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Supplementation With Carotenoids, Omega-3 Fatty Acids, and Vitamin E Has a Positive Effect on the Symptoms and Progression of Alzheimer's Disease

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Abstract.
Background: Preliminary work by our center has reported behavior and functional benefits in patients with Alzheimer's disease (AD) following targeted micronutritional supplementation.
Objective: To build on the existing exploratory research and investigate the impact of these micronutrients on the natural progression of AD in a randomized controlled trial.
Methods: Patients with mild-moderate AD consumed daily 1 g fish oil (of which 500 mg DHA, 150 mg EPA), 22 mg carotenoids (10 mg lutein, 10 mg *meso*-zeaxanthin, 2 mg zeaxanthin), and 15 mg vitamin E or placebo for 12 months in a double-blind, placebo-controlled, randomized clinical trial. Carotenoids, ω-3FAs, and vitamin E were quantified in blood. Carotenoids were also measured in skin. AD severity was measured using the mini-mental state examination and dementia severity rating scale tools. Behavior, mood, and memory were measured using an informant-based questionnaire.
Results: Following 12 months of supplementation, the active group ($n = 50$) compared to the placebo group ($n = 27$), demonstrated statistically significant improvements in skin carotenoid measurements, blood carotenoids, ω-3FAs, and vitamin E concentrations ($p < 0.05$, for all). The active group also performed better in objective measures of AD severity (i.e., memory and mood), with a statistically significant difference reported in the clinical collateral for memory ($p < 0.001$).
Conclusion: Exponential increases in the prevalence of AD and its relentless progressive nature is driving the need for interventions that help to ameliorate symptoms and improve quality of life in AD patients. Given the positive outcomes demonstrated in this trial, this combined micronutrient dietary supplement should be considered in the overall management of AD.

Keywords: Alzheimer's disease, antioxidants, carotenoids, clinical collateral, disease management, disease progression, nutrition, omega-3 fatty acids, vitamin E

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INTRODUCTION

Alzheimer's disease, the most common form of dementia, is a complex neurodegenerative disease with considerable heterogeneity in abnormalities in behavior, cognition, and day-to-day function. It is initially characterized by subtle declines in memory, attention, and language that progressively worsen over time. Behavioral changes (e.g., aggression, wandering) and psychological changes (e.g., anxiety, delusions, depressive mood) are also common manifestations of the disease as it progresses. These alterations in cognition, behavior, and function have a negative impact on the patient's quality of life and also has an impact on their carers/family [1]. To date, pharmaceutical interventions have had limited efficacy in the management of Alzheimer's disease.

Accumulating evidence from observational and interventional studies suggest that good nutrition (e.g., fruits, vegetables, fish) is important for optimizing cognition and reducing the risk, or delaying the onset, of Alzheimer's disease [2–6]. Nutrition-related challenges associated with Alzheimer's disease includes difficulty in meal planning and preparation because of functional and cognitive decline, difficulty in communicating hunger and nutritional needs and dysphagia as the disease progresses [7, 8]. Of note, lower circulating concentrations of specific nutrients including vitamins (e.g., folate, A, C, E, B^{12}, K), minerals (e.g., calcium, iron, zinc), carotenoids (plant-based pigments, e.g., lutein), and omega-3 fatty acids (namely DHA) have been more widely reported in patients with Alzheimer's disease in comparison to control groups [9–14]. Moreover, steeper rates of progression and decline have been reported among patients with a poorer nutritional status in comparison to Alzheimer's disease patients with a better nutritional profile [15, 16].

Our research concerns the connection between, and potential of, preventative micronutrients (specifically xanthophyll carotenoids, omega-3 fatty acids, and vitamin E) for Alzheimer's disease and enhancement of cognitive function [17]. The many related research findings, which support a now biologically plausible rationale, are summarized as follows: Carotenoids and omega-3 fatty acids are localized in brain tissue [18, 19]; Retinal carotenoids are related to brain carotenoid concentrations [20, 21]; Serum carotenoids and omega-3 fatty acids are related to brain concentrations of these compounds [18]; Individuals with high serum and macular pigment carotenoids exhibit better cognitive performance compared to individuals with low serum and macular pigment carotenoids [22, 23]; Healthy individuals supplemented with carotenoids and omega-3 fatty acids exhibit improvements in cognitive function [24, 25]; Patients with Alzheimer's disease have lower levels of carotenoids compared to age-matched controls [9]; Patients with mild cognitive impairment demonstrate improvements in global cognition following intervention with carotenoid and omega-3 fatty acids [26]. Of note, our preliminary data suggests that intervention with a combination of carotenoids and omega-3 fatty acids improves carotenoid levels and quality of life for patients with confirmed (mild/moderate) Alzheimer's disease [27, 28]. The current study, Memory Investigation with Nutrition for Dementia (Re-MIND) trial, was designed to investigate the impact of 12-month supplementation with carotenoids, omega-3 fatty acids and vitamin E on the natural progression of Alzheimer's disease.

MATERIALS AND METHODS

Study design

Re-MIND was a double-blind, placebo-controlled, block-randomized clinical trial. Patients aged 65+ years with mild to moderate stage Alzheimer's disease were identified as potentially suitable for enrolment based on a medical assessment performed by Consultant Geriatricians at the Age-Related Care Unit at University Hospital Waterford (Waterford, Ireland). A diagnosis of Alzheimer's disease was based on clinical assessment (including Montreal Cognitive Assessment), informant-based interviewing, and neuroimaging (to exclude stroke disease). Individuals were not invited to participate in the trial if they had consumed a carotenoid or omega-3 fatty acid supplement 3 months prior to enrolment; were unable to swallow capsules; had a diagnosis of depression (under active review or recent changes in medication); had previously confirmed stroke disease and/or infarct on a brain scan; achieved a Mini-Mental State Evaluation (MMSE) score >24; or an intact clock drawing test or semantic fluency test (i.e., naming more than 11 objects starting with the letter F in 1 minute) at the time of enrolment. Prior to enrolment, written informed consent was obtained from all individuals. Ethical approval was granted by the Research Ethics Committees of the Waterford Institute of Technology and University Hospital

Waterford (Waterford, Ireland) in May and October 2018, respectively. Re-MIND (trial registration number: ISRCTN11892249) adhered to the tenets of the Declaration of Helsinki, Article 7 of the Universal Declaration of Human Rights (1948), and the Belmont Report (1979). Re-MIND also followed the full code of ethics with respect to recruitment, testing and general data protection regulations as set out by the European Parliament and Council of the European Union.

