resonance imaging (MRI) was used in eight studies to measure neuroimaging markers. MRI alone was used in five studies (33, 35–38), and in combination with either diffusion tensor imaging (DTI) (29) or positron emission tomography (PET) in three studies (34, 39). Neuroimaging markers were assessed at one time point in four studies (29, 30, 33, 36–38), two time points in four other studies (34–36, 39), and three time points in one study (30).

Results of Main Outcome Analyses

A graphical overview of each of the DPs studied in relation to either their positive or null association to neuroimaging markers is provided within **Table 2**.

Mediterranean Diet and Structural Neuroimaging Markers

Six studies assessed the relationship between MeDi adherence and structural neuroimaging markers. These include grey matter volume (GMV), total brain volume (TBV), cortical thickness, white matter volume (WMV), white matter integrity (WMI), white matter hyperintensities (WMH) and cerebral infarcts.

Grey Matter Volumes

In total, four studies (29, 34, 36, 38) analysed the association between GMV and adherence to the MeDi. Two studies found no significant association between adherence to MeDi and GMV measured between 5 and 8.9 years later (29, 38). Two other studies (34, 36) included repeated measures of GMV. In the Lothian birth cohort, no significant association was found between adherence to the MeDi and GMV at baseline ($\beta = 0.182$; $SE = 0.553$; $P = 0.742$), follow-up ($\beta = 0.864$; $SE = 0.659$; $P = 0.191$), or change in GMV longitudinally across 3 years in older cognitively healthy adults ($\beta = 0.451$; $SE = 0.383$; $P = 0.240$) (36). Similarly, Berti and colleagues also found no significant differences between the high and low MeDi adherence and change in GMV over 3 years in middle-aged adults from the USA (34).

Total Brain Volumes

Two studies (36, 38) assessed TBV. One study (38) found MeDi adherence was not significantly associated with TBV measured at one time point 5 years following baseline. The other study (36) found no associations between MeDi adherence and TBV at either baseline or follow-up among older cognitively healthy adults, but lower MeDi adherence was significantly associated with greater reductions in TBV across 3 years ($\beta = 0.976$; $SE = 0.483$; $P = 0.044$) (36).

Cortical Thickness

Two prospective studies examined cortical thickness in relation to MeDi adherence (36, 39). In the Lothian birth cohort, MeDi adherence was not related to 3-year change in cortical thickness between 73 and 76 years ($\beta = 0.004$; $SE = 0.003$; $P = 0.198$) (36). Likewise, Walters and colleagues also found no significant association between adherence to the MeDi and thickness of the frontal cortex or the posterior cingulate cortex at baseline or follow-up (39). Nor was there a significant association between 2-year change in frontal or posterior cingulate cortical thickness and MeDi adherence (39).

White Matter Volumes

Two studies determined WMV in relation to MeDi adherence (29, 38). Neither found an association between MeDi adherence and WMV measured at an average of 5 or 8.9 years later (29, 38).

White Matter Integrity

Only one study (29) assessed WMI. The study found a significant association between increased adherence to the MeDi and reduced diffusivity values within specific areas of the white matter skeleton (the whole corpus callosum, anterior and posterior thalamic radiations, para cingulate gyrus cingulum, and parahippocampal fornix) among older French adults ($\geq$65 years). Better adherence to the MeDi was also associated with increased fractional anisotropy values in specific regions of the white matter skeleton (corpus callosum, anterior and posterior thalamic radiations) (29). Authors consequently explored further the associations between diffusivity and fractional anisotropy values and cognitive tests. As a result, significant associations were found between higher global cognitive scores and the DTI parameters significantly associated with the MeDi (Multivariable adjusted mean difference when comparing Q1 to Q5 of mean diffusivity = 0.35; 95% CI: 0.06, 0.65; P for trend = 0.002). Higher global cognitive scores were also significantly associated with the DTI parameters significantly associated with the MeDi when comparing individuals in quartile one to quartile five of fractional anisotropy values (Multivariable adjusted mean difference = 0.48; 95% CI: 0.19, 0.77; P for trend = 0.001) (29).

White Matter Hyperintensities and Cerebral Infarcts

One study examined the presence of WMH and cerebral infarcts (37). While MeDi was not related to the presence of WMH,

TABLE 2 | Graphical overview of each of the DPs studied in relation to their association to neuroimaging markers.

Structural neuroimaging markers assessed in relation to MeDi	
Grey matter volume (GMV)	(29) (38) (34) (36)
White matter volume (WMV)	(29) (38)
Total brain volume (TBV)	(38) (36)
Cortical thickness	(39) (36)
White matter hyperintensities (WMH)	(37)
Cerebral infarcts	(37)
White matter integrity (via DTI)	(29)
Functional neuroimaging markers assessed in relation to MeDi	
Glucose metabolism (via FDG-PET)	(34) (39)
Aβ load (via PiB-PET)	(34) (39) (30)
Structural neuroimaging markers assessed in relation to other DPs	
Hippocampal volume	(33) (35)
Colour codes:	
Positive association with neuroimaging markers measured at one time point.	Positive association with neuroimaging markers measured at two or more time points.
No association with neuroimaging markers measured at one time point.	No association with neuroimaging markers measured at two or more time points.

greater MeDi adherence was significantly associated with lower odds of cerebral MRI infarcts 5.8 years later (*OR* = 0.89; 95% CI = 0.80, 0.99; *P* = 0.04). This association was attenuated however, after exclusion of individuals with stroke (*n* = 86) and dementia (*n* = 46) (*OR* = 0.90; 95% CI = 0.80, 1.00; *P* = 0.07) (37).

Other Dietary Patterns and Structural Neuroimaging Markers

One study examined diet using the AHEI-2010 (33) and the other, PCA-derived prudent and western DPs (20) in relation to structural neuroimaging markers. Adherence to the AHEI-2010 was associated with increased total hippocampal volumes (HCV) measured 13 years later [β per each 1 SD increase in AHEI-2010 score (1 SD = 8.7 points) = 0.11; 95% CI: 0.02, 0.21]. Each 1 SD increase in AHEI-2010 score was associated with an increase of 92.5 mm^3 (*SE* = 42 mm^3) in total HCV, and when assessing each; an increase of 56.3 mm^3 (*SE* = 23 mm^3) in left HCV, and 36.2 mm^3 (*SE* = 22.7 mm^3) increase in right HCV (33). Similarly, better adherence to a prudent DP was significantly associated with larger left HCV, equal to 45.7 mm^3 (S.E = 22.9 mm^3). In contrast, a western DP was significantly associated with smaller left HCV, equal to 52.6 mm^3 (S.E = 26.6 mm^3). No associations were found between either *a posteriori* DP or right HCV (35). Change in left or right HCV across 4 years was also not significantly associated with the prudent DP ($\beta_{prudent \times time}$ = 20.8; *SE* = 24.4; *P* = 0.40) or western DP ($\beta_{western \times time}$ = 27.2; *SE* = 28.8; *P* = 0.34) (35).

Mediterranean Diet and Functional Neuroimaging Markers

Three studies assessed MeDi adherence in relation to functional neuroimaging markers, including brain glucose metabolism and Aβ load (30, 34, 39).

Brain Glucose Metabolism

Two studies used (18)F-fluorodeoxyglucose (FDG)-PET to assess glucose metabolism among middle-aged USA adults (34, 39). Low MeDi adherence was associated with significant reductions in the cerebral metabolic rate of glucose (CMRglc) specifically within the bilateral temporal cortex (*P* = 0.001) (34). Furthermore, the rate of CMRglc decline within both the temporal and posterior cingulate cortices among the group with low MeDi adherence was significantly faster than that of the high MeDi group (P interaction = 0.002). This equated to a decline of 3.83% (low MeDi group) (0.028 ± 0.049 units/year) compared to <1% (high MeDi group) (0.018 ± 0.054 SUVR units/year) per year from baseline (34). Similarly, Walters and colleagues also reported that lower adherence to the MeDi was significantly associated with a higher rate of FDG decline within the posterior cingulate cortex (β = 0.010; *SE* = 0.005; *P* = 0.043) but not in the frontal cortex (β = -0.012; *SE* = 0.007; *P* = 0.072) (39).

Amyloid Load

Three studies examined repeated measures of cerebral Aβ deposition up to 3 years (30, 34, 39). Two of the three studies found that decreased adherence to the MeDi was significantly associated with increased Aβ load among both middle-aged and older adults (30, 34). Lower MeDi adherence was associated with both higher Pittsburgh compound B (PiB) uptake at baseline and increased rate of PiB uptake across 3 years (*P* = 0.001) in middle-aged adults (34). Change in the low MeDi group was on average, 0.028 ± 0.031 SUVR units/year, equal to a 3% increase per year, compared to higher MeDi adherence which was on average, 0.009 ± 0.020 SUVR units/year, equal to a <1% increase per year (34). The Aβ accumulation rate within the AIBL cohort of older adults was higher than that of the cohort within the study by Berti and colleagues, at a rate of 0.050 SUVR units/year, meaning each 1-point increase in MeDi score was equal to 20% decrease in Aβ load per year (β = -0.01; S.E = 0.004; *P* = 0.0070)

(30). However, it should be noted this cohort consisted entirely of individuals defined as Aβ accumulators by authors. In contrast, Walters and colleagues found no significant association between MeDi adherence and changes in Aβ in the frontal cortex among middle-aged adults (β = -0.016; *SE* = 0.011; *P* = 0.104) (39).

Risk of Bias Assessment

An overview of the risk of bias assessment of all included studies is presented in **Table 3**. All studies included in the review were rated as medium (29, 30, 33, 35–39). Only one study achieved a high rating with a maximum total score of nine (34). The results of the quality assessment can be found in **Table 3**. Most studies were lacking within the selection domain, more specifically regarding ascertainment of exposure (due to most using self-reported assessment methods) and demonstration that the outcome was not present at baseline.

DISCUSSION

Summary of Overall Findings

This systematic review evaluates results from nine prospective studies including approximately 2,540 individuals. No intervention evidence was evaluated, as no RCTs were eligible for inclusion. Consequently, it is difficult to understand the application of neuroimaging markers within future dietary interventions, as we are reliant on evidence gathered from prospective observational studies Within included studies, the MeDi was examined as the DP exposure in the majority (n = 7), with only two studies examining other DPs. Findings for associations between the MeDi and structural neuroimaging markers were mixed from the small number of studies available. MeDi was consistently not associated with a change in volumes of key brain regions such as GMV, WMV, or cortical thickness but was associated with reduced risk of cerebral infarcts and lower total brain atrophy. One study also found evidence of a protective association between MeDi and WMI (29). Other DPs (AHEI-2010 and PCA-derived DPs) were linked to higher HCV (33, 35) which is particularly relevant for AD, given that hippocampal atrophy is a major AD characteristic that often predates clinical diagnosis (42–44). HCV is considered one of the earliest structural MRI markers for AD, with studies suggesting atrophy rates may deviate from normal ageing as far as 3–5.5 years prior to diagnosis (45, 46). The MeDi appeared to be more consistently protective against hypometabolism (34, 39) and Aβ load (30, 34) from mid-life to older age, which preceded any evidence of cognitive impairment. Such results are important, given that impairments in systemic metabolism (such as hypometabolism) and Aβ accumulation are pathophysiologic hallmarks of AD (47–49). However, current data is unable to discern the exact mechanisms behind these associations; specifically, whether a healthy DP helps by inhibiting Aβ accumulation/deposition, by improving Aβ clearance, or through both (30). Data in this area are currently limited, with few prospective studies examining Aβ load in relation to DPs among humans (30, 34, 39) consequently warranting further investigation.

TABLE 3 | Results of quality assessment of included studies using Newcastle-Ottawa Scale.

Authors, year	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at baseline	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur?	Adequacy of follow-up of cohorts	Overall rating
Akbaraly et al. (33)	★	★			★★	★	★	★	Medium
Berti et al. (34)	★	★	★	★	★★	★	★	★	High
Jacka et al. (35)	★	★		★	★★	★	★	★	Medium
Luciano et al. (36)	★	★			★★	★	★	★	Medium
Pelletier et al. (29)		★	★		★★	★	★	★	Medium
Rainey-Smith et al. (30)		★		★	★★	★	★	★	Medium
Scarmeas et al. (37)	★	★	★		★★	★	★	★	Medium
Walters et al. (39)	★	★		★	★★	★	★	★	Medium
Titova et al. (38)	★	★			★★	★	★	★	Medium

Overall, this systematic review of available prospective data suggests a protective association between healthy DPs and neuroimaging markers and confirms findings from a prior review involving prospective studies to 2017 (28) and other reviews involving both prospective and cross-sectional study evidence (25–27). The exact mechanisms responsible for how healthy DPs may be neuroprotective are not known but high-quality DPs such as the MeDi could improve vasculature functioning by reducing oxidative stress and inflammation. Better adherence to a healthy diet such as the MeDi, may serve to decrease the likelihood of vascular comorbidities including hypertension, metabolic syndrome, dyslipidaemia, and cardiopathy (50). It is proposed this subsequently promotes healthy brain ageing by reducing an individuals' risk of vascular-related brain pathologies. The main drivers of vascular preservation likely involve enhanced endothelial capacity, healthy cerebral blood flow and reduced inflammation and oxidative stress. The neuroimaging markers commonly associated with vascular pathologies relate to the potential progression of small vessel disease, such as the volume and enlargement of WMH, lesions, infarcts, and preceding this, loss of WMI (51). Previous cross-sectional studies have reported associations between greater MeDi adherence and reduced volume of WMH (52). A main predictor of WMH volume within this relationship, was suggested to be the ratio of monounsaturated fats to saturated fats within the MeDi (52). Greater fish consumption, as recommended by the MeDi, has also been independently related to reduced WMH volume and increased brain volumes (53–56). The vascular pathway is supported by findings of this review, as two studies found associations greater adherence to the MeDi and reduced appearance of vascular-related neuroimaging markers (specifically reduced odds of cerebral infarcts, and improved white matter tract integrity) (34, 37).

Loss of white matter microstructural integrity is also proposed to reflect both myelin breakdown and axonal damage (57, 58). The neurodegeneration hypothesis proposes pathological brain changes such as structural atrophy, formation, deposition of Aβ plaques and oxidative stress occur due to a combination of events. This includes axonal cytoskeleton degeneration, myelin degradation and an accumulation of reactive oxygen species. Findings from six studies included within this review lend support to a healthy DP for supporting axonal maintenance and protecting against neurodegeneration. For example, through reduced hippocampal and total brain atrophy over time (33, 35, 36), decreased Aβ deposition (30, 34) and slower declines in glucose metabolism within specific regions (34, 39). Data from several cross-sectional studies have also shown protective associations of healthy DPs on neuroimaging outcomes (22, 53, 59–62).

Healthy DPs such as the MeDi, AHEI-2010 and prudent DP derived a posteriori share similarities including high consumption of foods rich in polyphenols and antioxidants such as fruits/vegetables. Such compounds may exert protective effects by obstructing neuronal oxidation, maintaining cellular homeostasis and hence, limit abnormal intracellular responses such as senescence and alterations within brain plasticity (63–66). Furthermore, healthy DPs such as the MeDi, DASH and AHEI-2010 are suggested to reduce levels of inflammatory macrophage proteins, cytokines, and chemokines (67–70). Neuroinflammation plays a major role in both oxidative and neurodegenerative pathways, thus review outcomes support the role of healthy DPs such as the MeDi, AHEI-2010 and prudent DPs in decreasing neuroinflammation. In support of this, one study also found a western DP, often associated with systemic inflammation, was associated with decreased HCV (35).

Inconsistencies Within Study Methodology

This review also serves to highlight several inconsistencies within the designs of included studies when investigating associations between DPs and neuroimaging markers. This includes robustness of study design, potential biases within populations assessed, and measurement of neuroimaging markers, dietary intake and consequent DPs as discussed below.

