Alzheimer's & Dementia
Translational Research
& **Clinical Interventions**

RESEARCH ARTICLE

A 1-year randomized controlled trial of a nutritional blend to improve nutritional biomarkers and prevent cognitive decline among community-dwelling older adults: The Nolan Study

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Abstract

Introduction: This study aimed to test the efficacy of a nutritional blend (NB) in improving nutritional biomarkers and preventing cognitive decline among older adults.

Methods: A 1-year randomized, double-blind, multicenter, placebo-controlled trial with 362 adults (58.6% female, mean 78.3 years, SD = 4.8) receiving an NB or placebo. Erythrocyte ω-3 index and homocysteine concentrations were primary outcomes. Other outcomes included Patient-Reported Outcomes Measurement Information System (PROMIS) Applied Cognition-Abilities, composite cognitive score (CCS), Cognitive Function Instrument (CFI) self-assessment and study partner, hippocampal volume (HV), and Alzheimer's disease signature cortical thickness (CT).

Results: A total of 305 subjects completed the follow-up. Supplementation increased ω-3 index and decreased homocysteine, but did not affect CCS, CFI self-assessment, HV, and CT. Placebo improved and treatment did not change PROMIS at 1 month. Intervention showed a positive effect on CFI study partner.

Discussion: Although improving nutritional biomarkers, this 1-year trial with a multi-nutrient novel approach was not able to show effects on cognitive outcomes among older adults.

KEYWORDS
clinical trials, cognition, homocysteine, nutrition, older adults, omega-3

1 | INTRODUCTION

Cognitive decline (CD) has been reported consistently reported with aging across a range of cognitive domains including processing speed, attention, memory, and executive function.[1] Furthermore, CD is an early predictor of Alzheimer's disease (AD) and begins before the onset of dementia,[2] being associated with increased health care costs.[3] Therefore, interventions aiming at preventing CD may have major public health implications. In this sense, increased interest in identifying early predictors of CD has brought attention to potential biomarkers associated with cognitive outcomes. Among these candidates, elevated homocysteine (a marker of insufficient B-vitamin intake)[4] and low erythrocyte ω-3 polyunsaturated fatty acids (PUFA) have been considered modifiable risk factors for dementia and AD.[5–7]

Elevated homocysteine has been associated independently with CD, white matter damage, brain atrophy, and dementia.[5] It has been shown experimentally that, in young rats, elevated plasma homocysteine induced by deficient diets in dams directly stimulated apoptosis in selected brain areas and persistently affected neurobehavioral capacities.[8] Accordingly, proposed mechanisms for the deleterious effects of homocysteine on cognitive function include cerebrovascular effects, inhibition of methylation reactions, and activation of tau kinases, which may lead to regional brain atrophy, impaired synaptic function, and amyloid beta (Aβ) accumulation.[5] The neuroprotective properties of ω-3 PUFA also involve multiple pathways, such as favoring cerebral blood flow through improving vascular endothelial function and arterial stiffness, reducing oxidative stress in the brain, and modulating neuronal membrane fluidity.[9]

Preclinical and epidemiological evidence in favor of nutritional factors protecting cognitive function has suggested nutrition as a possible intervention pathway to prevent AD and CD, with several nutrients potentially affecting cognition.[4,9–14] However, trials supplementing individual nutrients yielded controversial results.[15] Considering multifactorial mechanisms involved in aging and the synergistic effect of nutrients and bioactive compounds, more recent evidence in human[16–18] and animal[19,20] studies, on the other hand, have suggested that combining nutrients may be a more promising strategy to prevent CD. In this sense, the Nolan Study was designed to test this novel approach, through a nutritional blend (NB) developed to serve as a source of multiple nutrients. This trial aimed to investigate the efficacy of a 1-year intervention with a NB on levels of erythrocyte ω-3 index and plasma homocysteine, as well as its effects on subjective and objective measures of cognitive function and on neuroimaging markers among community-dwelling older adults without dementia, but with subjective memory complaints. Effects were further assessed in exploratory analyses within sub-cohorts, stratified based on nutritional deficits and on apolipoprotein E (*APOE*) ε4 status.

2 | MATERIALS AND METHODS

2.1 | Study design and population

This double-blind, multicenter, randomized, placebo-controlled trial (RCT) was conducted in 18 centers in France with community-dwelling older adults. Recruitment of participants started in December 2016 and ended in January 2018. Follow-up ended in February 2019.