Randomization and Intervention

Study participants were assigned to the active or placebo group using block randomization with no stratification. Random allocation sequencing in block sizes of 12 and in a 2:1 active-placebo ratio was performed using a trial management system designed by the Nutrition Research Centre Ireland (NRCI) (see [26] for details) and overseen by a Statistician. Capsule dispensing was performed by a Research Assistant at the NRCI using the trial management system. The Research Nurse received a box of capsules from the Research Assistant which was subsequently given to the patient at the end of their study visit. Importantly, both the Research Nurse and study participants (and their primary carers) were blinded to the intervention. The intervention code was only revealed at study completion.

Participants were randomized to either the active intervention ($n = 50$) containing carotenoids (10 mg lutein, 10 mg *meso*-zeaxanthin, 2 mg, zeaxanthin), omega-3 fatty acids (1 g, of which 500 mg DHA and 150 mg EPA), and vitamin E (15 mg D-α-tocopherol) (now commercially known as Memory Health in the USA and ReMind in the UK and Europe) or placebo (sunflower oil) ($n = 27$) for 12 months (Fig. 1). Previous research has shown that the carotenoid formulation used in the present study is the most efficacious in terms of achieving a response in retinal tissue concentrations (which correlate with brain carotenoid concentrations) [29, 30]. Also, our works has demonstrated a further enhanced carotenoid response in blood when combined with fish oil [25, 31]. Of note, DHA is highly concentrated in retinal photoreceptors [32] and the grey matter of the brain [33], and therefore, a fish oil formulation with the highest DHA content achievable was chosen. Fifteen milligrams of vitamin E was chosen as it is the maximum amount allowed by the European Food Safety Authority. Doses were provided via three oval-sized capsules containing equal quantities of carotenoids, fish oil and vitamin E. Carotenoid and vitamin E concentrations were manufactured by Industrial Orgánica SA de CV (Monterrey, Mexico), while fish oil concentrations were manufactured by Epax® (*Å*lesund, Norway; product number: EPAX1050TG). The complete formula composition and the concentration of fatty acids of total lipids and carotenoids are available in Supplementary Table 1. Participants were instructed to consume three capsules per day and in one sitting with a meal. Carers were also informed of these instructions to enhance compliance. Frequent phone calls were made to further optimize compliance. Tablet counting was also performed at each follow-up visit to determine the overall level of compliance for both active and placebo groups. For each participant, the total number of capsules remaining at the end of the trial was divided by the total number of capsules issued for the trial. From this, a percentage was calculated. Study visits occurred at baseline and 12 months at the participants' residence in the presence of the participants' primary carer (i.e., immediate family member or close relative). Re-MIND commenced in November 2018 and concluded in June 2021.

Sample size calculation and outcome measures

Re-MIND was originally designed as a 24-month placebo-controlled, randomized clinical trial. As of 3 March 2020, 77 patients had been enrolled into the trial. In accordance with health guidelines from the Government of Ireland (due to the emergence of the COVID-19 pandemic), unforeseen challenges with recruitment, and recognition of the practicalities of conducting and concluding this clinical trial within the proposed timeline, no further patients were enrolled into the trial post 3 March 2020. Considering these challenges, the implications on the statistical power of this study are explored herein. The statistical power for this trial was based upon the MMSE as the primary outcome measure. The sample size required to achieve 80% power was recommended as $n = 120$ (active [$n = 80$]: placebo [$n = 40$]). The calculation of this power assessment assumed that MMSE score would decrease, on average, by 3.5 points per year [34], corresponding to a moderate Cohen's effect size, d, of approximately 0.5. After a 12-month intervention, a mean difference of 1.823 (95% CI*: [–1.674, 5.320]) in MMSE score was observed between intervention groups. As a result, a point estimate effect size of d = 0.3 (95% CI: [–0.267,0.865]) was observed between the active and placebo groups. Assuming

that the true effect size is in fact d = 0.3, a *post-hoc* statistical analysis suggested that the power of this study to detect a statistically significant difference between groups (with $n = 37$ subjects within the active group and $n = 18$ subjects in the placebo group) after 12 months was approximately 27%. Secondary outcome measures included change in the following variables: skin carotenoid concentrations; serum carotenoid and vitamin E concentrations, plasma omega-3 fatty acid concentrations; dementia severity rating scale score, clinical collateral, quality of life and frailty.

Measurement tools

Nutritional status

Skin carotenoid concentrations. Carotenoids are naturally occurring plant pigments that are ubiquitous throughout nature. They cannot be synthesized de novo by humans and so they must be obtained from the diet, largely through the leaves of edible plants and dark green, yellow, orange, and red vegetables and fruits. Primarily due to their antioxidant and inflammatory-resolution properties, carotenoids are known to play an important role in brain health [17]. Skin carotenoid concentrations were measured using the Pharmanex® BioPhotonic Scanner (Salt Lake City, UT, USA). This scanner measures carotenoid levels in human tissue at the skin surface using optical signals (resonant Raman spectroscopy) [35]. These signals identify the unique molecular structure of carotenoids, allowing their measurement without interference by other molecular substances. Participants placed a specific point (between the maximal and distal palmar creases, directly below the fifth finger) of their right hand (previously cleaned with hand sanitizer) in front of the scanner's low-energy blue light for 30 s. Following this, a skin carotenoid score (SCS) was generated, which provided an indication of the participants' overall carotenoid levels (ranging from zero to 90,000). A higher score was indicative of greater carotenoid intake. This technology has been previously validated for its safety and accuracy in measuring carotenoid status [36, 37].

Serum carotenoid, serum vitamin E, and plasma omega-3 fatty acid concentrations. Vitamin E is one of four essential fat-soluble vitamins. It is an important constituent of biological membranes where it contributes to membrane stability and helps to maintain cellular homeostasis. Due to its chemical structure, vitamin E is considered an important antioxidant and therefore important in mitigating the deleterious effects of oxidative damage in the brain [38, 39]. Non-fasting blood samples were collected at each study visit by standard venipuncture techniques. Carotenoids (lutein, zeaxanthin, and *meso*-zeaxanthin) and vitamin E (D-α-tocopherol) were extracted from serum and quantified using high performance liquid chromatography, as previously described [26].