Study Design

All studies included in this review were prospective cohort studies. Most studies were rated as medium quality, with the exception of one rated as high. None of the included studies were assessed as poor quality. Although the design of such prospective studies has several strengths, they cannot determine causality and may be biased toward residual confounding. All studies controlled for age and sex. Most studies controlled for important confounders that influence both diet and neurocognition, as eight (29, 30, 33–39) controlled for education, six (29, 30, 34, 36, 37, 39) controlled for APOE E4 status, and eight (29, 33–39) controlled for $\geq$1 measure of cardiovascular risk (i.e., diabetes, hypertension). However, only two studies controlled for socioeconomic status (SES) (33, 35). This is interesting, given the association between SES and cognition across the lifespan (71), in addition to the link between SES and diet (72). Although both studies which controlled for SES in this review found statistically significant findings to suggest an influence of DP on neuroimaging measures, future studies may wish to consider controlling for this factor within analyses. Finally, no included studies were RCTs. Subsequently, this means it is difficult to draw causal inferences from this review.

Populations Assessed

No study focused exclusively on an age category and therefore each included older adult populations (aged $\geq$ 60). Due to this, combined with conflicting findings overall it is difficult to draw conclusions as to whether associations may differ dependent upon the life stage. However, from the two studies that did include younger adult individuals (age $\geq$ 30), both found significant associations between MeDi and structural (specifically WMI) and functional neuroimaging markers (specifically Aβ and glucose metabolism) (34, 39). It has been suggested that the earliest pathological changes may be detected using neuroimaging markers between 15 and 30 years preceding symptom onset (42, 73, 74). As most studies captured older-aged individuals, it is difficult to understand how long an individual must have adhered to a DP, to influence changes in brain

ageing. The majority of studies were conducted among educated populations and only four studies reported ethnic status (mostly white populations except for WHICAP). Consequently, more studies are required in diverse populations, particularly those at higher risk of AD in later life.

Dietary Assessment

The limitations associated with methods of dietary intake measurement, specifically FFQs as the most frequently used method of assessment have previously been acknowledged (75, 76). This includes potential misreporting from the participant due to both recall bias and social desirability bias; as "healthy" foods are likely to be reported more frequently in comparison to "unhealthy" foods. Based on this, studies using FFQs to measure diet intake should aim to validate the obtained data to ensure a robust assessment of dietary intake using an alternative methodology such as combined measurement of various recovery-based nutrient biomarkers (e.g., doubly labelled water for energy intake or urinary nitrogen for protein intake) or another quantitative method as reference, such as food records or 24 h diet recalls (77, 78).

To validate subjective methods should be considered within studies to ensure a robust assessment of dietary intake. Furthermore, most studies within this review also failed to assess diet at multiple time points and relied on FFQ reporting from years prior to neuroimaging marker measurement. Older adulthood has previously been suggested to be a period where individuals may experience reduced diet stability (79–81). Therefore, it is difficult to rule out the risk of reverse causation within associations observed. Future studies should be conducted in earlier life, to understand how DPs may influence changes in neuroimaging markers across the adult life span.

Dietary Patterns Assessed

Most prospective studies to date have examined associations between the MeDi and neuroimaging markers, which can help elucidate potential neuroprotective mechanisms. However, differences were noted between the applications of the MeDi scoring system within the included studies. Although all studies applied a similar scoring index, there were interindividual study differences in food components, specific cut-offs for components (i.e., alcohol), and adjustments for energy intake. For example, Titova et al. (38) combined vegetables and legumes into one component, added potatoes to the cereals component, and replaced monounsaturated fats with polyunsaturated fats in the fats ratio component. Differences were also noted between studies in the scoring cut-offs for the alcohol component. Adaptations such as the above may contribute further towards inconsistent findings within this area; as it becomes difficult to understand if observed associations are due to the synergy within the MeDi pattern or due to its individual food components, which often differ between studies. Similarly, the AHEI-2010 score applied in one study within this review (33) was also adapted from the original score index by decreasing the recommended intake of alcohol, based on the evidence within the research topic. Furthermore, as previous studies focus on healthy DPs, there is a substantial lack of studies exploring the effects of unhealthy DPs on neuroimaging markers. Previous animal studies have illustrated that a western diet is associated with reduced cognitive functioning, due to reduced neuronal plasticity and higher levels of inflammation and oxidative stress (82, 83). This review found only one longitudinal study that investigated an unhealthy DP in relation to neuroimaging markers in humans. Further research should therefore address this gap and aim to understand the influence and mechanisms of poor-quality DPs on brain ageing, using neuroimaging markers. This would increase understanding of their effects on wider populations, such as those across westernised countries who often consume such diets.

Neuroimaging Marker Measurement

Only five studies assessed change in neuroimaging markers at two or more time points. The maximum length of time in any study between these two points was 4 years. Therefore, it is difficult to interpret to distinguish between normal vs. pathological brain changes and how this may associate with any given DP. The main modality of neuroimaging assessment across studies was via MRI (8 studies), with only three studies using other methodologies (i.e., PET and DTI) (30, 34, 39). However, it has been suggested that in pathological brain ageing, changes in Aβ deposition may occur first, followed by neurodegenerative changes such as Tau burden and structural brain atrophy (42, 84). Hence, this may help to explain why four (29, 30, 34, 39) of the eight studies in cognitively healthy adults found significant associations between adherence towards a DP and neuroimaging markers other than structural MRI. Another noteworthy finding from this review was the substantial lack of studies that assessed important sub-cortical brain regions such as the hippocampus that are vulnerable to AD. Previous animal studies have also suggested diet may impact hippocampal neurogenesis, by demonstrating a damaging role of saturated fats and refined sugar on hippocampal neurogenesis (85, 86). Interestingly, only two studies (33, 35) in this review investigated hippocampal volume. It would therefore be beneficial for future studies to adopt a multi-modal imaging approach; as this would enable us to understand the earliest and optimal window for nutritional interventions to support healthy brain ageing. The use of both modalities in combination with neuropsychological testing would also provide a comprehensive overview of structural and functional brain changes; how these correlate with cognitive performance and potential associations between DPs and the food components within that DP. Finally, it must be noted that this systematic review may be prone to bias as articles published in languages other than English were excluded. As a meta-analysis was not possible, it is also difficult to determine the extent of publication bias within the studies included.

CONCLUSION

This systematic review of prospective data suggests a protective association between healthy DPs and neuroimaging markers and agrees with prior reviews involving cross-sectional studies

(25–27). Evidence, while limited, suggests healthy DP's, such as the MeDi or AHEI-2010 may exert beneficial changes on both brain structure (reduced brain atrophy) and function (maintenance of glucose metabolism and reduced Aβ accumulation). Although the mechanisms underpinning these associations require further elucidation, both vascular and neurodegenerative processes are likely involved. This review highlighted the value of different neuroimaging techniques (i.e., MRI and PET) and markers in detecting and capturing subtle changes within the brain, which often occur prior to cognitive impairment. No RCTs were available for inclusion in this review and highlights a clear gap within the research field. Future intervention studies in cognitively healthy adults that include multi-modal neuroimaging measures would advance current knowledge on whether adopting a healthy diet impacts brain health. Undertaking such studies may be especially relevant and beneficial in reducing the likelihood of dementia among high-risk population groups, such as those with diabetes. Furthermore, future studies should be designed to adopt a life-span approach, conducting rigorous follow-up reviews with multi-modal neuroimaging techniques and validated dietary intake assessment at regular time points. This will enable a comprehensive depiction of how the length of adherence towards a specific DP may alter an individuals' brain ageing trajectory. Finally, although healthy DPs may offer neuroprotective benefits, the exact combination of foods and nutrients providing such benefits remain unknown. Consequently, future studies should also seek to address the paucity of research investigating DPs other than the MeDi.

AUTHOR CONTRIBUTIONS

RT, CM, and JW conceived and designed the study. RT conducted the search. RT and CM completed screening and extraction processes with any disagreements discussed in the presence of JW, RT, and RO'N and performed quality assessment. RT, CM, JW, RO'N, and FP contributed to drafting and completing the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.806006/full#supplementary-material

REFERENCES

1. Prince MJ, Wimo A, Guerchet MM, Ali GC, Wu Y-T, Prina M. *World Alzheimer Report 2015-The Global Impact of Dementia: An Analysis of Prevalence, Incidence, Cost and Trends*. London: Alzheimer's Disease International (2015).
2. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet.* (2020) 396:413–46. doi: 10.1016/S0140-6736(20)30367-6
3. Munoz-Garcia MI, Toledo E, Razquin C, Dominguez LJ, Maragarone D, Martinez-Gonzalez J, et al. "A priori" dietary patterns and cognitive function in the SUN project. *Neuroepidemiology.* (2020) 54:45–57. doi: 10.1159/000502608
4. Shakersain B, Santoni G, Larsson SC, Faxén-Irving G, Fastbom J, Fratiglioni L, et al. Prudent diet may attenuate the adverse effects of western diet on cognitive decline. *Alzheimers Dement.* (2016) 12:100–9. doi: 10.1016/j.jalz.2015.08.002
5. Tanaka T, Talegawkar SA, Jin Y, Colpo M, Ferrucci L, Bandinelli S. Adherence to a Mediterranean diet protects from cognitive decline in the invecchiare in Chianti study of aging. *Nutrients.* (2018) 10:2007. doi: 10.3390/nu10122007
6. Trichopoulou A, Kyrozis A, Rossi M, Katsoulis M, Trichopoulos D, La Vecchia C, et al. Mediterranean diet and cognitive decline over time in an elderly Mediterranean population. *Eur J Nutr.* (2015) 54:1311–21. doi: 10.1007/s00394-014-0811-z
7. Wengreen H, Munger RG, Cutler A, Quach A, Bowles A, Corcoran C, et al. Prospective study of dietary approaches to stop hypertension–and Mediterranean-style dietary patterns and age-related cognitive change: the Cache County study on memory, health and aging. *Am J Clin Nutr.* (2013) 98:1263–71. doi: 10.3945/ajcn.112.051276
8. Barberger-Gateau P, Raffaitin C, Letenneur L, Berr C, Tzourio C, Dartigues J-F, et al. Dietary patterns and risk of dementia: the three-city cohort study. *Neurology.* (2007) 69:1921–30. doi: 10.1212/01.wnl.0000278116.37320.52
9. Hosking DE, Eramudugolla R, Cherbuin N, Anstey KJ. MIND not Mediterranean diet related to 12-year incidence of cognitive impairment in an Australian longitudinal cohort study. *Alzheimers Dement.* (2019) 15:581–9. doi: 10.1016/j.jalz.2018.12.011
10. Morris MC, Tangney CC, Wang Y, Sacks FM, Bennett DA, Aggarwal NT. MIND diet associated with reduced incidence of Alzheimer's disease. *Alzheimers Dement.* (2015) 11:1007–14. doi: 10.1016/j.jalz.2014.11.009
11. Scarmeas N, Stern Y, Mayeux R, Manly JJ, Schupf N, Luchsinger JA. Mediterranean diet and mild cognitive impairment. *Arch Neurol.* (2009) 66:216–25.
12. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. Mediterranean diet and risk for Alzheimer's disease. *Ann Neurol.* (2006) 59:912–21.
13. Martínez-Lapiscina EH, Clavero P, Toledo E, Estruch R, Salas-Salvadó J, San Julián B, et al. Mediterranean diet improves cognition: the PREDIMED-NAVARRA randomised trial. *J Neurol Neurosurg Psychiatry.* (2013) 84:1318–25. doi: 10.1136/jnnp-2012-304792
14. Valls-Pedret C, Sala-Vila A, Serra-Mir M, Corella D, De la Torre R, Martínez-González MÁ, et al. Mediterranean diet and age-related cognitive decline: a randomized clinical trial. *JAMA Intern Med.* (2015) 175:1094–103. doi: 10.1001/jamainternmed.2015.1668
15. Martinez-Lapiscina EH, Clavero P, Toledo E, San Julian B, Sanchez-Tainta A, Corella D, et al. Virgin olive oil supplementation and long-term cognition: the PREDIMED-NAVARRA randomized, trial. *J Nutr Health Aging.* (2013) 17:544–52. doi: 10.1007/s12603-013-0027-6
16. Wade AT, Davis CR, Dyer KA, Hodgson JM, Woodman RJ, Keage HA, et al. A Mediterranean diet supplemented with dairy foods improves mood and processing speed in an Australian sample: results from the MedDairy randomized controlled trial. *Nutr Neurosci.* (2020) 23:646–58. doi: 10.1080/1028415X.2018.1543148
17. Chlebowski RT, Rapp S, Aragaki AK, Pan K, Neuhouser ML, Snetselaar LG, et al. Low-fat dietary pattern and global cognitive function: exploratory