Major inclusion criteria for the Nolan Study comprised age $\geq$70 years; self-reporting subjective memory complaints; and having a study partner to participate as a source of information. Major exclusion criteria were taking ω-3 PUFA supplements containing >200 mg of docosahexaenoic acid (DHA)/day over 6 months before inclusion; taking vitamin-B supplementation in the preceding 3 months; basic activities of daily living (ADL) score <4; Mini-Mental State Examination (MMSE) score <24; and a diagnosis of dementia. Complete inclusion and exclusion criteria are listed in Table S1. Treatment with anticoagulants or platelet aggregation inhibitors was not prohibited, but was carefully monitored, given that ω-3 PUFA can affect platelet function.

2.2 | Ethical disclosure

Subjects signed an informed consent. The trial protocol (NCT03080675) was approved by the Advisory Committee for Protection of Persons South West and Overseas II (CPP SOOM II) and the French Agency for the Safety of Medicines and Health Products (ANSM).

2.3 | Randomization and masking

Participants were randomly assigned (1:1) to one of the following groups: intervention (active supplementation with the NB) or control (placebo). (Details are provided in the Supplementary materials.)

RESEARCH IN CONTEXT

1. **Systematic review**: Preclinical and epidemiological evidence in favor of nutritional factors protecting cognition has suggested nutrition as a possible intervention pathway to prevent cognitive decline. However, trials supplementing single nutrients yielded controversial results. Recent evidence on human and animal studies has suggested that combining nutrients may be a more promising strategy.

2. **Interpretation**: Our study assessed the efficacy of 1-year nutritional blend supplementation on erythrocyte ω-3 index, homocysteine levels, and cognitive outcomes among older adults, and showed a positive effect of intervention in these biomarkers presumed to underlie an attenuation of cognitive decline during aging. Despite some observed effects in secondary outcomes, intervention was not able to prevent cognitive decline as measured by neuropsychological tests.

3. **Future directions**: Findings may help development of nutritional strategies for optimizing brain health, protecting cognition, and identifying individuals for whom supplementation may be more effective. Additional investigations are needed, especially trials with longer follow-up duration targeting individuals who are apolipoprotein E (APOE) ε4 carriers and those with nutritional deficiencies.

2.4 | Intervention

The intervention consisted in taking daily an NB characterized by two soft gel capsules (775 mg each) and one powdered sachet of ≈15 g (to be mixed in 120 mL of cold water), or by placebo equivalent doses. Total composition of a daily dose of the NB was established via an in-depth evaluation of available clinical data on each ingredient, and of previous studies using the NB in aged dogs[19] and cats,[20] and consisted of: thiamin (50 mg), riboflavin (15 mg), niacin (25 mg), pantothenic acid (23 mg), pyridoxine (18 mg), biotin (0.15 mg), folic acid (0.4 mg), cobalamin (0.5 mg), vitamin E (82.6 mg), vitamin C (500 mg), vitamin D (15 µg), choline (85 mg), selenium (80 µg), citrulline (3 g), eicosapentaenoic acid (EPA) (700 mg), and DHA (770 mg). (Additional information about capsules and powered drink composition is presented in the Supplementary materials.)

Subjects attended six in-person visits: screening, practice (when non-validated versions of cognitive tests, except MMSE, were administered to familiarize participants with procedures), baseline (beginning of the intervention), 1 month, 6 months, and 12 months. Additional phone calls were set up between visits at 3-month intervals to follow-up adverse events, product compliance (defined as good if taking ≥80% of scheduled product intake), and concomitant medication intake. No dietary restrictions were made, but participants were not allowed to take additional supplements containing B vitamins, DHA, and/or EPA.

2.5 | Outcomes: Nutritional biomarkers

Erythrocyte ω-3 index and plasma homocysteine concentrations were designated as the main outcomes based on evidence showing their capacity in underlying an attenuation in CD during aging.[5–7] They were measured at baseline, 6 months, and 1 year. Erythrocyte ω-3 PUFA was measured by capillary fast gas chromatography–Flame Ionization Detector (GC-FID) method. The ω-3 index was calculated as the sum of %DHA and %EPA, expressed as the percentage of total erythrocyte membrane fatty acids. Total plasma homocysteine was measured in µmol/L using a commercially available immunoassay kit (Architect 1L71, Abbott). Measurements were performed by trained professionals. (Further details are given in the Supplementary materials.)