Omega-3 fatty acids form the biological membranes of cells and their organelles and are necessary for storing energy. Given that omega-3 fatty acids (in particular, DHA) are key components of lipids in the brain, they have an important role in maintaining brain structure and function [40]. While the exact biological mechanisms by which omega-3 fatty acids confer cognitive benefits are not yet fully understood, the evidence to date suggests that their properties can primarily help to mitigate neuroinflammation [41, 42]. Their neuroprotective benefits may also be mediated indirectly through their established role as protectors against cardiovascular disease [43]. Omega-3 fatty acids (DHA and EPA) were extracted from plasma (see [26] for description). Fatty acid methyl esters (FAME) were prepared as explained previously [44]. FAME were quantified by GC coupled to flame ionization detector (GC-FID) with an Agilent 7890B Gas Chromatographer, using a Thermo 260M142P column (cyanopropylphenyl-based phase, 30 m length, 0.25 mm inner diameter and 0.25 m film thickness). Nitrogen was used as the carrier gas with a flow rate of 1.5 mL/min and an electronic pressure control at 20.8 psi. Temperature ramp started at 140°C and was held for 1 min, then followed by an increase of 6°C min^{-1} until 210°C, an increase of 2.5°C min^{-1} until 230°C and finally an increase of 10°C min^{-1} until 240°C, which was maintained for 5 min. Total run time was 26.7 min, with post run temperature at 50°C and maximum temperature at 250°C. FAME were identified by comparison with the authentic standard Mixture ME 1220 (Larodan). Quantification of FAME was performed by constructing a calibration line through the origin of the axes. The resulting slope was used as the response factor (RF). Following this procedure, methyl EPA was used to prepare a RF to quantify EPA, and methyl DHA was used to prepare a RF to quantify docosapentaenoic acid and DHA. Methyl docosanoate, methyl undecanoate, methyl heptadecanoate, methyl heneicosanoate, methyl tricosanoate, and methyl EPA were used to prepare calibration lines, and the resulting slopes were averaged and used as RF to quantify the rest of FAMEs.

Alzheimer's disease severity

The severity and progression of Alzheimer's disease was determined using the MMSE assessment tool and the Dementia Severity Rating Scale (DSRS). The MMSE has a high level of acceptance as a diagnostic instrument for screening for cognitive impairment in elderly populations, classifying the severity of cognitive impairment, and evaluating the progression of Alzheimer's disease [45]. This 30-point questionnaire measures orientation, short-term memory (recall), attention, language, and comprehension and motor skills. It was administered by the Research Nurse to determine Alzheimer's disease severity. A maximum score of 30 can be achieved, with a higher score indicative of better cognitive performance. Mean MMSE scores were also classified into normal (25–30), mild (21–24), moderate (10–20), or severe (0–9). The MMSE is short, easy to administer and has high test-retest reliability [46].

The DSRS is an 11-item, multiple choice, informant-based questionnaire used to assess disease severity (from the mildest to the most severe stages) and level of functional impairment. Areas assessed include memory, speech and language, recognition of family members, orientation to time, orientation to place, ability to make decision, social and community activity, home activity and responsibilities, personal care-cleanliness, eating, control of urination and bowels and ability to get from place to place. The questionnaire was administered to the primary carer by the Research Nurse. A maximum score of 54 can be achieved, with a lower score indicative of better functional ability and lower disease severity. DSRS scores can be classified into mild (0–18), moderate (19–36), or severe (37–54). The DSRS has demonstrated high concurrent validity with the clinical dementia rating scale (a commonly used dementia staging instrument) and the MMSE [47].

Quality of life

Quality of life quality was assessed using a 13-item questionnaire [48]. Separately, AD patients and their primary carer were asked by the Research Nurse to rate different aspects of the patients' quality of life using a 4-point Likert scale (1 = poor; 2 = fair; 3 = good; 4 = excellent). Areas of quality of life covered by the questionnaire include physical health, energy, mood, living situation, memory, close relationships, ability to perform activities, money, and how they feel about themselves and life in general. A minimum score of 13 and a maximum score of 52 can be achieved, with a higher score indicative of better quality of life. Previous studies have shown that Quality of Life in Alzheimer's disease questionnaire has good to excellent reliability [49], with Alzheimer's disease patients typically rating their quality of life higher than scores provided by their primary carer.

Frailty

Frailty has been variously described as an age-related syndrome of physiological decline that is characterized by marked vulnerability to adverse health outcomes, as well as a decline in functioning across multiple physiological systems that is accompanied by an increased vulnerability to stressors [50]. While associated with aging, frailty is not an inevitable consequence of the aging process, with up to three out of four older adults over 85 years remaining non-frail [51]. Therefore, a large proportion of older adults reach an advanced stage of life without ever developing frailty and we are only in the early stages of understanding why this is the case. The Clinical Frailty Scale was used by the Research Nurse to characterize and stratify individuals by level of vulnerability. Using a 9-point visual analogue scale and a clinical description, patients are graded on their degree of frailty which ranges from very fit (score of 1) to terminally ill (score of 9). Past studies have shown that the Clinical Frailty Scale was an independent predictor for all-cause 30-day mortality, in-patient mortality, and length of stay for hospitalized patients [52, 53]

Alzheimer's disease progression

Clinical collateral. Disturbances in mood and alterations in behavior are well established components of the multidomain definition of Alzheimer's disease. Capturing these changes can be complex and a detailed patient and collateral history from the family member or carer is key in identifying changes and their impacts [54]. Previous exploratory work by our center showed positive outcomes in memory, sight, and mood among patients with Alzheimer's disease following nutritional supplementation. These findings were based on non-structured telephone calls by a Clinical Nurse with the primary carer of each patient with Alzheimer's disease [28]. Building on this work, a 5-item questionnaire (with free text answers to facilitate documentation of carers' impressions on key clinical areas) was developed by a Consultant Geriatrician for the present study. Key clinical areas included observed changes in the patients memory, day-to-day function, and behavior/mood. These were chosen as they are common complaints discussed

(by patients and their carers) during clinical review. Free text answers provided by the carers were analyzed to capture changes in memory, ability to carry out every day activities and behavior/mood. Reports from the carers were analyzed by three members of the research team and consensus was achieved as to whether the reported symptoms improved, remained unchanged or deteriorated. The analysis was conducted in a masked fashion (i.e., the team members did not know participants intervention status, active or placebo). The structured interview questions (not validated to date) (see the Supplementary Material) were administered to the primary carer by the Research Nurse.

Demographic, health, and lifestyle data

Demographic, health, and lifestyle data, medical history and medication use were recorded via questionnaire. All demographic data was recorded in the presence of the primary carer, including dietary recall. Weekly consumption of carotenoid-rich foods (eggs, broccoli, corn, and dark green leafy vegetables) was recorded using a short dietary questionnaire (developed by Professor Elizabeth Johnson) Perry et al. [55]. Weekly consumption of foods rich in omega-3 fatty acids (herring, sardines, mackerel, salmon [fresh and tinned], tuna [fresh and tinned], halibut, rainbow trout, haddock, cod, linseed oil, and flaxseeds) were also recorded using a dietary questionnaire developed by the NRCI (not validated to date). The dietary scores generated from each food frequency questionnaire were weighted for frequency of intake and for bioavailability of the respective nutrients within these foods. The Malnutrition Universal Screening Tool (MUST) was used to identify individuals who were malnourished, at risk of malnutrition or obese [56]. Following the 5-step assessment individuals were scored from zero to 2. Individuals that score zero are classified as low risk of malnutrition. Individuals that obtained a score of 1 or 2 are classified as medium and high risk of malnutrition, respectively. Height and weight measurements were recorded to calculate body mass index (BMI) (kg/m^2). Waist circumference (cm) was obtained by placing a tape measure horizontally around the participants waist, just above the hipbone.