analyses of the women's health initiative (WHI) randomized dietary modification trial. *EClinicalMedicine.* (2020) 18:100240. doi: 10.1016/j.eclinm.2019.100240
18. Kwok T, Lam L, Sea M, Goggins W, Woo J. A randomized controlled trial of dietetic interventions to prevent cognitive decline in old age hostel residents. *Eur J Clin Nutr.* (2012) 66:1135–40. doi: 10.1038/ejcn.2012.117
19. Marseglia A, Xu W, Fratiglioni L, Fabbri C, Berendsen AA, Bialecka-Debek A, et al. Effect of the NU-AGE diet on cognitive functioning in older adults: a randomized controlled trial. *Front Physiol.* (2018) 9:349. doi: 10.3389/fphys.2018.00349
20. Wade AT, Davis CR, Dyer KA, Hodgson JM, Woodman RJ, Keage HA, et al. A Mediterranean diet with fresh, lean pork improves processing speed and mood: cognitive findings from the MedPork randomised controlled trial. *Nutrients.* (2019) 11:1521. doi: 10.3390/nu11071521
21. Jack CR Jr, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol.* (2010) 9:119–28. doi: 10.1016/s1474-4422(09)70299-6
22. Gu Y, Brickman AM, Stern Y, Habeck CG, Razlighi QR, Luchsinger JA, et al. Mediterranean diet and brain structure in a multiethnic elderly cohort. *Neurology.* (2015) 85:1744–51. doi: 10.1212/WNL.0000000000002121
23. Mosconi L, Murray J, Tsui WH, Li Y, Davies M, Williams S, et al. Mediterranean diet and magnetic resonance imaging-assessed brain atrophy in cognitively normal individuals at risk for Alzheimer's disease. *J Prev Alzheimers Dis.* (2014) 1:23–32.
24. Vassilaki M, Aakre JA, Syrjanen JA, Mielke MM, Geda YE, Kremers WK, et al. Mediterranean diet, its components, and amyloid imaging biomarkers. *J Alzheimers Dis.* (2018) 64:281–90. doi: 10.3233/JAD-171121
25. Rodrigues B, Asamane EA, Magalhães R, Sousa N, Thompson JL, Santos NC. The association of dietary patterns with cognition through the lens of neuroimaging-a systematic review. *Ageing Res Rev.* (2020) 63:101145. doi: 10.1016/j.arr.2020.101145
26. Gauci S, Young LM, Arnoldy L, Lassemillante A-C, Scholey A, Pipingas A. Dietary patterns in middle age: effects on concurrent neurocognition and risk of age-related cognitive decline. *Nutr Rev.* (2021) 0:1–31. doi: 10.1093/nutrit/nuab047
27. Jensen DEA, Leoni V, Klein-Flügge MC, Ebmeier KP, Suri S. Associations of dietary markers with brain volume and connectivity: a systematic review of MRI studies. *Ageing Res Rev.* (2021) 70:101360. doi: 10.1016/j.arr.2021.101360
28. Chen X, Maguire B, Brodaty H, O'Leary F. Dietary patterns and cognitive health in older adults: a systematic review. *J Alzheimers Dis.* (2019) 67:583–619.
29. Pelletier A, Barul C, Féart C, Helmer C, Bernard C, Periot O, et al. Mediterranean diet and preserved brain structural connectivity in older subjects. *Alzheimers Dement.* (2015) 11:1023–31. doi: 10.1016/j.jalz.2015.06.1888
30. Rainey-Smith SR, Gu Y, Gardener SL, Doecke JD, Villemagne VL, Brown BM, et al. Mediterranean diet adherence and rate of cerebral Aβ-amyloid accumulation: data from the Australian imaging, biomarkers and lifestyle study of ageing. *Transl Psychiatry.* (2018) 8:1–7. doi: 10.1038/s41398-018-0293-5
31. van den Brink AC, Brouwer-Brolsma EM, Berendsen AA, van de Rest O. The Mediterranean, dietary approaches to stop hypertension (DASH), and Mediterranean-DASH intervention for neurodegenerative delay (MIND) diets are associated with less cognitive decline and a lower risk of Alzheimer's disease—a review. *Adv Nutr.* (2019) 10:1040–65. doi: 10.1093/advances/nmz054
32. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses*. Oxford: Oxford University Press (2000).
33. Akbaraly T, Sexton C, Zsoldos E, Mahmood A, Filippini N, Kerleau C, et al. Association of long-term diet quality with hippocampal volume: longitudinal cohort study. *Am J Med.* (2018) 131:1372–81.e4. doi: 10.1016/j.amjmed.2018.07.001
34. Berti V, Walters M, Sterling J, Quinn CG, Logue M, Andrews R, et al. Mediterranean diet and 3-year Alzheimer brain biomarker changes in middle-aged adults. *Neurology.* (2018) 90:e1789–98. doi: 10.1212/WNL.0000000000005527
35. Jacka FN, Cherbuin N, Anstey KJ, Sachdev P, Butterworth P. Western diet is associated with a smaller hippocampus: a longitudinal investigation. *BMC Med.* (2015) 13:215. doi: 10.1186/s12916-015-0461-x
36. Luciano M, Corley J, Cox SR, Hernández MCV, Craig LC, Dickie DA, et al. Mediterranean-type diet and brain structural change from 73 to 76 years in a Scottish cohort. *Neurology.* (2017) 88:449–55. doi: 10.1212/WNL.0000000000003559
37. Scarmeas N, Luchsinger JA, Stern Y, Gu Y, He J, DeCarli C, et al. Mediterranean diet and magnetic resonance imaging–assessed cerebrovascular disease. *Ann Neurol.* (2011) 69:257–68.
38. Titova OE, Ax E, Brooks SJ, Sjögren P, Cederholm T, Kilander L, et al. Mediterranean diet habits in older individuals: associations with cognitive functioning and brain volumes. *Exp Gerontol.* (2013) 48:1443–8. doi: 10.1016/j.exger.2013.10.002
39. Walters MJ, Sterling J, Quinn C, Ganzer C, Osorio RS, Andrews RD, et al. Associations of lifestyle and vascular risk factors with Alzheimer's brain biomarker changes during middle age: a 3-year longitudinal study in the broader New York City area. *BMJ Open.* (2018) 8:e023664. doi: 10.1136/bmjopen-2018-023664
40. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med.* (2003) 348:2599–608. doi: 10.1056/NEJMoa025039
41. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, et al. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr.* (2012) 142:1009–18. doi: 10.3945/jn.111.157222
42. Jack CR Jr, Knopman DS, Jagust WJ, Petersen RC, Weiner MW, Aisen PS, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* (2013) 12:207–16. doi: 10.1016/S1474-4422(12)70291-0
43. Barnes J, Bartlett JW, van de Pol LA, Loy CT, Scahill RI, Frost C, et al. A meta-analysis of hippocampal atrophy rates in Alzheimer's disease. *Neurobiol Aging.* (2009) 30:1711–23. doi: 10.1016/j.neurobiolaging.2008.01.010
44. Jack CR Jr, Shiung MM, Gunter JL, O'Brien PC, Weigand SD, Knopman DS, et al. Comparison of different MRI brain atrophy rate measures with clinical disease progression in AD. *Neurology.* (2004) 62:591–600. doi: 10.1212/01.wnl.0000110315.26026.ef
45. Ridha BH, Barnes J, Bartlett JW, Godbolt A, Pepple T, Rossor MN, et al. Tracking atrophy progression in familial Alzheimer's disease: a serial MRI study. *Lancet Neurol.* (2006) 5:828–34. doi: 10.1016/S1474-4422(06)70550-6
46. Schott JM, Fox NC, Frost C, Scahill RI, Janssen JC, Chan D, et al. Assessing the onset of structural change in familial Alzheimer's disease. *Ann Neurol.* (2003) 53:181–8. doi: 10.1002/ana.10424
47. Edison P, Archer H, Hinz R, Hammers A, Pavese N, Tai Y, et al. Amyloid, hypometabolism, and cognition in Alzheimer disease: an [11C] PIB and [18F] FDG PET study. *Neurology.* (2007) 68:501–8. doi: 10.1212/01.wnl.0000244749.20056.d4
48. Landau SM, Mintun MA, Joshi AD, Koeppe RA, Petersen RC, Aisen PS, et al. Amyloid deposition, hypometabolism, and longitudinal cognitive decline. *Ann Neurol.* (2012) 72:578–86. doi: 10.1002/ana.23650
49. Lowe VJ, Weigand SD, Senjem ML, Vemuri P, Jordan L, Kantarci K, et al. Association of hypometabolism and amyloid levels in aging, normal subjects. *Neurology.* (2014) 82:1959–67. doi: 10.1212/WNL.0000000000000467
50. Siervo M, Shannon OM, Llewellyn DJ, Stephan BC, Fontana L. Mediterranean diet and cognitive function: from methodology to mechanisms of action. *Free Radic Biol Med.* (2021) 176:105–17. doi: 10.1016/j.freeradbiomed.2021.09.018
51. Bennett IJ, Madden DJ, Vaidya CJ, Howard DV, Howard JH Jr. Age−related differences in multiple measures of white matter integrity: a diffusion tensor imaging study of healthy aging. *Hum Brain Mapp.* (2010) 31:378–90. doi: 10.1002/hbm.20872
52. Gardener H, Scarmeas N, Gu Y, Boden-Albala B, Elkind MS, Sacco RL, et al. Mediterranean diet and white matter hyperintensity volume in the Northern Manhattan Study. *Arch Neurol.* (2012) 69:251–6. doi: 10.1001/archneurol.2011.548
53. Staubo SC, Aakre JA, Vemuri P, Syrjanen JA, Mielke MM, Geda YE, et al. Mediterranean diet, micronutrients and macronutrients, and MRI measures of cortical thickness. *Alzheimers Dement.* (2017) 13:168–77. doi: 10.1016/j.jalz.2016.06.2359

54. Raji CA, Erickson KI, Lopez OL, Kuller LH, Gach HM, Thompson PM, et al. Regular fish consumption and age-related brain gray matter loss. *Am J Prev Med.* (2014) 47:444–51. doi: 10.1016/j.amepre.2014.05.037
55. Bowman GL, Silbert L, Howieson D, Dodge H, Traber M, Frei B, et al. Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. *Neurology.* (2012) 78:241–9.
56. Virtanen JK, Siscovick DS, Longstreth WT, Kuller LH, Mozaffarian D. Fish consumption and risk of subclinical brain abnormalities on MRI in older adults. *Neurology.* (2008) 71:439–46. doi: 10.1212/01.wnl.0000324414.12665.b0
57. Bartzokis G. Age-related myelin breakdown: a developmental model of cognitive decline and Alzheimer's disease. *Neurobiol Aging.* (2004) 25:5–18.
58. Bennett IJ, Madden DJ. Disconnected aging: cerebral white matter integrity and age-related differences in cognition. *Neuroscience.* (2014) 276:187–205. doi: 10.1016/j.neuroscience.2013.11.026
59. Karstens AJ, Tussing-Humphreys L, Zhan L, Rajendran N, Cohen J, Dion C, et al. Associations of the Mediterranean diet with cognitive and neuroimaging phenotypes of dementia in healthy older adults. *Am J Clin Nutr.* (2019) 109:361–8. doi: 10.1093/ajcn/nqy275
60. Mosconi L, Walters M, Sterling J, Quinn C, McHugh P, Andrews RE, et al. Lifestyle and vascular risk effects on MRI-based biomarkers of Alzheimer's disease: a cross-sectional study of middle-aged adults from the broader New York city area. *BMJ Open.* (2018) 8:e019362. doi: 10.1136/bmjopen-2017-019362
61. Matthews DC, Davies M, Murray J, Williams S, Tsui WH, Li Y, et al. Physical activity, Mediterranean diet and biomarkers-assessed risk of Alzheimer's: a multi-modality brain imaging study. *Adv J Mol Imaging.* (2014) 4:43–57. doi: 10.4236/ami.2014.44006
62. Croll PH, Voortman T, Ikram MA, Franco OH, Schoufour JD, Bos D, et al. Better diet quality relates to larger brain tissue volumes: the Rotterdam study. *Neurology.* (2018) 90:e2166–73. doi: 10.1212/wnl.0000000000005691
63. Bhullar KS, Rupasinghe H. Polyphenols: multipotent therapeutic agents in neurodegenerative diseases. *Oxid Med Cell Longev.* (2013) 2013:891748. doi: 10.1155/2013/891748
64. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev.* (2009) 2:270–8. doi: 10.4161/oxim.2.5.9498
65. Lau FC, Shukitt-Hale B, Joseph JA. The beneficial effects of fruit polyphenols on brain aging. *Neurobiol Aging.* (2005) 26:128–32. doi: 10.1016/j.neurobiolaging.2005.08.007
66. Youdim KA, Spencer JP, Schroeter H, Rice-Evans C. Dietary flavonoids as potential neuroprotectants. *Biol Chem.* (2002) 383:503–19. doi: 10.1515/bc.2002.052
67. McGrattan AM, McGuinness B, McKinley MC, Kee F, Passmore P, Woodside JV, et al. Diet and inflammation in cognitive ageing and Alzheimer's disease. *Curr Nutr Rep.* (2019) 8:53–65. doi: 10.1007/s13668-019-0271-4
68. Soltani S, Chitsazi MJ, Salehi-Abargouei A. The effect of dietary approaches to stop hypertension (DASH) on serum inflammatory markers: a systematic review and meta-analysis of randomized trials. *Clin Nutr.* (2018) 37:542–50. doi: 10.1016/j.clnu.2017.02.018
69. Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults. *J Am Coll Cardiol.* (2004) 44:152–8. doi: 10.1016/j.jacc.2004.03.039
70. Akbaraly TN, Shipley MJ, Ferrie JE, Virtanen M, Lowe G, Hamer M, et al. Long-term adherence to healthy dietary guidelines and chronic inflammation in the prospective Whitehall II study. *Am J Med.* (2015) 128:152–60.e4. doi: 10.1016/j.amjmed.2014.10.002
71. Hackman DA, Farah MJ, Meaney MJ. Socioeconomic status and the brain: mechanistic insights from human and animal research. *Nat Rev Neurosci.* (2010) 11:651–9. doi: 10.1038/nrn2897
72. Parrott MD, Shatenstein B, Ferland G, Payette H, Morais JA, Belleville S, et al. Relationship between diet quality and cognition depends on socioeconomic position in healthy older adults. *J Nutr.* (2013) 143:1767–73. doi: 10.3945/jn.113.181115
73. Petersen RC, Wiste HJ, Weigand SD, Fields JA, Geda YE, Graff-Radford J, et al. NIA-AA Alzheimer's disease framework: clinical characterization of stages. *Ann Neurol.* (2021) 89:1145–56. doi: 10.1002/ana.26071
74. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med.* (2012) 367:795–804. doi: 10.1056/NEJMoa1202753
75. Kristal AR, Peters U, Potter JD. *Is it Time to Abandon the Food Frequency Questionnaire?*. Philadelphia, PA: AACR (2005).
76. Byers T. Food frequency dietary assessment: how bad is good enough? *Am J Epidemiol.* (2001) 154:1087–8. doi: 10.1093/aje/154.12.1087
77. Carroll RJ, Midthune D, Subar AF, Shumakovich M, Freedman LS, Thompson FE, et al. Taking advantage of the strengths of 2 different dietary assessment instruments to improve intake estimates for nutritional epidemiology. *Am J Epidemiol.* (2012) 175:340–7. doi: 10.1093/aje/kwr317
78. Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, et al. Structure of dietary measurement error: results of the OPEN biomarker study. *Am J Epidemiol.* (2003) 158:14–21. doi: 10.1093/aje/kwg091
79. Ward KA, Prentice A, Kuh DL, Adams JE, Ambrosini GL. Life course dietary patterns and bone health in later life in a British birth cohort study. *J Bone Miner Res.* (2016) 31:1167–76. doi: 10.1002/jbmr.2798
80. Ding D, Grunseit AC, Chau JY, Vo K, Byles J, Bauman AE. Retirement—a transition to a healthier lifestyle?: evidence from a large Australian study. *Am J Prev Med.* (2016) 51:170–8. doi: 10.1016/j.amepre.2016.01.019
81. Ali-Kovero K, Pietiläinen O, Mauramo E, Jäppinen S, Rahkonen O, Lallukka T, et al. Changes in fruit, vegetable and fish consumption after statutory retirement: a prospective cohort study. *Br J Nutr.* (2020) 123:1390–5. doi: 10.1017/S0007114520000136
82. Stranahan AM, Norman ED, Lee K, Cutler RG, Telljohann RS, Egan JM, et al. Diet–induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle–aged rats. *Hippocampus.* (2008) 18:1085–8. doi: 10.1002/hipo.20470
83. Molteni R, Barnard R, Ying Z, Roberts CK, Gomez-Pinilla F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience.* (2002) 112:803–14. doi: 10.1016/s0306-4522(02)00123-9
84. Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, et al. NIA–AA research framework: toward a biological definition of Alzheimer's disease. *Alzheimers Dement.* (2018) 14:535–62. doi: 10.1016/j.jalz.2018.02.018
85. Kanoski SE, Davidson TL. Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity. *Physiol Behav.* (2011) 103:59–68. doi: 10.1016/j.physbeh.2010.12.003
86. Pistell PJ, Morrison CD, Gupta S, Knight AG, Keller JN, Ingram DK, et al. Cognitive impairment following high fat diet consumption is associated with brain inflammation. *J Neuroimmunol.* (2010) 219:25–32. doi: 10.1016/j.jneuroim.2009.11.010
87. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71

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An Alkaline Protease-Digestion of Silkworm Powder Enhances Its Effects Over Healthspan, Autophagy, and Mitochondria Function in a Rotenone-Induced *Drosophila* Model

*Linh Xuan Mai[1†], Sang-Kug Kang[2†], You-Young Jo[2†], Phuong Nguyen[3], A-Young Kim[3], Kee-Young Kim[2], Nam-Suk Kim[2] and Young Ho Koh[1,3]**

[1] Department of Biomedical Gerontology, Hallym University Graduate School, Chuncheon-si, South Korea, [2] Industrial Insect and Sericulture Division, National Institute of Agricultural Science, Wanju-gun, South Korea, [3] Ilsong Institute of Life Science, Hallym University, Seoul, South Korea

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Zoltan Sarnyai,
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***Correspondence:**
Young Ho Koh
kohyh@hallym.ac.kr

[†]*These authors share first authorship*

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Background: Recent studies have reported that steamed and freeze-dried mature silkworms, also known as HongJam, have various health-promoting effects.

Objective: The goal of this study was to elucidate changes in the various health-promoting effects of HongJam, after its digestion with a food-grade protease.

Materials and Methods: We examined whether healthspan-promotion and rotenone-induced loss of motor-control prevention effects were enhanced in *Drosophila* fed with food-grade alkaline protease-digested HongJam compared to those fed with non-digested HongJam. The differences in mitochondrial functions, chemical susceptibilities, and activations of signal transduction pathways between *Drosophila* supplemented with various feed were examined to elucidate the molecular and biochemical basis of healthspan-promotion and locomotor-improvement effects of protease-digested HongJam.