Statistical analysis

The statistical package IBM SPSS version 28 was used for all analyses and a 5% significance level applied. Mean MMSE score was the primary outcome measure of this study. All continuous data were reported as mean (±standard deviation [SD]). No adjustment was made for multiple comparisons. Standard statistical tests, such as the independent samples *t*-test for quantitative variables, and the contingency table χ^2 test for categorical variables, were used to compare active and placebo groups at baseline. Repeated measures analysis of variance was used for the between-group comparisons of change in continuous variables over time (baseline and 12-month follow up). Paired samples *t*-test were used for within-group comparison of change in outcome variables, where appropriate. For ordinal data, the Wilcoxon Signed Rank test was used for within-group differences over time. Chi-square test for categorical variables (e.g., change in MMSE/clinical collateral category), were used to compare active and placebo groups after 12-month follow up. No variables were controlled for during statistical analysis, as in this RCT all baseline variables were comparable (with the exception of plasma zeaxanthin) which did not change alter the outcome(s) of our experiment. Subjects who failed to complete the 12-month follow-up assessment were not included in final between-group analysis.

One patient carer was no longer willing to commit to the trial, while another carer felt that their family member was consuming too many tablets (i.e., daily medication plus Re-MIND capsules). Three patients requested to be withdrawn from the trial: one patient reported symptoms of nausea while another patient felt the supplements were contributing to hair loss. Upon trial completion, details of the intervention code revealed that these participants were enrolled into the placebo group. The final patient that requested to be removed from the trial felt that the supplements were worsening their irritable bowel condition. Upon trial completion, details of the intervention code revealed that this participant was enrolled into the active group. Reason for dropout was not recorded for 1 participant. Thus, an attrition rate of 26% was recorded (24% attrition rate for the active group and 30% attrition rate for the placebo group).

RESULTS

Baseline results

Demographic, health, and lifestyle data of active and placebo intervention groups were statistically

Table 1A
Demographic, health, and lifestyle data of active and placebo intervention groups at baseline

Variable	Active ($n = 38$)	Placebo ($n = 19$)	Sig.
Age (y)	78.63 ± 7.21	79.74 ± 5.30	0.556
Sex ([*n*]; [% female])	25 (65.8%)	11 (57.9%)	0.560
Education (y)	16.17 ± 2.40	16.00 ± 2.17	0.805
BMI (kg/m^2)	27.18 ± 5.26	25.24 ± 3.31	0.109
BMI category ([*n*]; [%])			
Underweight	0	0	0.600
Normal	15 (41.7%)	8 (47.1%)	
Overweight	10 (27.8%)	6 (35.3%)	
Obese	11 (30.6%)	3 (17.6%)	
Waist circumference (cm)	100.16 ± 14.38	96.16 ± 12.70	0.310
Physical activity (min/week)	165.00 ± 218.28	182.11 ± 174.28	0.768
Smoking ([*n*]; [%])			0.709
Never	26 (68.4%)	11 (57.9%)	
Past	10 (26.3%)	7 (36.8%)	
Current	2 (5.3%)	1 (5.3%)	
Alcohol consumption ([*n*]; [%])			0.777
Never	19 (50.0%)	10 (52.6%)	
Special occasions	10 (26.3%)	3 (15.8%)	
1-2 times/month	1 (2.6%)	2 (10.5%)	
1-2 times/week	6 (15.8%)	3 (15.8%)	
Everyday	2 (5.3%)	1 (5.3%)	
Medications	6.29 ± 3.06	5.89 ± 2.51	0.636
Co-morbidities ([*n*]; [% yes])			
Hypertension	30 (78.9%)	16 (84.2%)	0.735
Peripheral arterial disease	3 (7.9%)	1 (5.3%)	1.000
Diabetes	7 (18.4%)	2 (10.5%)	0.703
Ocular Disease	12 (31.6%)	6 (31.6%)	0.878
COPD	0	1 (5.3%)	0.333

Data displayed are mean ± standard deviation for numeric data and actual number and percentages for categorical data; Education, age (years) left formal education; BMI, body mass index; BMI category,<18.5 = underweight, 18.5–24.9 = normal, 25–29.9 = overweight, 30–34.9 = obese, >35 extremely obese); Medications, the number of prescribed medications consumed; Ocular diseases, cataracts, glaucoma, or other; COPD, chronic obstructive pulmonary disease; Physical activity, defined as any voluntary bodily movement produced by skeletal muscles that requires energy expenditure, was measured in minutes per week; Smoking status, never = smoked <100 cigarettes in lifetime), past = smoked 100 cigarettes in lifetime and none in the past year, current = smoked 100 cigarettes in lifetime and at least 1 cigarette in the last year). Alcohol consumption was measured in unit intake per week. One unit of alcohol (10 mL) was the equivalent to one of the following: a single measure of spirits (ABV 37.5%); half a pint of average-strength (4%) lager; two-thirds of a 12 mL glass of average-strength (12%) wine; half a 175 mL glass of average-strength (12%) wine; a third of a 250 mL glass of average-strength (12%) wine. Education data missing for 2 individuals in the active group and 1 individual in the placebo group. BMI data missing for 2 individuals in the active group and 2 individuals in the placebo group. Waist circumference data missing for 1 individual in the active group. Medications data missing for 3 individuals in the active group.

comparable ($p > 0.05$, for all) at baseline (see Table 1A). In terms of relevant study nutrition data (see Table 1B), serum zeaxanthin concentrations were higher in the active group at baseline ($p = 0.01$). All other nutrition variables were statistically comparable. Table 1C summarizes Alzheimer's disease severity, quality of life, and frailty data. All variables were statistically comparable between active and placebo intervention groups ($p > 0.05$). Of note, and in accordance with inclusion criteria, all individuals in the trial were of mild or moderate Alzheimer's disease severity (as per the MMSE measurement tool). Four patients in the active group and one patient in the placebo group obtained a baseline MMSE score that was within the "normal" category. Of note, 8.2% of participants lived in residential care (i.e., nursing home, convent, or supported accommodation), whereas the majority of participants (91.8%) lived at home and were supported by the primary carer. These patients were deemed to fulfil inclusion