Results: We first found that the healthspan-promotion effect of HongJam digested with a food-grade protease was different depending on the silkworm variety used for its production. Digestion with food-grade protease into White-Jade HongJam (WJ) as prepared from the White-Jade silkworm variety that spins white cocoons did not enhance its functionality. However, compared to Golden-Silk HongJam (GS), a food-grade protease-digested Golden-Silk HongJam (GSD) produced from the Golden-Silk silkworm variety that spins yellow cocoons, it further promoted the healthspan in a *Drosophila* model. By conducting a series of studies to reveal the molecular and biochemical basis for healthspan-promoting effects, we found that GS and GSD similarly enhanced mitochondrial activity, but GSD activated autophagy signaling more than GS. In addition, GSD feed (GSDf)-, GSD supernatant feed (GSDsupf)-, and GSD precipitate feed (GSDprecf)-reared *Drosophila* were also found to have increased resistance to an autophagy inhibitor compared to that of normal feed- or GS feed-reared *Drosophila*.

Furthermore, we found that the rotenone-induced loss of motor control prevention effect was superior for GSDsup compared to GS, GSD, or GSDprec. This result may have occurred because GSDsup has more phenolic compounds and antioxidant activities than other samples.

Conclusion: GSDsup contained more digested small peptides and free phytochemicals than other samples due to the digestion of proteins with a food-grade protease. Thus, GSDsup leads to further healthspan-promoting and locomotor-improvement effects than GS, GSD, or GSDprec.

Keywords: HongJam, protease, mitochondria, autophagy, healthspan, rotenone-induced Parkinson's disease

INTRODUCTION

Since silk moths were bred by humans starting 5,000 years ago, they have provided silk fibers for making fabrics and their pupae have been a source of high-quality proteins and fats for humans for a long time (1). In addition, silkworm eggshells, excrements, larval molts, *Bombycis Corpus cum Batryticatus*, and silk moth extracts have been used as traditional Oriental medicines for treating various diseases, such as diabetes, hypertension, fever, stroke, and cerebral infarct (2). Since the 1990s, investigations supporting the scientific bases for various health improvement effects of silkworms and their byproducts have been attempted. As a result, the various health-promoting effects of silkworms and their byproducts recorded in ancient traditional Oriental medicine books for many years have been supported through preclinical and/or clinical studies in recent years. Representative effects include the hypoglycemic effect of freeze-dried 5th instar 3rd-day silkworms (3–5), the sexual function-enhancing effect of male silk moth extracts (6), and the memory enhancement effect of silk Fibroin protein hydrolysates (7, 8). Recently, a processing method has been developed that makes it possible to consume mature silkworms containing enlarged silk glands (9, 10). After the 5th instar on the 3rd-day, silkworms start to develop silk glands. The 5th instar 7th- or 8th-day silkworms, known as mature silkworms, have degenerated internal organs and enlarged silk glands filled with silk fiber proteins (9). Therefore, mature silkworms must have various health improvement effects originating from their diverse functional nutrients (2). In fact, steamed and freeze-dried mature silkworms, also known as HongJam, have been reported to have memory enhancements in mild cognitive impairment rodent models (11, 12), preventing the onset of rotenone-induced loss of motor control (13–15), gastrointestinal protection (16, 17), liver function improvement (18), skin whitening (19), and promotion of the lifespan and healthspan (10, 13, 14).

Since *Drosophila* has an anatomically separated brain, evolutionarily conserved important signal transduction pathways in its genome, short lifespan, and established various research methods for measuring locomotor ability, it has been used as an important animal model for investigating aging and longevities (20, 21). Healthspan was applied for the first time in the study of aging using the *Caenorhabditis elegans* model, and the most relevant to healthy aging of *C. elegans* is mobility (22). Similarly, *Drosophila* also needs to fly or walk to survive, so the most important factor for healthy aging is locomotor ability (13, 14, 23, 24). Therefore, maintenance of voluntary locomotor ability has been used as the most important factor in determining the healthspan of *C. elegans* (22) and *Drosophila* (10, 13, 24). In previous studies, we have shown that *Drosophila* fed with HongJam showed promotion of lifespan and healthspan (10, 13, 14).

Parkinson's disease (PD) is a progressive degenerative disorder of the central nervous systems caused by genetic defects and/or environmental risk factors in humans. Behavioral symptoms of PD are including slowness of movement, tremor, rigidity, and difficulty with walking. The pathological hallmark of PD is the death of dopaminergic neurons in human brains (25). Among various animal models for PD, *Drosophila* has been used for investigating molecular and cellular mechanisms underlying PD caused by genetic defects or environmental risk factors. For example, null mutations of Parkin, DJ-1, or LRKK2 in *Drosophila* faithfully replicated behavioral symptoms and loss of dopaminergic neurons in brains. In addition, rotenone or paraquat known to cause PD in humans also induced PD in *Drosophila* (26, 27). The molecular etiologies observed in PD patients and animal models included abnormalities in unfolded protein response (UPR), autophagy, mammalian target of rapamycin, and mitochondrial function (28, 29). In previous studies, we have shown that *Drosophila* fed with HongJam showed prevention of the onset of rotenone-induced loss of motor control (10, 13, 15). However, it is not still investigated whether HongJam can enhance UPR, autophagy, Target of rapamycin (Tor), and mitochondrial functions in *Drosophila*.

While we analyzed the nutritional compositions and health enhancement effects between HongJam produced with various pulverization methods, we found that the health enhancement effects of HongJam depended on the sizes of particles and recovery rates (9, 30, 31). Even though smaller HongJam particles gave rise to more effects, the currently available pulverization method for generating approximately 1 μm-sized HongJam particles induced the loss of quite an amount of HongJam particles because of mechanical defects (19).

HongJam consists of approximately 70% crude proteins, 15% crude fatty acids, 3% crude ash, and 2% phytochemicals and vitamins (10, 19, 30, 32). Recently, the Fibroin in silk fibers could reportedly be hydrolyzed by food-grade proteolytic enzymes (33). The FoodPro® alkaline protease (FP® AP) was efficiently used to hydrolyze the silk fiber Fibroin. Thus, we

investigated whether digesting HongJam with FP® AP can enhance healthspan promotion and rotenone-induced loss of motor control prevention effects of HongJam.

MATERIALS AND METHODS

Rearing Mature Silkworms and Producing HongJam

White-Jade and Golden-Silk varieties of *Bombyx mori* were raised on mulberry leaves at the campus of the National Institute of Agricultural Science (NIAS), Wanju-gun, Jeolla-buk do, South Korea. HongJam, also known as steamed and freeze-dried mature silkworms, was produced as previously published (9, 30). Voucher specimens of White-Jade HongJam (WJ) and Golden-silk HongJam (GS) were deposited in the *Bombyx mori* Quality Maintenance and Storage Laboratory, Division of Industrial Insect and Sericulture, NIAS, Wanju-gun, Jeollabuk-do, South Korea.

Digestion of HongJam With a Food-Grade Protease

A previously published protocol was used (34). In brief, to prepare enzymatically digested WJ (WJD) and GS (GSD), 150 g of WJ or GS was mixed with 75 ml of FP® AP (DuPont Industrial Biosciences, Brabrand, Denmark). After dH_2O was added to the mixtures to make 1 L of solution, an enzyme digestion was performed in a rotary stirrer at 55°C for 24 h. The FP® AP was deactivated by boiling at 90°C for 10 min. WJD and GSD were harvested and separated with supernatants and precipitates by centrifuging at 13,000 rpm for 10 min. The supernatants and precipitates were freeze-dried, and the weight ratio of dried supernatants to precipitates was 7:3.

Size Exclusion Chromatography of Various Samples

To determine the biophysical characteristics of samples, size exclusion chromatography was performed using an AKTA fast protein liquid chromatography system (AKTA FPLC, GE Healthcare, Chicago, IL, United States) equipped with a Superose^{TM} 6 10/300 column (GE Healthcare) equilibrated with 0.1 M phosphate buffer (pH 7.2, 150 mM NaCl). A gel filtration standard markers kit (protein molecular weight 12.4∼200.0 kDa, Merck KGaA, Darmstadt, Germany) was used to calibrate a Superose^{TM} 6 10/300 column. The void volume (Vo) of the Superose^{TM} 6 10/300 column was 7.5 ml. The ratios of elution volume/Vo of 200, 150, 66, 29, and 12.4 kDa protein standards were 1.988, 2.095, 2.174, 2.399, and 2.537, respectively. Radio immuno-precipitation assay (RIPA) buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5 mM EGTA, 1.0% NP-40, 1.0% sodium deoxycholate, 0.1% SDS, 140 mM NaCl, Merck) containing Halt^{TM} protease inhibitor cocktail (ThermoFisher Scientific, Waltham, MA, United States) was used to extract proteins from samples. 0.3 ml (5.0 mg/ml) of samples were injected into AKTA FPLC and then separated for 70 min with a flow rate of 1.0 ml/min. UV absorbance changes at 215, 254, and 280 nm were monitored to reveal amounts of digested peptides, other bio-molecules with ring structures, and proteins, respectively, in samples.

Drosophila Lifespan and Healthspan Assays

The Canton-S strain of *Drosophila melanogaster* obtained from the Bloomington *Drosophila* Stock Center (Indiana University, Bloomington, IN, United States) was used to test whether the lifespan and healthspan of *Drosophila* supplemented with WJ, WJD, GS, or GSD were altered. *Drosophila* was raised with normal feed [Nf (1.0 L dH_2O, 7.7 g agar, 62.4 g dried yeast, 40.8 g corn starch, 84.0 g glucose, 13.0 ml molasses, and 12.5 ml mold inhibitor)] or HongJam feeds containing 6.24 g of WJ, WJD, GS, or GSD in Nf. The life expectancy and healthspans were investigated as previously published (10, 13, 24). The healthspan in *Drosophila* was defined as the period with voluntary movement ability in previous reports.

To examine the effects of GSD supernatant (GSDsup) and GSD precipitate (GSDpepc) on *Drosophila* lifespans and healthspans, 6.24 g of GSDsup or GSDprec was mixed with Nf to make GSDsup feed (GSDsupf) or GSDprec feed (GSDprecf). The *Drosophila* were raised with Nf, GS feed (GSf), GSD feed (GSDf), GSDsupf, or GSDprecf, and the numbers of live flies and active flies were counted as previously published (10, 13, 24).

Assays for Activities of Mitochondrial Complexes I∼IV

The activities of mitochondrial complexes (MitoCom) I∼IV in 5-, 10-, 15-, or 20-day-old *Drosophila* reared with Nf, GSf, or GSDf were measured as previously published (11, 12). Ten *Drosophila* adults were ground in mitochondria lysis buffer (0.25 M sucrose, 5 mM Tris-HCl, 2 mM EGTA, and 1% BSA, pH 7.4), and then the debris was removed by three layers of medical gauze (Dae Han Medical Supply Co. LTD, Chuncheon-si, South Korea). Filtered lysates were centrifuged at 150× *g* for 5 min at 4°C and the supernatants were collected and then centrifuged at 900× *g* for 10 min at 4°C to collect the mitochondrial pellets. The pellets were resolved with 200 μl of lysis buffer and then divided into two tubes: one tube containing 155 μl for MitoCom I∼III and the other tube containing 45 μl of a mitochondrial sample with 5 μl of 10 mM n-D-β-D maltoside for MitoCom IV. All activities of MitoCom I∼IV were normalized by using the activities of *Drosophila* reared with Nf.

Chemical Susceptibility Assay for *Drosophila*

After treatment with various signal transduction pathway modulators, the changes in the survival rates of *Drosophila* reared with diverse feeds were investigated to elucidate the signaling altered by GS or GSD. Dithiothreitol [DTT, an endoplasmic reticulum (ER) stress inducer, Merck], fipronil [a gamma-aminobutyric acid A-type receptor ($GABA_A$-R) channel blocker, Tokyo Chemical Industry], H_2O_2 (an oxidative stress inducer, Merck), LiCl (an autophagy inducer, DaeJung), and 3-methyladenine (3-MA, an autophagy inhibitor, Merck) were used

to modulate various signal transduction pathways. *Drosophila* were reared with various feeds containing GS, GSD, GSDsup, or GSDprep, and then one hundred adults (50 females and 50 males) were exposed to various signal transduction modulators mixed with 1.5% agar with 0.5 M sucrose and incubated at 28.0 ± 1.0°C. The numbers of live flies were counted every 3 days, and then those live flies were transferred to new tubes containing fresh feed with modulators.

Real-Time Quantitative PCR Protocol

The expression changes of 36 genes related to the unfolded protein response (UPR), autophagy, and the target of rapamycin (Tor)/AKT/phosphatidylinositol 3-kinase (PI3K) were investigated by performing real-time quantitative PCR (RT–qPCR). In addition, the expression changes of six gustatory receptors (Gr), Gr64af, were investigated. The heads and bodies of 7- or 15-day-old adult flies were collected to extract the total RNA using TRIzol reagents according to the manufacturer's protocol. After the quality of the total RNA was determined using the A260/A280 and 28S rRNA/18S rRNA ratios, DNase I (Promega, Madison, WI, United States) was used to remove genomic DNA contaminants, and then cDNA was synthesized using Superscript IV (Thermo Fisher Scientific). The DNA sequences of the oligomers and PCR conditions are listed in **Supplementary Table 1**. RT-qPCRs using SYBR green master mix (Thermo Fisher Scientific) were performed using an ARIA MX RT-PCR machine and software (Agilent, Santa Clara, CA, United States). Three biological replications were performed for each analysis. The previously published $2^{-\Delta\Delta CT}$ method was used to quantify the relative expression levels of the genes.

Gene-to-Gene Interaction Analysis

The STRING database (DB)[1] (35) was used to perform a gene-to-gene interaction analysis of differentially expressed genes (DEGs) in the heads and bodies of 7- or 15-day-old *Drosophila*.

Survival Analysis for Rotenone-Induced *Drosophila* Model

To investigate the effects of GS, GSD, GSDsup, or GSDprec on the onset and progression of rotenone-induced loss of motor control in a *Drosophila* model, Nf-, GSf-, GSDf-, GSDsupf-, or GSDprecf-reared *Drosophila* were treated with 0.2 M rotenone in 0.1% dimethyl sulfoxide (DMSO), 1.5% agar, and 0.5 M sucrose (Dae-Jung). One hundred age-matched adult *Drosophila* (50 females and 50 males) were collected and then exposed to 0.2 M rotenone. For the controls, Nf-, GSf-, GSDf-, GSDsupf-, or GSDprecf-reared *Drosophila* were exposed to only 0.1% DMSO in 1.5% agar and 0.5 M sucrose. The number of live *Drosophila* was counted every 3 days. Kaplan-Meier survival analyses were performed as described below.

Determining the Phytochemicals in GS, GSD, GSDsup, and GSDprec

To quantify the amounts of flavonoids or polyphenolic compounds in various samples, the samples were mixed with 80% methanol (MeOH, vol/vol) and then shaken for 90 min at 150 rpm using a rotary shaker (IS971R, Jeio Tech, DaeJeon, South Korea). Mixed solutions were centrifuged to obtain their supernatants. Following filtration with a syringe filter (0.2 μm pore size, Sartorius AG, Gottingen, Germany) to remove small debris, the amounts of total phenolic compounds and flavonoids were measured as previously published (11, 14, 36). The amounts of phytochemicals in GSD, GSDsup, and GSDprec were normalized by using those of GS.

Antioxidant Activity Assays

The antioxidant activities of GS, GSD, GSDsup, and GSDprep were examined by performing a 1,1-di-phenyl-2-picryl-hydrazyl (DPPH) assay and a ferric-reducing ability of plasma (FRAP) assay as previously published (11, 14, 36). The antioxidant activities in GSD, GSDsup, and GSDprec were normalized by using those of GS.

Statistical Analysis

For comparing mitochondrial activities, amounts of phytochemicals, and antioxidant activities, one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) *post-hoc* analysis were performed using Microsoft Excel for Windows 10 (Microsoft, Redmond, WA, United States) as previously published (11, 12). All results were presented as the mean ± the standard error of the mean.