Table 1B
Nutritional data of active and placebo intervention groups at baseline

Variable	Active ($n = 38$)	Placebo ($n = 19$)	Sig.
SCS	23,594 ± 8,131	23,286 ± 10,299	0.914
SCS category ([n]; [%])			0.106
Low	28 (87.5%)	9 (64.3%)	
Moderate	4 (12.5%)	5 (35.7%)	
High	0	0	
Serum lutein	0.14 ± 0.05	0.12 ± 0.06	0.115
Serum zeaxanthin	0.05 ± 0.01	0.04 ± 0.01	**0.014**
Serum meso-zeaxanthin	0	0	–
Serum vitamin E	25.33 ± 5.47	23.95 ± 4.48	0.359
Plasma DHA	173.03 ± 48.71	147.89 ± 59.44	0.103
Plasma EPA	99.49 ± 60.87	77.82 ± 31.60	0.164
FFQ LZ intake	13.58 ± 9.71	12.89 ± 10.45	0.808
FFQ omega intake	1.33 ± 1.34	1.17 ± 1.99	0.716
MUST score	0	0	–

Data displayed are mean ± standard deviation; SCS, skin carotenoid score; Serum lutein, zeaxanthin and meso-zeaxanthin concentrations are expressed in μmol/L; Plasma docosahexaenoic acid and eicosapentaenoic acid concentrations expressed in μmol/L; FFQ, self-reported intake using a food frequency questionnaire; MUST, malnutrition universal screening tool. Skin carotenoid data missing for 6 individuals in the active group and 5 individuals in the placebo group. Serum carotenoid and vitamin E data missing for 2 individuals in the active group and 1 individual in the placebo group. Plasma DHA data missing for 2 individuals in the active group and 1 individual in the placebo group. Plasma EPA data missing for 3 individuals in the active group and 1 individual in the placebo group.

Table 1C
Cognitive function, quality of life, dementia severity, and frailty data of active and placebo intervention groups at baseline

Variable	Active ($n = 38$)	Placebo ($n = 27$)	Sig.
Alzheimer's disease severity			
MMSE	20.63 ± 3.44	19.05 ± 3.63	0.114
MMSE category ([n]; [%])			
Normal	3 (7.9%)	0	0.401
Mild	19 (50.0%)	9 (47.4%)	
Moderate	16 (42.1%)	10.06 ± 6.37	
Severe	0	0	
DSRS	13.49 ± 7.73	10 (52.6%)	0.109
DSRS category ([n]; [%])			0.476
Mild	28 (75.7%)	16 (88.9%)	
Moderate	8 (21.6%)	2 (11.1%)	
Severe	1 (2.7%)	0	
Quality of life			
Patient perspective	36.32 ± 4.72	36.47 ± 5.14	0.911
Carer perspective	33.06 ± 6.38	36.43 ± 5.32	0.086
Frailty			
Clinical frailty score	4.08 ± 1.57	3.63 ± 1.71	0.328

Data displayed are mean ± standard deviation; MMSE, Mini-Mental State Examination (normal = 25–30, mild = 21–24, moderate = 10–20, severe = 0–9); DSRS, Dementia Severity Rating Scale. Dementia severity rating scale data missing for 1 individual in the active group and 1 individual in the placebo group. Quality of Life data from carer perspective missing for 2 individuals in the active group and 5 individuals in the placebo group.

Table 2
Repeated measures analysis of variance illustrating the change in nutrition variables over 12 months between active and placebo intervention groups

Variable	Active intervention				Placebo intervention				
	n	Baseline M ± SD	12 months M ± SD	%Δ	*n*	Baseline M ± SD	12 months M ± SD	%Δ	T x G Sig.
SCS	30	23,533 ± 7,619	37,000 ± 12,879	+57	14	23,286 ± 10,299	26,857 ± 14,174	+15	0.003
Serum L	36	0.144 ± 0.053	0.848 ± 0.649	+489	17	0.122 ± 0.062	0.117 ± 0.067	–4	<0.001
Serum Z	36	0.045 ± 0.014	0.076 ± 0.045	+69	17	0.036 ± 0.013	0.040 ± 0.018	+11	0.006
Serum MZ	36	0	0.079 ± 0.083	–	17	0	0	0	<0.001
Serum vit. E	36	25.326 ± 5.469	39.560 ± 11.766	+56	17	24.507 ± 3.903	23.163 ± 5.059	–6	<0.001
Plasma DHA	36	173.03 ± 48.71	308.82 ± 77.59	+79	17	150.86 ± 60.05	157.02 ± 62.01	+4	<0.001
Plasma EPA	35	99.49 ± 60.87	140.25 ± 47.75	+41	17	80.81 ± 29.83	83.77 ± 51.09	+4	0.037

Data displayed are mean ± SD; %Δ: 12-month visit minus baseline visit expressed as a percentage; Outcome, Interpretation of direction of result (i.e., improved, declined or remained unchanged over time); T × G, Time × Group interaction effect; SCS, skin carotenoid score (measured using the Pharmanex BioPhotonic Scanner). Serum lutein, zeaxanthin, meso-zeaxanthin, and vitamin E concentrations are expressed in μmol/L; Plasma docosahexaenoic acid and eicosapentaenoic acid concentrations expressed in μmol/L.

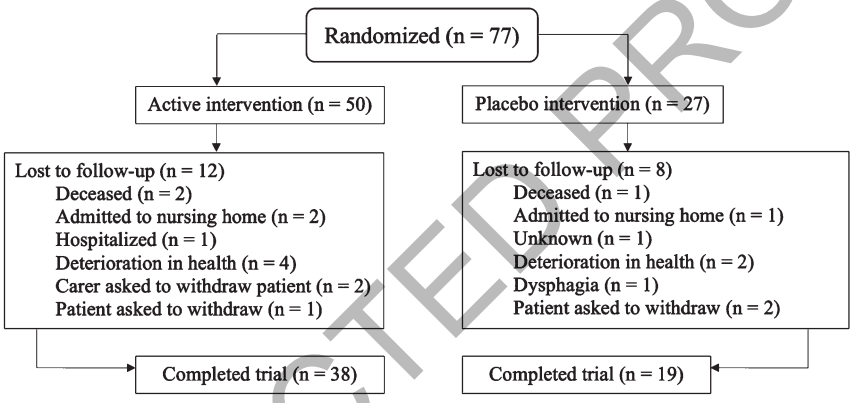

Fig. 1. Consolidated standards of reporting trials flow diagram for Re-MIND.

criteria for the trial following a review of clinical letters and neuroimaging data by a Consultant Geriatrician.

Level of compliance

On average, the level of compliance to the intervention was 82% among individuals in the active group ($n = 33$) and 79% in the placebo group ($n = 19$). Level of compliance was statistically comparable between both groups.