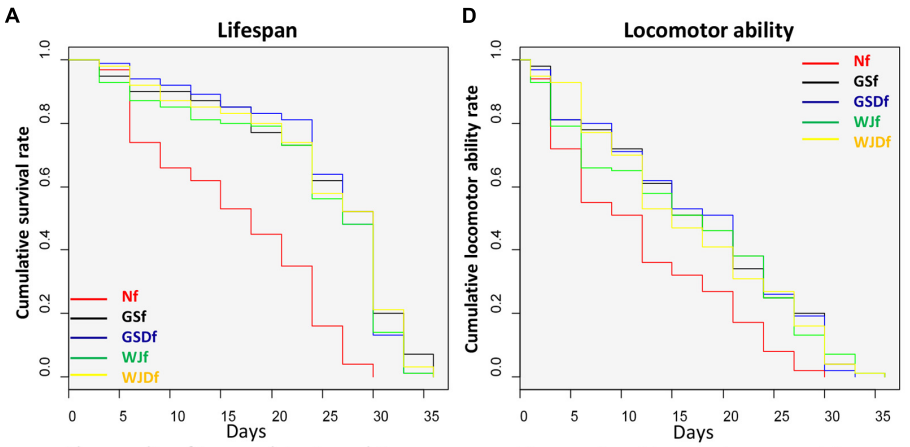
Kaplan-Meier survival estimations were performed to draw the survival or locomotor ability curves of *Drosophila* fed with different feeds or treated with various chemicals. In addition, Cox proportional hazard regression analyses and the log-rank tests were performed to obtain hazard ratios (HRs) and 95% confidential intervals (CIs), and *p*-values, respectively. Kaplan-Meier survival estimations, the log-rank tests, and Cox proportional hazard regression analyses were performed using the R program (version 4.0.3; The R Foundation) as previously published (12, 37).

RESULTS

Digestion of HongJam With a Food-Grade Protease Altered Its Biophysical Characteristics

The size exclusion chromatograms of RIPA extracts of GS, GSD, GSDsup, or GSDprec revealed unique biophysical characteristics of samples (**Supplementary Figure 1**). The chromatogram of a RIPA extract of GS had two separate parts (**Supplementary Figure 1A**). The size of the 1st part was smaller than that of the 2nd part. In contrast, the chromatogram of a RIPA extract of GSD showed the tiny 1st part and the large 2nd part (**Supplementary Figure 1B**). In addition, the chromatograms of a RIPA extract of GSDsup showed a unique pattern. The narrow and pointed 1st part appeared close to the large 2nd part (**Supplementary Figure 1C**). This unique chromatogram of GSDsup suggested that digestion of HongJam with a food-grade protease might cause digestion of high molecular weight

[1] https://string-db.org/

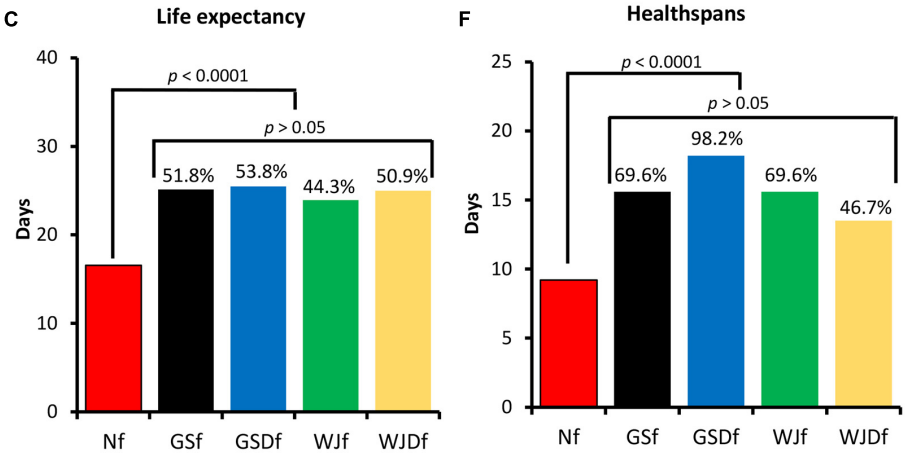


B The results of Log-rank tests and Cox proportional hazard regression analyses

Food	GSf	GSDf	WJf	WJDf
p value	2×10^{-16}	3×10^{-16}	2×10^{-15}	2×10^{-16}
HR (95% CI)	0.240 (0.178 ~ 0.324)	0.296 (0.222 ~ 0.397)	0.306 (0.229 ~ 0.411)	0.258 (0.192 ~ 0.347)

E The results of Log-rank tests and Cox proportional hazard regression analyses

Food	GSf	GSDf	WJf	WJDf
p value	9×10^{-6}	2×10^{-5}	2×10^{-5}	4×10^{-5}
HR (95% CI)	0.527 (0.397 ~ 0.698)	0.542 (0.409 ~ 0.718)	0.542 (0.408 ~ 0.719)	0.258 (0.192 ~ 0.347)



G

Food	Nf	GSf	GSDf	WJf	WJDf
Life expectancy (days)	16.56	25.14	25.47	23.91	24.99
Healthspan (days)	9.2	15.6	18.2	15.6	13.5
Ratio (%) of Healthspan/ Life expectancy	55.6	62.1	71.6	65.2	54.0

FIGURE 1 | The life expectancy and healthspan of *Drosophila* reared with various feeds. **(A)** The lifespans of *Drosophila* reared with GS feed (GSf), GSD feed (GSDf), WJ feed (WJf), or WJD feed (WJDf) were extended compared with that of Normal feed (Nf)-reared *Drosophila*. **(B)** When the life expectancy of *Drosophila* reared with GSf, GSDf, WJf, or WJDf was compared with that of Nf-reared *Drosophila*, the hazard ratios (HRs) were significantly reduced ($p < 0.005$). **(C)** Compared to Nf-reared *Drosophila*, the average lifespans of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* were increased. **(D)** Compared with Nf-reared *Drosophila*, the voluntary locomotor abilities of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* were significantly increased. The period of *Drosophila* retained voluntary locomotor ability was defined as the healthspan. **(E)** The HRs of GSf-, GSDf-, WJf-, and WJDf-reared *Drosophila* were significantly reduced ($p < 0.005$). **(F)** The healthspans of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* were significantly increased. **(G)** The ratios of life expectancies and healthspans were higher in GSDf-, GSf-, and WJf- but lower in WJDf-reared *Drosophila* than in Nf-reared *Drosophila*.

proteins to low molecular weight peptides. The chromatogram of a RIPA extract of GSDprec differed from others. The 1st part was larger than those of other samples (**Supplementary Figure 1D**). This result indicated that peptidoglycans, major components of larval cuticles, might be digested by a food-grade protease, and decomposed high molecular weight molecules

were released from GSDprec while extracting with a RIPA buffer. The unique chromatograms of samples might ensure the quality of the samples.

Further Extension of Healthspan in *Drosophila* Supplemented With GSD

The lifespans and healthspans of *Drosophila* reared with WJf, WJDf, GSf, or GSDf were compared with those of Nf-reared *Drosophila* (**Figure 1**). All the *Drosophila* reared with WJf, WJDf, GSf, and GSDf showed a statistically significant decrease in HRs (**Figures 1A,B**). Compared with Nf-reared *Drosophila*, the HRs of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* were 0.240, 0.296, 0.306, or 0.258, respectively. However, there was no significant difference between the various HongJam feed-reared groups. When the life expectancy was converted into the average lifespan, the average lifespan of Nf-reared *Drosophila* was 16.56 days, while the average lifespans of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* were 25.14 days (51.8% up), 25.47 days (53.8% up), 44.4% to 23.91 days (44.4% up), or 24.99 days (50.9% up), respectively (**Figure 1C**).

When the locomotion ability was examined to calculate the healthspan of *Drosophila*, we found that the locomotion ability of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* was significantly increased compared to that of Nf-reared *Drosophila*. The HRs of GSf-, GSDf-, WJf-, and WJDf-reared *Drosophila* were significantly decreased to 0.527, 0.542, 0.542, and 0.553, respectively, compared to those of Nf-reared *Drosophila* ($p < 0.005$, **Figures 1D,E**). In previous studies, the healthspan of *Drosophila* was defined as the point at which 50% of the individuals had locomotor ability (10, 13, 14). Although the healthspan of Nf-reared *Drosophila* was only 11.9 days, those of GSf-, GSDf-, WJf-, or WJDf-reared *Drosophila* were extended to 15.6 days (69.6% up), 18.2 days (98.2% up), 15.6 days (69.6% up), or 13.5 days (46.7% up), respectively (**Figure 1F**).

As the ratio of healthspans to life expectancies increased, the period of suffering from disease decreased (22, 38), so this ratio is one of the important factors to confirm health promotion. The ratio of the healthspan to the average lifespan of Nf-reared *Drosophila* was 55.6%, whereas that of GSf-, GSDf-, or WJf-cultured *Drosophila* increased up to 62.1, 71.6, or 65.2%, respectively. However, that of WJDf-reared *Drosophila* decreased to 54.0% (**Figure 1G**). The ratio of healthspan/life expectancy was deteriorated in WJD compared to WJ. These results showed that GS and GSD were more effective at increasing the healthspan than WJ and WJD. Thus, further research was conducted using GS and GSD.

Enhancement of MitoCom I∼IV Activities in *Drosophila* by GS and GSD

In previous studies, we showed that *Drosophila* and mice fed GS showed enhanced mitochondrial function (11, 12). Thus, we investigated whether the activities of MitoComs I∼IV in *Drosophila* reared with GSf or GSDf were different from those of Nf-reared *Drosophila*. The MitoCom I activities were highest in GSf-reared *Drosophila* from Day 5 to Day 15 but in GSDf-reared *Drosophila* on Day 20 (**Supplementary Figure 2A**). Similarly, the MitoCom II activities were highest in GSf-reared *Drosophila* from Day 5 to Day 15. On Day 20, GSf- and GSDf-reared *Drosophila* had significantly higher MitoCom II activities than Nf-reared *Drosophila* (**Supplementary Figure 2B**). The activity of MitoCom III was highest in GSf-reared *Drosophila* on Day 5 and Day 15, and GSDf-reared *Drosophila* had the highest activity on Day 10. On Day 20, both GSf- and GSDf-reared *Drosophila* showed higher activity than Nf-reared *Drosophila* (**Supplementary Figure 2C**). The activity of MitoCom IV in GSf- and GSDf-reared *Drosophila* was significantly higher on Day 10 than that in Nf-reared *Drosophila*. On the other days, GSf-reared *Drosophila* had the highest MitoCom IV activity (**Supplementary Figure 2D**).

These results suggested that the enhanced activities of MitoCom I∼IV might be in part the molecular basis for the extended life expectancy and healthspans in GSf- and GSDf-reared *Drosophila*.

GS and GSD Spatiotemporally Differentially Regulate Autophagy, Tor, and UPR Signaling

In previous studies, we showed that the onset and progression of rotenone-induced loss of motor control in *Drosophila* was prevented by GS. UPR, autophagy, mTor signaling, and mitochondrial function were shown to be involved with longevities and loss of motor control (28, 29). Thus, we investigated the DEGs in the heads and bodies of 7- or 15-day-old *Drosophila* reared with Nf, GSf, or GSDf.

In comparison with the expression of genes in the heads of 7-day-old Nf-reared *Drosophila*, the expression of 11 or 6 genes in the heads of 7-day-old GSf-reared *Drosophila* was significantly reduced or increased, respectively (**Supplementary Table 2**). Five out of 7 DEGs (Atg1, Atg2, Atg4a, Atg5, and Hsc70-4) associated with autophagy signaling or 6 DEGs (Atf6, Gp93, Grp170, crc, Hsc70-3, and Xbp 1) in UPR signaling were downregulated, while the expression of AKT1 in Tor signaling and three Grs (Gr64d, Gr64f, and Gr64e) were upregulated compared with those of Nf-reared *Drosophila* (**Figure 2A**). Similarly, compared with the body of 7-day-old Nf-reared *Drosophila*, the expression of 15 genes decreased and only one increased in the body of 7-day-old GSDf-reared *Drosophila* (**Supplementary Table 2**). Eight DEGs in autophagy signaling (Atg1, Atg4, Atg5, Atg7, Atg8a, Atg12, Atg13, and Hsc70-4), one DEG in Tor signaling (Pi3K59F), and 6 DEGs in UPR signaling (Atf6, crc, Gp93, Hsc70-3, PEK, and Xbp1) were all downregulated, and only Gr64e was upregulated (**Figure 2B**). When comparing GSf- and GSDf-reared *Drosophila*, two DEGs in autophagy signaling (Atg2 and Atg7) were upregulated, and 2 DEGs in Tor signaling (Pi3K59F and Akt1), UPR signaling (Xbp1 and PEK) and Grs (Gr64d and Gr64f) were downregulated (**Figure 2C**). More DEGs were found in the bodies of 7-day-old *Drosophila* reared with GSf or GSDf than in Nf-reared *Drosophila* (**Supplementary Table 2** and **Figures 2D–F**). Compared with Nf-reared *Drosophila*, 19 down- and three upregulated DEGs were observed in GSf-reared *Drosophila*. Eight out of 10 DEGs in autophagy signaling (Atg1, Atg2, Atg4a, Atg5, Atg8a, Atg13, Hsc70-4, and Hsc70-5), one

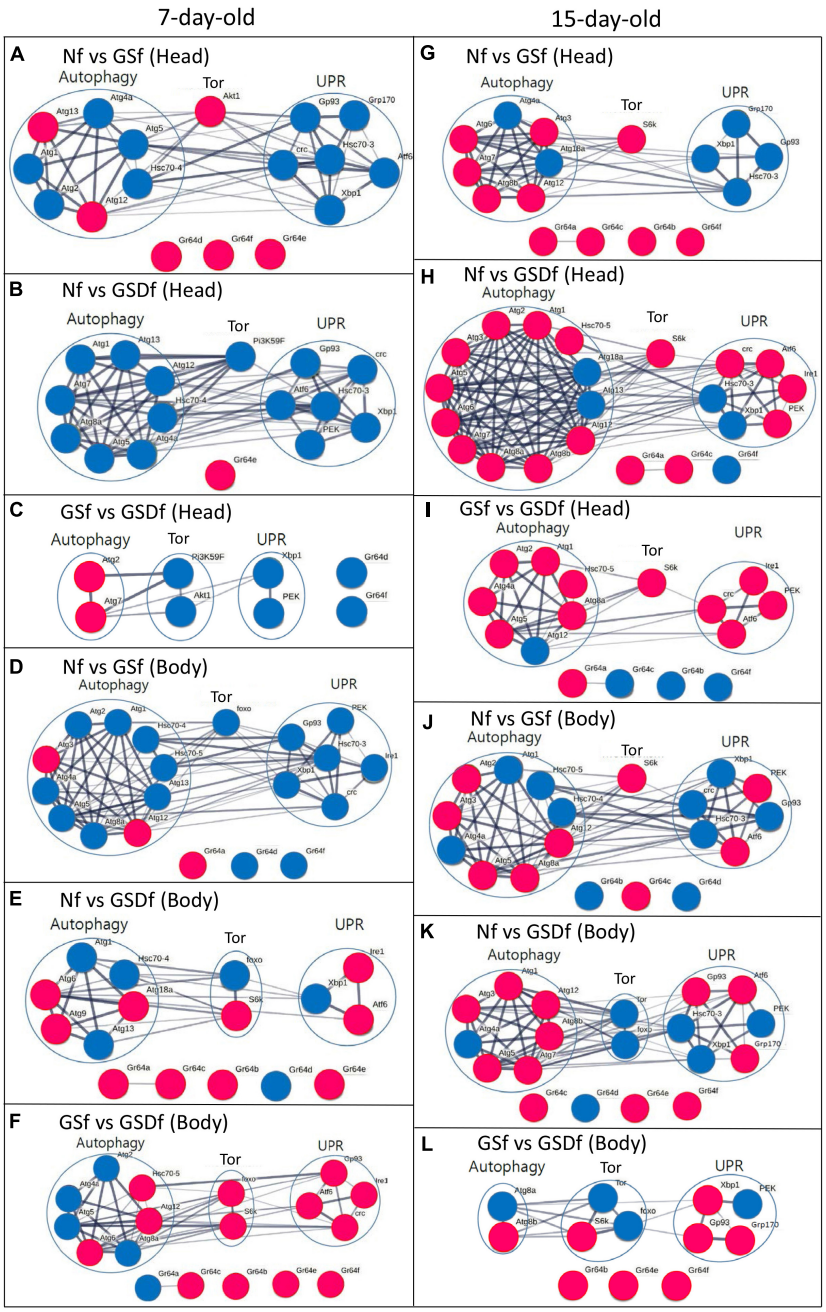


FIGURE 2 | Results of gene-to-gene interaction analysis of differentially expressed genes (DEGs) in autophagy-, Unfolded protein response (UPR)-, and Tor-signaling in the heads and bodies of 7- or 15-day-old Nf-, GSf-, and GSDf-reared *Drosophila*. Red circles indicate upregulated DEGs, while blue circles indicate downregulated DEGs. DEGs in the heads of 7-day-old GSf- **(A)** or GSDf-reared *Drosophila* **(B)** compared with those of Nf-reared *Drosophila*. DEGs in the heads of 7-day-old GSDf-reared *Drosophila* compared with those of GSf-reared *Drosophila* **(C)**. DEGs in the body of 7-day-old GSf- **(D)** or GSDf-reared *Drosophila* **(E)** compared with that of Nf-reared *Drosophila*. DEGs in the heads of 7-day-old GSDf-reared *Drosophila* compared with those of GSf-reared *Drosophila* **(F)**. DEGs in the heads of 15-day-old GSf- **(G)** or GSDf-reared *Drosophila* **(H)** compared with that of Nf-reared *Drosophila*. DEGs in the heads of 15-day-old GSDf-reared *Drosophila* compared with those of GSf-reared *Drosophila* **(I)**. DEGs in the bodies of 15-day-old GSf- **(J)** or GSDf-reared *Drosophila* **(K)** compared with those of Nf-reared *Drosophila*. DEGs in the bodies of 15-day-old GSDf-reared *Drosophila* compared with that of GSf-reared *Drosophila* **(L)**.