Observed change in micronutritional status

Table 2 (and Fig. 2 as an example) summarizes the observed change in nutrition variables for both groups following the 12-month intervention period. Individuals in the active intervention group exhibited statistically significant improvements in SCS in comparison to patients in the placebo group (57% improvement versus 15% improvement for active and placebo groups, respectively). In terms of biochemical response, individuals receiving the active intervention exhibited statistically significant improvements in serum carotenoid concentrations (lutein, zeaxanthin, and meso-zeaxanthin) serum vitamin E concentrations and plasma omega-3 fatty acid (EPA and DHA) concentrations in comparison to individuals receiving placebo ($p < 0.05$, for all).

Using our dietary assessment questionnaire, we report no significant change in fish consumption for either group over the intervention period. However, interestingly, carotenoid intake assessed using this questionnaire increased significantly in the active group and not in the placebo group. To investigate further, we performed a repeated measures of ANOVA to study the change in serum carotenoids, while adjusting for change in dietary carotenoid intake and found that Intervention remained highly statistically significant ($p < 0.001$). Additionally, we created a general

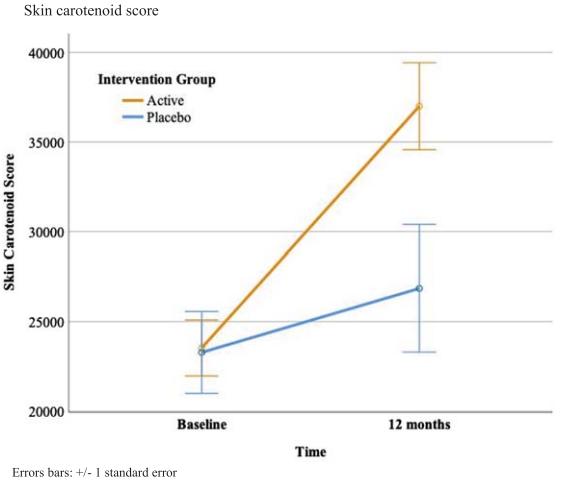

Fig. 2. Line graph illustrating change in skin carotenoid concentrations over 12 months between active and placebo groups.

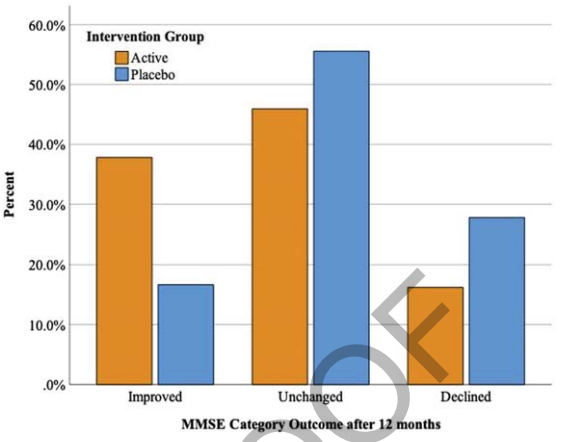

Fig. 3. Observed change in MMSE category after 12 months for active and placebo groups. Calculation of percentages were computed from the number of patients within each intervention group that improved, remained unchanged or declined in their MMSE category.

linear model using change in dietary carotenoid score and Intervention (active or placebo) as predictors. It was found that Intervention was a statistically significant predictor of serum lutein at 12 months ($p = 0.002$), while change in dietary score was not a significant predictor of serum lutein ($p = 0.202$). In other words, while we did find an increase in dietary carotenoid score in the active group and not in the placebo group, the improvement detected in serum lutein was due to the supplement intervention and not change in diet in the active group. This analysis is extremely interesting for this patient group, as it suggests an improvement in lifestyle/wellness in the active group, seen with improvement in dietary consumption of eggs, broccoli, corn, and dark green leafy vegetables (foods rich in carotenoids).

Observed change in Alzheimer's disease severity

The rate of decline in mean (±SD) MMSE score after 12 months was statistically similar in both groups, albeit greater in the placebo group. On average, individuals in the active group declined by 0.19 (±4.65) points after 12 months while individuals in the placebo group declined, on average, by 0.22 (±4.54) points after 12 months ($p = 0.980$). A trend towards a statistically significant improvement in MMSE category was observed among individuals receiving the active intervention ($p = 0.074$). Thirty-eight percent ($n = 14$) of patients in this group exhibited an improvement in MMSE category, while 46% remained unchanged and 16% declined (see Fig. 3). Of the 14 individuals that exhibited an improvement in MMSE category after 12 months, 9 moved from a mild to normal MMSE category and 5 moved from a moderate to mild MMSE category. Among individuals in the placebo group, 16.7% ($n = 3$) improved in MMSE category, 55.5% remained unchanged and 27.8% declined (comparison between groups using χ^2 analysis, $p = 0.480$). Of note, 12-month MMSE data were missing for two patients (one from each intervention group). These individuals completed their follow-up assessment via telephone only (i.e., no subsequent home visit assessment was performed).

The rate of decline in mean (±SD) DSRS score after 12 months was statistically similar in both groups, albeit greater in the placebo group. On average, individuals in the active group declined by 3.78 (±7.57) points after 12 months while individuals in the placebo group declined, on average, by 4.78 (±7.50) points after 12 months ($p = 0.649$). A statistically significant change in dementia severity category was recorded in both the active ($p = 0.021$) and placebo groups ($p = 0.046$) after 12 months. Just over two thirds (67.6% [$n = 25$]) of patients in the active group remained in the same dementia severity category at follow-up. Twenty seven percent ($n = 10$) of patients in the active group recorded a decline in dementia severity, 9 of which moved from a mild to moderate severity category and 1 moved from the moderate to severe category. Among individuals in the placebo group, 77% remained unchanged and 22.2% moved from the mild to moderate category (i.e., declined).

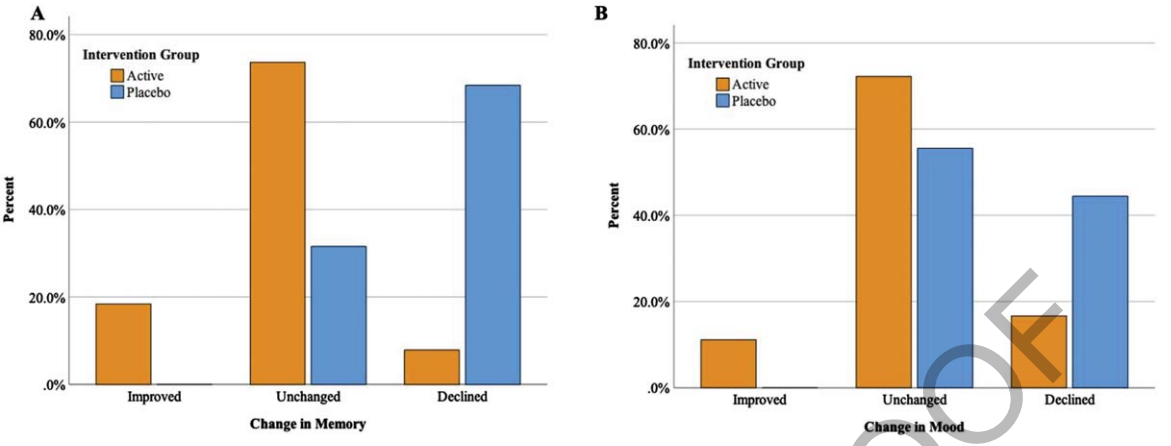

Fig. 4. Observed change in clinical collateral memory and mood scores after 12 months. Calculation of percentages were computed from the number of patients within each intervention group that improved, remained unchanged or declined in their memory and mood categories, respectively.

Observed changed in Alzheimer's disease progression

A statistically significant difference between active and placebo groups was observed after 12 months for the clinical collateral memory score ($p < 0.001$) (see Fig. 4A). In the active group, the majority of carers (73.7%) reported no change in the patients' memory. Eighteen percent of carers reported an improvement in the patients' memory while 7.9% of carers reported a decline. In the placebo group, 68.4% of carers reported a decline in the patients' memory while 31.6% reported no change.

A change in the clinical collateral mood score was also observed after 12 months. The recorded p value was borderline for statistical significance ($p = 0.055$). In the active intervention group, 72.2% of carers reported no change in the patients mood, 11.1% reported an improvement, and 16.7% reported a decline in mood. In the placebo group, 55.6% of carers reported no change in mood while the remainder (44.4%) reported a decline (see Fig. 4B).

Quality of life and frailty

On average, patients in the active intervention group self-reported a 2.22 ± 4.79 point improvement in their perceived quality of life, while patients receiving placebo self-reported an average improvement of 1.78 ± 4.31 points ($p = 0.741$). On average, carers of patients in the active group reported an improvement 0.56 ± 6.05 points while the carers of patients in the placebo group reported an average decline of 1.71 ± 7.48 points ($p = 0.271$). No Time or Time*Group interaction effects were observed for the clinical frailty score ($p > 0.05$, for both).

Impact of COVID-19

Independent Samples T-Tests and Wilcoxon Signed Ranks for continuous and categorical data, respectively were used to investigate the potential impact of COVID-19 on 12-month follow-up data. Data were split by date of data collection, i.e., data collected pre (before March 15, 2020) and post (after March 15, 2020) the pandemic. Forty-two patients completed their 12-month follow-up assessment pre the pandemic and 35 completed their follow-up visit post the pandemic. Active and placebo groups were examined separately. For both active and placebo groups, there were no statistically significant differences in pre- and post-COVID-19 scores (continuous and categorical data) for disease severity, quality of life or functional ability. Therefore, there was no evidence to suggest that the pandemic had an impact on the 12-month data collected for these outcome measures.

The memory score within the clinical collateral questionnaire did not differ significantly pre and post COVID-19 ($p = 0.252$ and $p = 0.659$ for active and placebo groups, respectively). However, among individuals in the active intervention the mood score within the clinical collateral questionnaire was significantly different between pre and post COVID-19 subgroups ($p = 0.007$), whereas there was no significant difference observed in the placebo group ($p = 0.346$). The biggest difference noted between the

groups (pre versus post COVID-19) occurred in the "declined" category, with 37.5% more carers reporting a decline in patients' mood for the post COVID-19 group ($n = 16$) compared to the pre COVID-19 group ($n = 20$).

DISCUSSION

Summary of findings

Following 12-month nutritional supplementation, individuals in the active group demonstrated statistically significant improvements in skin carotenoid concentrations and blood concentrations of carotenoids, omega-3 fatty acids, and vitamin E in comparison to individuals consuming placebo. Moreover, greater declines in Alzheimer's disease severity (as per the MMSE category) and Alzheimer's disease progression (as per the clinical collateral mood and memory scores) were observed among individuals consuming the placebo in comparison to individuals consuming the active nutritional intervention.

The importance of improvements in micronutritional status in Alzheimer's disease

Generally speaking, individuals with Alzheimer's disease are at increased risk of malnutrition, due to changes in cognition and ability to function [57]. This includes over-nutrition (i.e., relying on calorie-dense convenience foods, low in micronutritional value) or under-nutrition (i.e., insufficient caloric intake of macronutrients such as protein or micronutrients such as vitamins and minerals). Also, it is known that adherence to healthy dietary patterns (e.g., Mediterranean, Okinawan, Nordic diets), as well as greater consumption of specific nutrients, are associated with better cognitive outcomes and a reduced risk of Alzheimer's disease in later life [2, 17, 58]. Previous studies have shown that individuals with Alzheimer's disease are deficient in specific micronutrients including carotenoids (namely lutein and zeaxanthin), DHA, and vitamin E [9, 12, 14].

As noted above, these specific micronutrients have been quantified in brain tissue [19, 40] and are deemed important for brain health primarily due to their antioxidant and inflammatory- properties [39, 41, 59]. Intervention studies involving patients with Alzheimer's disease have illustrated that these individuals do respond to micronutritional intervention (in terms of increases in circulating concentrations of the respective nutrients). Moreover, progression of Alzheimer's disease was slower among patients that consumed a carotenoid plus omega-3 fatty acid combination where carers reported functional benefits in memory, sight, and mood. Unfortunately, any observed improvements in nutritional status do not always translate into improvements in outcomes related to cognition, function, or behavior among/in this population group [60]. This is likely due to the complexities of Alzheimer's disease and the considerable heterogeneity in its clinical manifestation, as well as a high level of heterogeneity between studies in their design, duration, and outcomes of interest. In the present study, however, a trend towards an improvement in MMSE category was observed among individuals in the active group, while a greater proportion of people receiving placebo exhibited a greater decline in Alzheimer's disease severity (versus the active group). In addition, progression of Alzheimer's disease was reported to be less severe/slower among individuals consuming the active nutritional intervention, with carers reporting functional benefits in memory and mood. In contrast, more carers of patients receiving the placebo reported declines in memory and mood. These findings support previous exploratory work (noted above) by our research group [28], but in the context of a double-bind, placebo-controlled, randomized clinical trial.