DEG in Tor signaling (foxo), all six DEGs in UPR signaling (crc, Gp93, Hsc70-3, Ire1, PEK, and Xbp1), and two out of three Grs (Gr64d and Gr64f) were downregulated (**Figure 2D**). By contrast, more DEGs were upregulated in GSDf-reared *Drosophila* than in Nf-reared *Drosophila*. Three out of six DEGs in autophagy signaling (Atg1, Atg13, and Hsc70-4), one out of two Tor

TABLE 1 | Altered susceptibility of GS feed (GSf)-, GSD feed (GSDf)-, GSD supernatant feed (GSDsupf)-, and GSD precipitate feed (GSDprecf)-reared *Drosophila* to various signal transduction pathway modulators.

Chemicals		DTT[3]		Fipronil		H_2O_2		LiCl	3-MA[4]
		ER stress inducer		$GABA_A$-R blocker		Oxidative stressor		Autophagy inducer inhibitor	
Concentration		50 mM	0.1 M	10 nM	50 nM	0.3%	1.5%	50 mM	20 mM
GS	HR[1]	1.2510	0.7160 ↓	0.6959 ↓	0.7080 ↓	4.274 ↑	1.571 ↑	0.9383	0.8055
	p-value	>0.05	<0.05	<0.05	<0.05	<0.005	<0.005	>0.05	>0.05
	95% CI[2]	0.9476∼1.1651	0.5418∼0.9426	0.5234∼0.9252	0.5304∼0.945	3.099∼5.893	1.183∼2.085	0.5794∼1.013	0.6039∼1.0744
GSD	HR[1]	0.4767 ↓	0.5022 ↓	0.9669	0.8669	1.923 ↑	2.0986 ↑	0.7662	0.1705 ↓
	p-value	<0.005	<0.005	>0.05	>0.05	<0.005	<0.005	>0.05	<0.005
	95% CI[2]	0.3518∼0.646	0.3783∼0.6667	0.7301∼1.280	0.6562∼1.145	1.438∼2.571	1.579∼2.790	0.5794∼1.013	0.1218∼0.2387
GSDsup	HR[1]	1.6024 ↑	1.348 ↑	0.7029 ↓	0.6414 ↓	2.897 ↑	0.9339	0.957	0.1761 ↓
	p-value	<0.01	<0.05	<0.05	<0.005	<0.01	>0.05	>0.05	<0.005
	95% CI[2]	1.209∼2.123	1.018∼1.784	0.5295∼0.9329	0.4806∼0.8559	1.097∼1.931	0.7099∼1.244	0.7383∼1.2892	0.1254∼0.2473
GSDprec	HR[1]	0.8329	1.323 ↑	0.9445	1.447 ↑	1.454 ↑	1.9732 ↑	0.6511 ↓	0.1729 ↓
	p-value	>0.05	<0.05	>0.05	>0.01	<0.005	<0.005	<0.005	<0.005
	95% CI[2]	0.625∼1.110	1.000∼1.750	0.7153∼1.2482	1.0938∼1.9143	2.141∼3.919	1.488∼2.616	0.4911∼0.8631	0.1230∼0.2431

↑, *significantly increased.*
↓, *significantly decreased.*
[1]*HR, Hazard ratio;* [2]*95% CI, 95% confidential interval;* [3]*DTT, Dithiothreitol;* [4]*3-MA, 3-methyladenine.*

signaling (foxo), one out of three DEGs in UPR signaling (Xbp1), and one out of five DEGs in Grs (Gr64d) were downregulated (**Figure 2E**). When GSf- and GSDf-reared *Drosophila* were compared, three out of seven DEGs in autophagy signaling (Atg6, Atg12, and Hsc70-5), two DEGs in Tor signaling (foxo and S6k), all four UPR signaling (Atf6, crc, Gp93, and Ire1), and four out of five DEGs in Grs (G64b, Gr64c, Gr64e, Gr6f) were upregulated. Except for the Grs, the DEGs showed very strong intra- and inter-signaling interactions (**Figures 2A–F**).

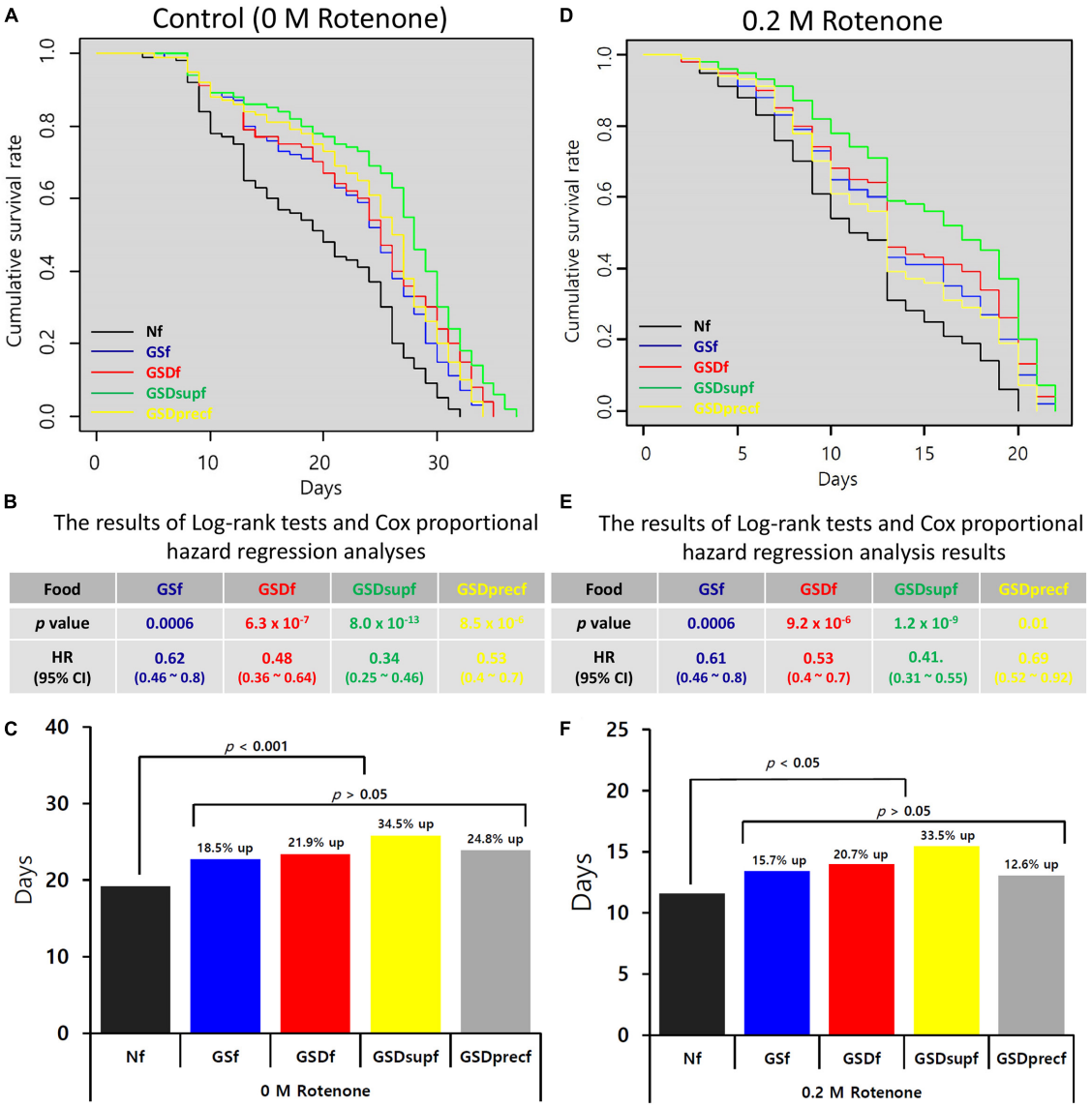
The expression patterns of DEGs in the heads and bodies of 15-day-old *Drosophila* reared with GSf or GSDf compared to those of Nf-reared *Drosophila* were different from those of 7-day-old *Drosophila* (**Supplementary Table 3** and **Figures 2G–L**). Compared with the heads of 15-day-old Nf-reared *Drosophila*, five out of seven DEGs in autophagy signaling (Atg3, Atg6, Atg7, Atg8b, and Atg12), one DEG in Tor signaling (S6k), and all four Grs (Gr64a, Gr64b, Gr64c, and Gr64f) were upregulated, while all four DEGs in UPR signaling (Hsc70-3, Grp170, Gp93, and Xbp1) were downregulated (**Figure 2G**). More DEGs were revealed when GSDf-reared *Drosophila* was compared with Nf-reared *Drosophila* (**Figure 2H**). Ten out of 12 DEGs in autophagy signaling (Atg1, Atg2, Atg3, Atg5, Atg6, Atg7, Atg8a, Atg8b, and Atg12), one DEG in Tor signaling (S6k), and four out of six DEGs in UPR signaling (Atf6, crc, Ire1, and PEK) were upregulated in the heads of 15-day-old GSDf-reared *Drosophila*. When GSf- and GSDf-reared *Drosophila* were compared, six out of seven DEGs in autophagy signaling (Atg1, Atg2, Atg4a, Atg5, Atg8a, and Hsc70-5), one DEG in Tor signaling (S6k), all four DEGs in UPR signaling (Atf6, crc, Ire1, and PEK), and one out of four DEGs (Gr64a) in Grs were upregulated (**Figure 2I**). When the expression of genes in the body of 15-day-old GSf-reared *Drosophila* was compared with that of Nf-reared *Drosophila*, five out of nine DEGs in autophagy signaling (Atg2, Atg3, Atg5, Atg8a, and Atg12), one DEG in Tor signaling (S6k), two out of six DEGs in UPR signaling (Atf6 and PEK), and one out of three DEGs in Grs (Gr64c) were upregulated (**Figure 2J**). In the case of GSDf-reared *Drosophila*, one out of seven DEGs in autophagy signaling (Atg4a), two DEGs in Tor signaling (Tor and foxo), three out of six DEGs in UPR signaling (Hsc70-3, PEK, and Xbp1), and one out of four DEGs in Grs (Gr64d) were downregulated (**Figure 2K**). In addition, when GSf- and GSDf-reared *Drosophila* were compared, one out of two DEGs in autophagy signaling (Atg8b), one out of three DEGs in Tor signaling (S6k), three out of four DEGs in UPR signaling (Gp93, Grp170, and Xbp1), and all three DEGs in Grs (Gr64c, Gr64e, and Gr64f) were upregulated (**Figure 2L**). Except for the DEGs in the Grs, there were strong intra- and inter-signaling interactions among DEGs (**Figures 2G–L**).

Taken together, the DEG analysis results showed that GS and GSD had different spatiotemporal effects on the heads and bodies of 7- and 15-day-old *Drosophila*. Since GSDf-reared *Drosophila* had more up-regulated DEGs in autophagy and UPR signaling than GSf-reared *Drosophila*, the functional nutrients in enzyme-digested GSD might be more easily absorbed than those in GS, resulting in enhancing autophagy and UPR signaling in 15-day-old *Drosophila*.

GS and GSD Alter the Susceptibility of *Drosophila* to Modulators of Various Signal Transduction Pathways

Drosophila reared with GSf and GSDf showed significantly extended life expectancies and healthspans (**Figure 1**). To investigate which signal transduction pathways were altered, we investigated the alteration of sensitivities in *Drosophila* to the modulators of various signal transduction pathways (**Table 1**).

After the *Drosophila* were treated with the ER stress inducer DTT at 50 and 100 mM in GSf-, GSDf-, GSDsupf-,



Food	GSf	GSDf	GSDsupf	GSDprecf
p value	0.0006	6.3×10^{-7}	8.0×10^{-13}	8.5×10^{-6}
HR (95% CI)	0.62 (0.46 ~ 0.8)	0.48 (0.36 ~ 0.64)	0.34 (0.25 ~ 0.46)	0.53 (0.4 ~ 0.7)

Food	GSf	GSDf	GSDsupf	GSDprecf
p value	0.0006	9.2×10^{-6}	1.2×10^{-9}	0.01
HR (95% CI)	0.61 (0.46 ~ 0.8)	0.53 (0.4 ~ 0.7)	0.41. (0.31 ~ 0.55)	0.69 (0.52 ~ 0.92)

FIGURE 3 | Prevention of the onset and progression of rotenone-induced loss of motor control by GS, GSD, GSDsup, or GSDprec. **(A)** The lifespans of GSf, GSDf, GSDsupf, or GSDprecf-reared *Drosophila* were extended compared with that of Nf-reared *Drosophila*. **(B)** In comparison with Nf-reared *Drosophila*, the HRs of *Drosophila* reared with GSf, GSDf, GSDsupf, or GSDprecf were significantly reduced, when they were not treated with rotenone. **(C)** The average lifespans of GSf-, GSDf-, GSDsupf-, or GSDprecf-reared *Drosophila* were more promoted than that of Nf-reared *Drosophila*. **(D)** When *Drosophila* was exposed to 0.2 M rotenone, the lifespans of GSf, GSDf, GSDsupf, or GSDprecf-reared *Drosophila* were extended compared with that of Nf-reared *Drosophila*. **(E)** The HRs of GSf-, GSDf-, GSDsupf-, and GSDprecf-reared *Drosophila* were significantly reduced. **(F)** The average lifespans of GSf-, GSDf-, GSDsupf-, and GSDprecf-reared *Drosophila* were more promoted than that of Nf-reared *Drosophila*.

and GSDprecf-reared *Drosophila*, the survival rate was compared to that of Nf-reared *Drosophila* (**Table 1**). When GSf-reared *Drosophila* were exposed to DTT, HR (0.7160, $p < 0.05$) was significantly reduced only when exposed to 100 mM DTT. The HRs of GSDf-reared *Drosophila* exposed to 50 mM (0.4767, $p < 0.005$) or 100 mM DTT (0.5022, $p < 0.005$) were significantly reduced. However, GSDsupf-reared *Drosophila* exposed to 50 mM (1.6024, $p < 0.005$) and 100 mM DTT (1.348, $p < 0.05$) showed significantly increased HRs. In addition, GSDprecf-reared *Drosophila* exposed to 100 mM DTT only showed a significantly increased HR (1.323, $p < 0.05$).

Drosophila reared with various feeds showed altered HRs when exposed to 10 nM or 50 nM of the $GABA_AR$ blocker fipronil (**Table 1**). The HRs of GSf-reared *Drosophila* exposed to 10 nM (0.6959, $p < 0.05$) or 50 nM fipronil (0.7080, $p < 0.05$) were significantly reduced. Although the HRs of GSDf-reared *Drosophila* exposed to fipronil did not show any difference from those of Nf-reared *Drosophila*, GSDsupf-reared *Drosophila* exposed to 10 nm (0.7029, $p < 0.05$) or 50 nm fipronil (0.6414,

$p < 0.005$) showed significantly reduced HRs. By contrast, GSDprecf-reared *Drosophila* showed a significantly increased HR when exposed to 50 nm fipronil (1.447, $p < 0.01$).

For the oxidative stressor H_2O_2, the HRs of 0.3% (4.274, $p < 0.005$) or 1.5% H_2O_2 (1.571, $p < 0.05$)-exposed GSf-reared *Drosophila* were significantly increased. Similarly, the HRs of 0.3% (1.454, $p < 0.005$) or 1.5% H_2O_2 (1.9732, $p < 0.005$) were significantly increased in GSDf-reared *Drosophila*. However, GSDsupf-reared *Drosophila* only showed a significantly increased HR when exposed to 0.3% H_2O_2 (2.897, $p < 0.01$).

When exposed to the autophagy inducer 50 mM LiCl, the HR (0.6511, $p < 0.005$) of GSDprecf-reared *Drosophila* was significantly reduced. By contrast, when exposed to the autophagy inhibitor 20 mM 3-MA, the HRs of GSDf- (0.1705, $p < 0.005$), GSDsupf- (0.1761, $p < 0.005$), and GSDprecf (0.1729, $p < 0.005$) were significantly reduced.

Taken together, these results suggested that the signal transduction pathways affected by GS and GSD might be different. Nevertheless, consistent with the DEG analysis results, GSD-, GSDsup-, and GSDprec-reared *Drosophila* revealed enhanced resistance to an autophagy inhibitor.

Preventing the Onset of Rotenone-Induced Loss of Motor Control in GSf-, GSDf-, GSDsupf-, and GSDprecf-Reared *Drosophila*

Rotenone is a widely used natural plant protection agent that is extracted from the roots of *Derris* spp., *Lonchocarpus* spp., *Tephrosia* spp., and *Mundulea* spp. Although rotenone is widely used in eco-friendly or organic farming, an important side effect observed in humans and animals exposed long-term to rotenone is loss of motor control because this compound is a MitoCom I inhibitor (39). In previous studies, we showed that GS could prevent the onset and progression of rotenone-induced loss of motor control in *Drosophila* (13, 14). Thus, we tested whether GSD, GSDsup, or GSDprec could prevent the onset and progression of loss of motor control in *Drosophila* (**Figure 3**). In addition to GSf-reared *Drosophila* (HR = 0.61), the HRs of GSDf- (0.48), GSDsupf- (0.34), and GSDprecf-reared *Drosophila* (0.53) were significantly reduced ($p < 0.001$, **Figures 3A,B**). Compared with that of Nf-reared *Drosophila* (19.15 days), the average lifespans of GSf-, GSDf-, GSDsupf-, and GSDprecf-reared *Drosophila* were 22.69 days (18.5% up), 23.34 days (21.9% up), 25.75 days (34.5% up), and 23.89 days (24.8% up), respectively (**Figure 3C**). When *Drosophila* was exposed to 0.2 M rotenone, compared with Nf-reared *Drosophila*, the HRs of GSf- (0.61), GSDf- (0.53), GSDsupf- (0.41), and GSDprecf-reared *Drosophila* (0.69) were significantly reduced ($p < 0.001$, **Figures 3D,E**). Compared with that of Nf-reared *Drosophila* exposed to 0.2 M rotenone (11.58 days), the average lifespans of GSf-, GSDf-, GSDsupf-, or GSDprecf-reared *Drosophila* exposed to 0.2 M rotenone were 13.4 days (15.7% up), 13.98 days (20.7% up), 15.46 days (33.5% up), and 13.04 days (12.6% up), respectively (**Figure 3F**). This result suggested that GSDsupf-reared *Drosophila* exhibited the superior prevention effect of rotenone-induced loss of motor control.

More Total Phenolic Compounds and Antioxidant Activities in GSD and GSDsup

To investigate the effect of FP® AP treatment on the quantity and antioxidant activity of phytochemicals and small molecules present in GS, 80% MeOH extracts of GS, GSD, GSDsup, and GSDprec were used. There were non-significant differences in the total flavonoid amounts in the 80% MeOH extracts (GS = 100.0 ± 9.07%, GSD = 106.7 ± 8.57%, GSDsup = 96.3 ± 5.79, GSDprec = 98.8 ± 3.90, $p > 0.05$, **Figure 4A**). By contrast, the relative total phenolic compounds of GSD (278.7 ± 2.25%), GSDsup (295.0 ± 4.06%), and GSDprec (245.7 ± 2.01%) were significantly increased compared to those of GS (100.0 ± 0.43%) ($p < 0.005$, **Figure 4B**).

The antioxidant effects of the GS, GSD, GSDsup, and GSDprec extracts were examined by using DPPH and FRAP assays. The DPPH radical scavenging activity was significantly increased with GSD extract (158.3 ± 0.82%), GSDsup extract (150.9 ± 1.30%), and GSDprec extract (131.3 ± 1.23%) compared with GS extract (100 ± 2.54%) ($p < 0.005$, **Figure 4C**). By contrast, the FRAP activities were significantly increased in the GSD extract (160.2 ± 4.05%) and GSDsup extract (188.6 ± 3.27%) but decreased significantly with the GSDprec extract (79.4 ± 0.91%) compared to the GS extract (100.0 ± 2.49%) ($p < 0.005$, **Figure 4D**). These results suggest that the increased antioxidant effect observed in the GSD extract is probably due to the increased phenolic compounds in the GSDsup extract. In addition, the increased amount of total phenolic compounds and antioxidant effect in GSD, GSDsup, and GSDprec compared to GS (**Figure 4**) might be used together with FPLC chromatograms (**Supplementary Figure 1**) to assure the quality of the samples.

DISCUSSION

More than 1,900 species of edible insects worldwide are consumed as food and feed that supplies protein and lipids (40–42). Compared to traditional livestock, edible insects called mini-livestock have epidemiological advantages for use as foods and feeds, because there are no common infectious disease pathogens between insects and humans or vertebrates. By contrast, feeding livestock with the same or other species byproducts is known to cause severe diseases that can be transmitted to humans. More than 60% of human diseases originate from vertebrate animals, including domestic livestock, pets, and wild animals (43). Therefore, edible insects can be used as a portion of major food and feed sources to cope with infectious diseases that threaten humanity now and in the future. Furthermore, in addition to being used as food or feed, certain edible insects may have special health-promoting effects. One of the most important examples is the mulberry silkworm, the longest-reared mini-livestock by humans (1, 2). Prior to the 1990s, silkworm pupae, a byproduct of the process of producing silk fibers from cocoons, were primarily used as protein and fat sources. By scientifically investigating the health-promoting effects of various sericulture products mentioned in ancient Oriental medical documents since the 1990s, various health enhancement effects

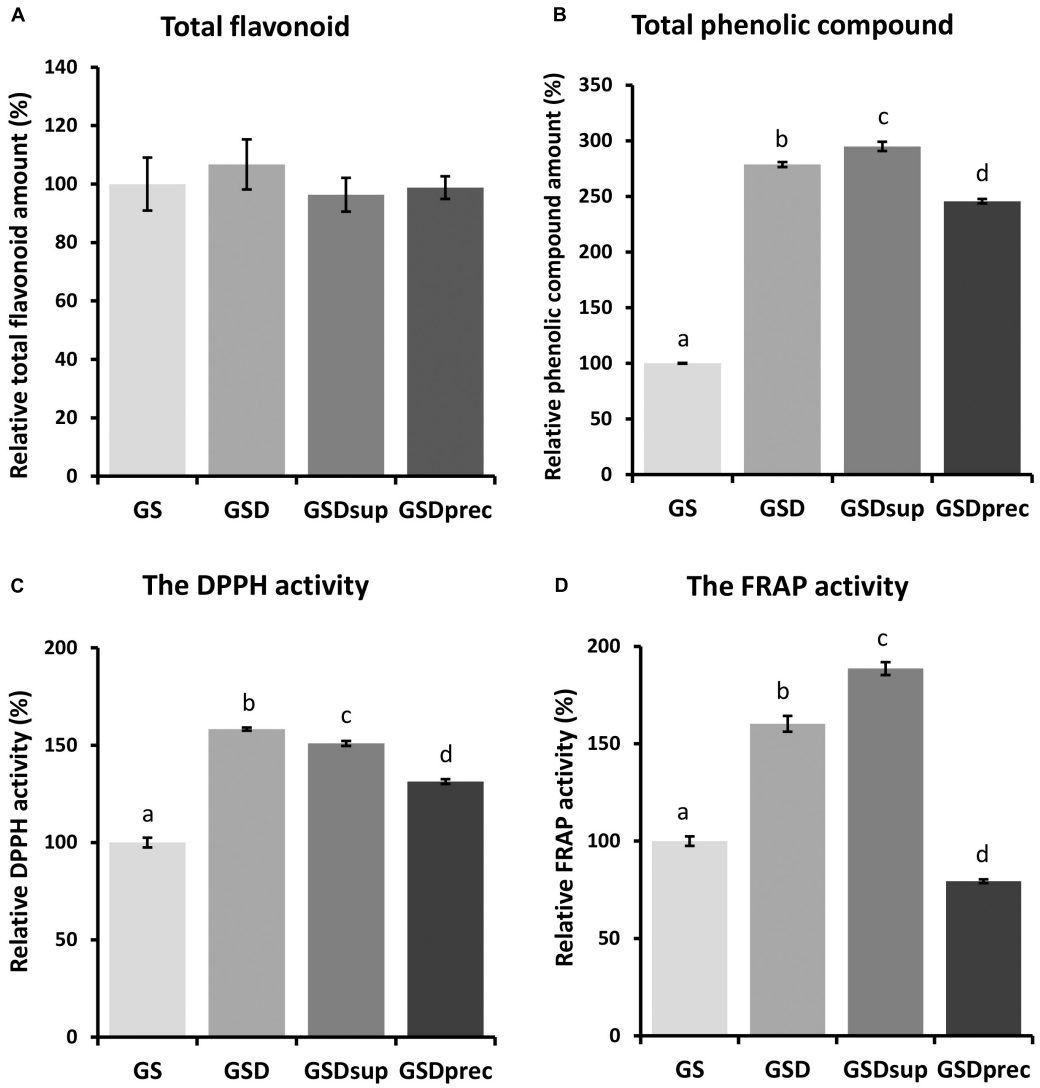


FIGURE 4 | The amounts of phytochemicals and antioxidant activities of 80% methyl alcohol (MeOH) extracts of GS, GSD, GSDsup, and GSDprec. **(A)** There were non-significant differences in the amounts of total flavonoids among the 80% MeOH extracts of GS, GSD, GSDsup, and GSDprec [$F_{(3,19)} = 0.38698$, $p = 0.76$]. **(B)** There were significant differences in the amounts of total phenolic compounds among the 80% MeOH extracts of GS, GSD, GSDsup, and GSDprec [$F_{(3,19)} = 1229.14$, $p = 4.0 \times 10^{-19}$]. **(C)** There were significant differences in the 1,1-di-phenyl-2-picryl-hydrazyl (DPPH) radical scavenger activities among the 80% MeOH extracts of GS, GSD, GSDsup, and GSDprec [$F_{(3,19)} = 261.98$, $p = 8.3 \times 10^{-14}$]. **(D)** There were significant differences in the ferric-reducing ability of plasma (FRAP) activities among the 80% MeOH extracts of GS, GSD, GSDsup, and GSDprec [$F_{(3,19)} = 304.9$, $p = 2.5 \times 10^{-14}$]. The letters above the error bars represent significant differences as determined by one-way ANOVA and Tukey's HSD *post-hoc* test.

of silkworm products have been revealed (1, 2, 42). People can eat meats, poultry, fish, eggs, etc., which are products of traditional livestock without any aversion. However, there are great preference differences among people for edible insects. Thus, research has been conducted toward producing edible insects as general food, health functional food, or food for special medical purposes after reducing aversion due to shape or smell through various treatment processes rather than the raw materials (2).

The advantage of silkworms compared to other edible insects is that their body wall is very thin and soft, so they can be consumed whole by steaming and freeze-drying (9). In previous studies, we have shown that the size of the final powder is related to the health-promotion effects of HongJam. When a hammer mill, a natural stone roller mill, or an air-jet mill were used, the average size of the final powders that could be obtained was approximately 120 μm, 10 μm, or 1 μm, respectively (9, 30, 31). Although there was a significant difference in the health-promotion effects and contents of nutrients between HongJam powder sizes of 120 μm and 10 μm (30), there was no significant difference in the contents of nutrients between HongJam powder sizes of 10 μm and 1 μm (19). The reason that there were no differences in the health-promotion effects and the nutritional compositions between HongJam powder sizes

of 10 μm and 1 μm is probably that the powders containing fatty acids are lost because of the mechanical characteristics of an air jet mill used for pulverizing. These results suggested it is necessary to develop a method of making the size of HongJam powders smaller without the loss of certain nutrients or transforming them into a form that might be easily absorbed by humans or animals. Therefore, the most important contribution from this study was providing the molecular and biochemical evidence of how the treatment of food-grade protease enhances the health-promotion effects of HongJam powder with a size of 10 μ m.

Interestingly, we found that the effect of food-grade protease treatment on HongJam was different depending on the silkworm variety when comparing the healthspan-promoting effect. There was no difference in the healthspan-promoting effect between WJ and WJD made with the white-colored cocoon silkworm variety, while a significant healthspan-promoting effect was present in GSD compared to GS made with the yellow-colored cocoon silkworm variety (**Figure 1**). In a previous study, we reported that GS had a superior memory improvement effect in mild cognitive impairment rodent models compared to WJ (11). Since we reported that the memory improvement effect in the mild cognitive impairment model by GS was due to the enhancement of mitochondrial functions, the enhancement of mitochondrial functions of GS- and GSD-reared *Drosophila* was compared in this study (**Figure 2**). However, unexpectedly, there was no significant difference in the mitochondrial function-enhancing effect between GS and GSD. These results suggested the possibility that the healthspan-promoting effect of GSD can be achieved by activating certain signal transduction pathways in addition to mitochondrial function enhancement. To support this hypothesis, we conducted a study on the signal transduction mechanisms related to healthspan promotion or disease inhibition, such as Tor, autophagy, and UPR signaling (44). As expected, we confirmed that the expression of autophagy- and UPR-associated genes increased more in GSDf-reared *Drosophila* (**Figures 3**, **4**). This result was further reinforced by the increased resistance of GSDf-, GSDsupf-, and GSDprecf-reared *Drosophila* to the autophagy inhibitor 3-MA (**Table 1**).

Another important finding from this study was that GSDsup had an excellent inhibitory effect on the onset of rotenone-induced loss of motor control compared to GS, GSD, or GSDprec (**Figure 3**). The reason this result is important is that, for proteases, large molecular weight–proteins that make up silk fibers, such as Fibroin, may be degraded into small molecular weight peptides, and phytochemicals that are strongly bound to the silk fibers may be dissociated and then rapidly absorbed into the body, resulting in excellent healthspan promotion and inhibition of the onset of rotenone-induced loss of motor control. Consistent with our hypothesis, we confirmed in this study that GSDsup had the highest total polyphenol content and antioxidant activity (**Figure 4**) and more small molecular weight peptides than GS, GSD, or GSDprec (**Supplementary Figure 1**). In addition, we have shown that digestion of freeze-dried 5th instar 3rd-day larval powders with food-grade proteases increased small molecular weight peptides in the previous study (33), supporting our results.