The findings from the Re-MIND trial highlight the benefits of micronutritional enrichment on the symptoms and natural progression of Alzheimer's disease. This is important, as past studies have shown steeper rates of decline and clinical progression among patients with a poorer nutritional status in comparison to patients with a better nutritional profile [15, 16]. Findings from the present study also suggest that supplementation with targeted micronutrition can play an important role in the management of Alzheimer's disease, with better performance in objective measures of severity and, from a clinical perspective, in areas related to memory and mood were observed among individuals consuming the nutritional supplement. Indeed, recent guidelines from the European Society for Clinical Nutrition and Metabolism recommends that nutritional care and support is integrated into dementia management [61]. Our work supports the use of targeted micronutrients, that are locally present in neural tissue, and have proven antioxidant and anti-inflammatory properties, resulting in improved outcomes in Alzheimer's disease [17, 62]. For example, lutein activates the endogenous antioxidant defenses through the Nrf2 pathway, thus reducing the levels of inflammatory mediators

usually related to neurodegenerative disorders [63]. DHA is an integral part of the retina and the brain [33]. The physicochemical characteristics of this fatty acid make it essential in vision and cognition, as it facilitates cell membrane regeneration in locations where enhanced fluidity is needed, such as in the photoreceptors and in neuronal synapsis [32, 64]. Although sufficient production and metabolism of DHA in healthy adults is disputed [65, 66], it seems clear that it is disrupted in Alzheimer's disease patients [67, 68]. Finally, vitamin E is found in the brain and is an established antioxidant that protects cells from damage associated with oxidative stress [69]. Vitamin E also possesses anti-inflammatory properties [70]. Vitamin E is considered one of the most important antioxidants in the brain primarily due to the high levels of its transporter (α-TTP), which is responsible for the regulation and distribution of this molecule [39]. Further, the protein TAP has also been suggested to play a role in brain vitamin E accumulation, as demonstrated by Zimmer et al. [71].

Strengths and limitations

Strengths of the Re-MIND trial include its double-blind, placebo-controlled randomized design, which is deemed the gold standard for evaluating the effectiveness of interventions. In addition, the implementation of robust inclusion and exclusion criteria ensured a clean dataset (e.g., only patients with Alzheimer's disease, and no other form of dementia, were enrolled into the trial). Another strength of Re-MIND included the high level of compliance of the research participants to the intervention. Overall, the nutritional supplement was well-tolerated, and patients consuming it experienced little side effects (see Fig. 1). To date, most of the research in Alzheimer's disease has concentrated on pharmacological agents for its treatment and management, namely cholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and N-methyl-D-aspartate receptor antagonists (memantine). More recently, Aducanumab was approved by the U.S. Food and Drug Administration (FDA) and is designed to target and reduce amyloid-β plaques that contribute to cell death and tissue loss in the brain. While the goal of these pharmacological agents is to improve the symptoms of Alzheimer's disease, and more recently to target the underlying biology of the disease (i.e., Aducanumab), these treatment options can have considerable side effects such as headaches, dizziness, nausea, vomiting, weight loss, frequent urination, and sleepiness. These in turn can reduce compliance to these treatments and also have a negative impact on the quality of life of patients with Alzheimer's disease. As research continues (e.g., exploring monotherapies and combination therapies [72]) to advance drug treatments for Alzheimer's disease, focus is also being directed to complementary and alternative/non-pharmacological therapies for the treatment and management of Alzheimer's disease (e.g., lifestyle modification, music therapy, cognitive intervention [73, 74]). The present study highlights the opportunity for dietary intervention as a non-pharmacological therapy to assist in the management of Alzheimer's disease. The intervention had a positive impact on outcomes related to Alzheimer's disease severity and progression; was safe and well-tolerated; and importantly, matched the ingredient label claim (see Supplementary Table 1). The interpretation, analysis, and generalizability of results from Re-MIND were limited due to the lack of statistical power in the trial. To ensure sufficient statistical power, Re-MIND aimed to recruit 120 patients with mild to moderate stage Alzheimer's disease. As noted previously (Materials and Methods section), the unforeseen challenges with recruitment and the emergence of the COVID-19 pandemic hindered volunteer recruitment and data collection for the trial. The small sample size also precluded the examination of potential relationships between observed changes in nutritional status and outcomes related to Alzheimer's disease severity and progression, clinical collateral, quality of life and frailty.

Using the MMSE tool to calculate Alzheimer's disease progression rates when designing clinical trials is considered a standardized approach [34, 75]. For the present study, an average decline of 3.5 points per year in mean MMSE score was chosen as the progression estimate. However, after a 12-month intervention, a mean difference of only 1.823 was observed in the sample. While this resulted in an underestimation of Alzheimer's disease progression in the sample (which in turn affected the sample power), it is important to acknowledge that there is considerable variability in progression rates among individuals with Alzheimer's disease. It is also important to note that MMSE decline is non-linear. In recognition of the high heterogeneity of Alzheimer's disease progression, consideration to alternative predictor variables should be given when designing intervention trials for Alzheimer's disease patients in the future. For example, a combination of measurement tools that assess cognitive and functional

outcomes to better predict progression rates or to use MMSE grouping variables, as performed by Doody and colleagues [76].

Finally, the carer collateral in this study highlighted positive outcomes in terms of mood and memory. Re-MIND is also the first study of its kind to produce these positive outcomes in the context of a randomized clinical trial. Our study highlights the importance of taking a clinical collateral history, consistent with good clinical practice. However, we acknowledge that this type of assessment questionnaire has not yet been validated for research use. Of note, the mood score within the clinical collateral questionnaire was significantly different between pre and post COVID-19 subgroups. Carers reported a greater decline in the patients' mood post COVID-19 than pre COVID-19. This may have contributed to the marginally significant result recorded for this variable. Despite these limitations, Re-MIND has demonstrated improvements in skin carotenoid concentrations and circulating concentrations of carotenoids (lutein, zeaxanthin, *meso*-zeaxanthin) omega-3 fatty acids (EPA and DHA) and vitamin E (D-α-tocopherol) in blood, as well as trends towards slower rates of Alzheimer's disease severity and progression following the consumption of nutritional supplement containing these respective nutrients.

Conclusion

The exponential increase in the prevalence of Alzheimer's disease and its relentless progressive nature is driving the need for interventions that help to ameliorate symptoms in patients and aid in the overall management of the disease. The evidence from the present study highlights the benefits of targeted micronutritional enrichment on the natural progression and management of Alzheimer's disease. Dietary intervention with a combination of carotenoids, omega-3 fatty acids and vitamin E can improve these micronutrients for patients with mild to moderate stage Alzheimer's disease. Importantly, improving these specific micronutrients suggest a positive impact on symptoms and patient outcomes, with better performance in objective measures of severity, as well as performing better from a clinical perspective in areas related to memory and mood. Given the positive outcomes demonstrated in this trial, this combined micronutrient dietary supplement should be considered in the overall management of Alzheimer's disease.

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SUPPLEMENTARY MATERIAL

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