Interestingly, recent studies have reported that phytochemicals in food inhibit the onset of PD by activating autophagy signaling (29, 45–47). In a previous study, we reported HongJam contains significant amounts of phytochemicals such as rutin, quercetin, isoquercetin, kaempferol, and astragalin (14, 36). Since quercetin glycosides (47), kaempferol (45, 48), or astragalin (49) are reported to have inhibitory effects on the onset of PD in rodent models and/or cellular models, it is speculated that various types of functional nutrients with small molecular weights present in GSDsup are more rapidly absorbed into the body and delivered to the brain, thereby exhibiting excellent inhibitory effects during the onset of rotenone-induced loss of motor control.

In summary, the reason GSDsup was more effective in healthspan promotion and prevention of the onset of rotenone-induced loss of motor control compared to other samples is that GSDsup may contain more free phytochemicals and small molecular weight peptides that enhance autophagy signaling and mitochondrial function of *Drosophila* than other samples. Nevertheless, further clinical and preclinical researches are needed to confirm the prevention or treatment of PD through activation of autophagy signaling by phytochemicals or other small molecular weight molecules in GSDsup.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LXM, S-KK, Y-YJ, and PN performed experiments, analyzed data, and made **Figures 1–4**, **Table 1**, and **Supplementary Data**. A-YK, K-YK, and N-SK provided HongJam, performed experiments, and wrote manuscript. YHK designed and conceived experiments, got funded, analyzed data, and wrote manuscript. All authors reviewed the manuscript and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.808295/full#supplementary-material

REFERENCES

1. Koh YH. The memory enhancement and healthspan extension effects of HongJam. *Policy Rep Natl Inst Korean Med Dev.* (2020) 5:22–33. doi: 10.1016/j.jep.2021.114520
2. Kim KY, Osabutey AF, Nguyen P, Kim SB, Jo YY, Kweon HY, et al. The experimental evidences of steamed and freeze-dried mature silkworm powder as the calorie restriction mimetics. *Int J Indust Entomol.* (2019) 39:1–8. doi: 10.7852/ijie.2019.39.1.1
3. Ryu KS, Lee HS, Chung SH, Kang PD. An activity of lowering blood-glucose levels accoring to preparative conditions of silkworm powder. *J Seric Entomol Sci.* (1997) 39:79–85.
4. Ryu KS, Lee HS, Kim KY, Kim MJ, Kang PD, Chun SN, et al. Anti-diabetic effects of the silkworm (*Bombyx mori*.) extracts in the db/db mice. *Planta Med.* (2012) 78:I458. doi: 10.1055/s-0032-1321145
5. Ryu KS, Lee HS, Kim KY, Kim MJ, Sung GB, Ji SD, et al. 1-deoxynojirimycin content and blood glucose-lowering effect of silkworm (*Bombyx mori*) extract powder. *Int J Indust Entomol.* (2013) 27:237–42. doi: 10.7852/ijie.2013.27.2.237
6. Oh HG, Lee HY, Kim JH, Kang YR, Moon DI, Seo MY, et al. Effects of male silkworm pupa powder on the erectile dysfunction by chronic ethanol consumption in rats. *Lab Anim Res.* (2012) 28:83–90. doi: 10.5625/lar.2012.28.2.83
7. Kang YK, Lee BY, Bucci LR, Stohs SJ. Effect of a Fibroin enzymatic hydrolysate on memory improvement: a placebo-controlled, double-blind study. *Nutrients.* (2018) 10:233. doi: 10.3390/nu10020233
8. Kang YK, Lee WJ, Kang BH, Kang HN. Memory-enhancing fffects of silk fibroin-derived peptides in scopolamine-treated mice. *J Microbiol Biotechn.* (2013) 23:1779–84. doi: 10.4014/jmb.1308.08059
9. Ji SD, Kim NS, Lee JY, Kim MJ, Kweon H, Sung G, et al. Development of processing technology for edible mature silkworm. *J Seric Entomol Sci.* (2015) 53:38–43. doi: 10.7852/jses.2015.53.1.38
10. Ji SD, Nguyen P, Yoon SM, Kim KY, Son JG, Kweon HY, et al. Comparison of nutrient compositions and pharmacological effects of steamed and freeze-dried mature silkworm powders generated by four silkworm varieties. *J Asia Pacific Entomol.* (2017) 20:1410–8. doi: 10.1016/j.aspen.2017.10.010
11. Nguyen P, Kim KY, Kim AY, Choi BH, Osabutey AF, Park YH, et al. Mature silkworm powders ameliorated scopolamine-induced amnesia by enhancing mitochondrial functions in the brains of mice. *J Funct Foods.* (2020) 67:103886. doi: 10.1016/j.jff.2020.103886
12. Nguyen P, Kim KY, Kim AY, Kang S, Osabutey AF, Jin H, et al. The additive memory and healthspan enhancement effects by the combined treatment of mature silkworm powders and Korean angelica extracts. *J Ethnopharmacol.* (2021) 281:114520.
13. Nguyen P, Kim KY, Kim AY, Kim NS, Kweon H, Ji SD, et al. Increased healthspan and resistance to Parkinson's disease in *Drosophila* by boiled and freeze-dried mature silk worm larval powder. *J Asia Pacific Entomol.* (2016) 19:551–61. doi: 10.1016/j.aspen.2016.05.003
14. Choi BH, Ji SD, Son JG, Nguyen P, Kim KY, Park YH, et al. Phytochemicals and silk proteins in mature silkworm powders responsible for extended life expectancy and enhanced resistances to Parkinson's disease. *J Asia Pacific Entomol.* (2017) 20:1425–33. doi: 10.1016/j.aspen.2017.10.011
15. Ji SD, Son JG, Koh YH. Some skeletons in the mature silkworm larvae: not only spinning silk threads but also preventing Parkinson's disease. *J Alzheimers Dis Parkisonism.* (2016) 6:7. doi: 10.4172/2161-0460.1000289
16. Lee DY, Cho JM, Yun SM, Hong KS, Ji SD, Son JG, et al. Comparative effect of silkworm powder from 3 *Bombyx mori* varieties on ethanol-induced gastric injury in rat model. *Int J Indust Entomol.* (2017) 35:14–21. doi: 10.7852/ijie.2017.35.1.14
17. Yun SM, Cho JM, Hong KS, Lee DY, Ji SD, Son JG, et al. Gastroprotective effect of mature silkworm, *Bombyx mori* against ethanol-induced gastric mucosal injuries in rats. *J Funct Foods.* (2017) 39:279–86. doi: 10.1016/j.jff.2017.10.036
18. Hong KS, Yun SM, Cho JM, Lee DY, Ji SD, Son JG, et al. Silkworm (*Bombyx mori*) powder supplementation alleviates alcoholic fatty liver disease in rats. *J Funct Foods.* (2018) 43:29–36. doi: 10.1016/j.jff.2018.01.018
19. Ji SD, Kim SB, Kim KY, Kim NS, Kim SW, Jo YY, et al. Contents of nutrients in ultra-fine powders of steamed and lyophilized mature silkworms generated by four silkworm varieties. *J Asia Pacific Entomol.* (2019) 22:969–74. doi: 10.1016/j.aspen.2019.07.009
20. Proshkina EN, Shaposhnikov MV, Sadritdinova AF, Kudryavtseva AV, Moskalev AA. Basic mechanisms of longevity: a case study of *Drosophila* pro-longevity genes. *Ageing Res Rev.* (2015) 24:218–31. doi: 10.1016/j.arr.2015.08.005
21. Piper MDW, Partridge L. *Drosophila* as a model for ageing. *Biochim Biophys Acta Mol Basis Dis.* (2018) 1864:2707–17. doi: 10.1016/j.bbadis.2017.09.016
22. Bansal A, Zhu LJ, Yen K, Tissenbaum HA. Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. *Proc Nat Acad Sci USA.* (2015) 112:E277–86. doi: 10.1073/pnas.1412192112
23. Koh YH. *Drosophila* as a model organism for investigating molecular and cellular etiologies underlying complex neurological disorders in humans. *J Asia Pacific Entomol.* (2006) 9:75–84.
24. Gaitanidis A, Dimitriadou A, Dowse H, Sanyal S, Duch C, Consoulas C. Longitudinal assessment of health-span and pre-death morbidity in wild type *Drosophila*. *Aging.* (2019) 11:1850–73. doi: 10.18632/aging.101880
25. Blesa J, Przedborski S. Parkinson's disease: animal models and dopaminergic cell vulnerability. *Front Neuroanat.* (2014) 8:155. doi: 10.3389/fnana.2014.00155
26. Whitworth AJ. *Drosophila* models of Parkinson's disease. *Adv Genet.* (2011) 73:1–50. doi: 10.1016/B978-0-12-380860-8.00001-X
27. Duty S, Jenner P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br J Pharmacol.* (2011) 164:1357–91. doi: 10.1111/j.1476-5381.2011.01426.x
28. Hou X, Watzlawik JO, Fiesel FC, Springer W. Autophagy in Parkinson's disease. *J Mol Biol.* (2020) 432:2651–72. doi: 10.1016/j.jmb.2020.01.037
29. Balakrishnan R, Azam S, Cho DY, Kim IS, Choi DK. Natural phytochemicals as novel therapeutic strategies to prevent and treat Parkinson's disease: current knowledge and future perspectives. *Oxid Med Cell Longev.* (2021) 2021:6680935. doi: 10.1155/2021/6680935
30. Ji SD, Son JG, Kim S, Kim NS, Kim KY, Kweon H, et al. Production techniques to improve the quality of steamed and freeze-dried mature silkworm larval powder. *Int J Indust Entomol.* (2017) 34:1–11. doi: 10.7852/ijie.2017.34.2.17
31. Kim SB, Kim KY, Ji SD, Kim SW, Kim NS, Jo YY, et al. Effect of pulverizing method on the particle size of matured silkworm powder. *Int J Indust Entomol.* (2018) 37:105–8. doi: 10.7852/ijie.2018.37.2.105
32. Ji SD, Kim NS, Kweon H, Choi BH, Yoon SM, Kim KY, et al. Nutrient compositions of *Bombyx mori* mature silkworm larval powders suggest their possible health improvement effects in humans. *J Asia Pacific Entomol.* (2016) 19:1027–33. doi: 10.1016/j.aspen.2016.08.004
33. Joung JA, Park MN, You JY, Song BJ, Choi JH. Application of food-grade proteolytic enzyme for the hydrolysis of regenerated silk fibroin from *Bombyx mori*. *J Chem.* (2018) 2018:1285823. doi: 10.1155/2018/1285823
34. Bae SM, Jo YY, Lee KG, Kim HB, Kweon H. Antioxidant activity of silkworm powder treated with protease. *Int J Indust Entomol.* (2016) 33:78–84. doi: 10.7852/ijie.2016.33.2.78
35. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res.* (2021) 49:D605–12. doi: 10.1093/nar/gkaa1074
36. Choi BH, Ji SD, Jeong JH, Kim KY, Koh YH. Quantification and comparison of functional phytochemicals in steamed and freeze-dried mature silkworm powders and freeze-dried mulberry leaves. *Int J Indust Entomol.* (2017) 35:89–96. doi: 10.7852/ijie.2017.35.2.89
37. Kim AY, Seo JB, Kim WT, Choi HJ, Kim SY, Morrow G, et al. The pathogenic human torsin A in *Drosophila* activates the unfolded protein response and increases susceptibility to oxidative stress. *BMC Genomics.* (2015) 16:338. doi: 10.1186/s12864-015-1518-0
38. Hansen M, Kennedy BK. Does longer lifespan mean longer healthspan? *Trends Cell Biol.* (2016) 26:565–8. doi: 10.1016/j.tcb.2016.05.002
39. Gupta RC. Rotenone. 3rd ed. In: Wexler P editor. *Encyclopedia of Toxicology*. (Oxford: Academic Press) (2014). doi: 10.1016/B978-0-12-386454-3.00194-9
40. van Huis A, Itterbeeck JV, Klunder H, Mertens E, Halloran A, Muir G, et al. *Edible Insects Future Prospects for Food and Feed Security*. Rome: Food and Agriculture Organization of the United Nations (2013).

41. van Huis A. Potential of insects as food and feed in assuring food security. *Ann Rev Entomol.* (2013) 58:563–83. doi: 10.1146/annurev-ento-120811-153704
42. Park SJ, Kim KY, Baik MY, Koh YH. Sericulture and the edible-insect industry can help humanity survive: insects are more than just bugs, food, or feed. *Food Sci Biotechnol.* (2022) 31:657–668. doi: 10.1007/s10068-022-01090-3
43. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. *Nature.* (2008) 451:990–3. doi: 10.1038/nature06536
44. Aman Y, Schmauck-Medina T, Hansen M, Morimoto RI, Simon AK, Bjedov I, et al. Autophagy in healthy aging and disease. *Nat Aging.* (2021) 1:634–50. doi: 10.1038/s43587-021-00098-4
45. Pan X, Liu X, Zhao H, Wu B, Liu G. Antioxidant, anti-inflammatory and neuroprotective effect of kaempferol on rotenone-induced Parkinson's disease model of rats and SH-S5Y5 cells by preventing loss of tyrosine hydroxylase. *J Funct Foods.* (2020) 74:104140. doi: 10.1016/j.jff.2020.104140
46. Tamtaji OR, Hadinezhad T, Fallah M, Shahmirzadi AR, Taghizadeh M, Behnam M, et al. The therapeutic potential of quercetin in Parkinson's disease: insights into its molecular and cellular regulation. *Curr Drug Targets.* (2020) 21:509–18. doi: 10.2174/1389450120666191112155654
47. Magalingam KB, Radhakrishnan A, Ramdas P, Haleagrahara N. Quercetin glycosides induced neuroprotection by changes in the gene expression in a cellular model of Parkinson's disease. *J Mol Neurosci.* (2015) 55:609–17. doi: 10.1007/s12031-014-0400-x
48. Calderon-Montaño JM, Burgos-Moron E, Perez-Guerrero C, Lopez-Lazaro M. A review on the dietary flavonoid kaempferol. *Mini Rev Med Chem.* (2011) 11:298–344. doi: 10.2174/138955711795305335
49. Riaz A, Rasul A, Hussain G, Zahoor MK, Jabeen F, Subhani Z, et al. Astragalin: a bioactive phytochemical with potential therapeutic activities. *Adv Pharmacol Sci.* (2018) 2018:9794625. doi: 10.1155/2018/9794625

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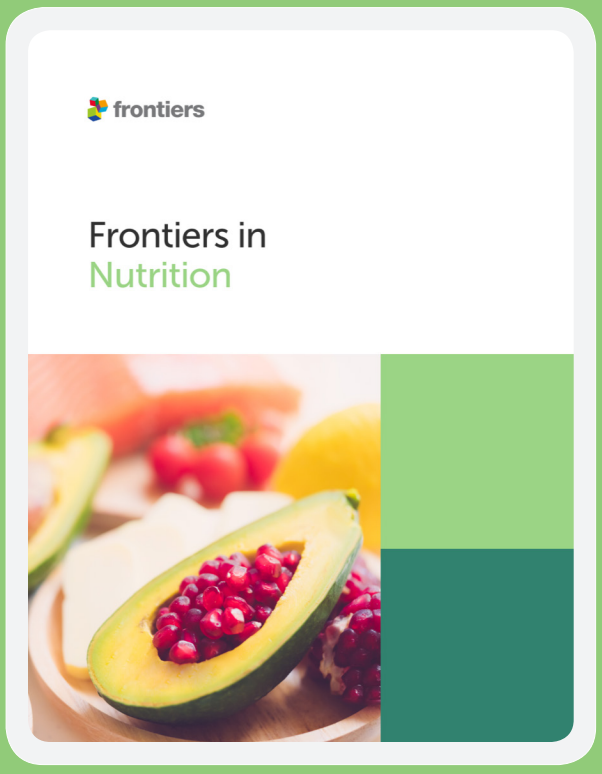

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