2.6 | Outcomes: Cognitive tests

Secondary outcomes included the composite cognitive score (CCS); the Patient-Reported Outcomes Measurement Information System (PROMIS) Applied Cognition-Abilities instrument version 1.0; and the Cognitive Function Instrument (CFI) self-assessment test. The CCS was based on the following neuropsychological outcomes: sum of free recall and of total recall from the Free and Cued Selective Reminding Test (FCSRT); MMSE orientation score (10 items); Wechsler Adult Intelligence Scale (WAIS)-IV coding test (Digit Symbol Substitution); 2-minute Category Naming Test (CNT). Combined, these four tests assess timed executive function, episodic memory and global cognition. A Z-score was calculated for each test and the CCS was defined as the sum of Z-scores divided by four (higher scores indicating better function).[21] (Further information about the cognitive outcomes is displayed in Supplementary materials.)

Besides these a priori defined secondary outcomes, the CFI study partner questionnaire was included as an exploratory outcome. It includes the same 14 questions as the CFI self-assessment, but answered by the participant's partner. The CFI instruments and the CCS were assessed at baseline, 6 months, and 1 year.

2.7 | Outcomes: Brain imaging

Secondary outcomes included brain measures assessed by magnetic resonance imaging (MRI): hippocampal volume (HV) (right, left and total) and AD signature cortical thickness (CT). The AD signature CT was composed of four different regions of interest (ROIs): entorhinal, inferior temporal, middle temporal, and fusiform, and calculated as the sum of CT multiplied by volume estimates of each hemisphere and each ROI, divided by the sum of volume of all ROIs. MRI was performed on a voluntary basis among a subgroup at baseline ($n = 284$) and 1 year ($n = 183$), using the same imaging protocols and processing pipelines

from the Alzheimer's Disease Neuroimaging Initiative (ADNI) (http://adni.loni.usc.edu/methods/mri-tool/mri-analysis). (Details about MRI are available in Supplementary materials.)

2.8 | *APOE* ε4 status and nutritional deficits

The effect of intervention was also studied in exploratory analyses according to apolipoprotein E (*APOE*) ε4 status (carrier vs noncarrier) and to the presence of nutritional deficits at baseline: low ω-3 index (≤5.69%, lowest quartile)[22,23]; high homocysteine (>14 μmol/L)[23,24]; and low 25 hydroxyvitamin D concentrations (25(OH)D; <20 ng/mL).[23,25] For this purpose, a whole blood sample ≥1 mL was collected at baseline for DNA analyses including *APOE* ε4 status. 25(OH)D concentrations were determined using a commercially available immunoassay kit (Architect 5P02, Abbott). Intervention was also tested by combining the nutritional deficits (≥2 vs <2).

2.9 | Statistical analysis

Descriptive statistics (means and SD, frequencies, and percentages) were used. Efficacy analyses were done on a modified intention-to-treat (mITT) basis (i.e., including all randomly assigned participants with outcomes measured at baseline who completed at least one post-baseline visit). Linear mixed-effects regression (with a random subject intercept and, if significant, a random linear slope and a random center intercept) adjusted on baseline data were performed in the mITT population to determine the effect of intervention compared to placebo on outcomes. For each model, the following fixed effects were included: baseline value, intervention group, time (continuous variable), and interaction between group and time. All available data were included in the models (baseline, 6 months, and 12 months). Analyses on HV were adjusted additionally for baseline total intracranial volume. For CFI, an imputation by the worst modality ("1") was made in case of one missing item, what allowed to recover between 10% and 13% of the subjects at each visit. Exploratory analyses according to *APOE* ε4 status and nutritional deficits were performed. The Statistical Analysis Software (SAS) version 9.4 (Cary, NC, USA) was used. Statistical significance was set as 5%.

The Nolan Study was designed originally to last for 4 years but was restricted posteriorly to 1 year because of funding limitations. Due to the limited duration, the primary outcome of the trial was changed from CD measured by a CCS to the aforementioned nutritional biomarkers. (Sample size calculation is available in the Supplementary materials.)

3 | RESULTS

3.1 | Characterization of the sample and adherence to intervention

Baseline characteristics of participants according to randomized groups are presented in Table 1. From the 362 participants originally

enrolled (mean 78.3 years, SD = 4.8; 58.6% female), 57 dropped out during follow-up (*n* = 26 intervention, *n* = 31 control), corresponding to a mean drop-out rate of 15.7%. Thus 305 participants completed the trial (*n* = 154 intervention, *n* = 151 control) (Figure 1).

Mean compliance to intervention for sachet and capsule consumption after 1 year was, respectively, 79.0% and 81.4% (active group), and 78.5% and 81.8% (control group). Good compliance (≥80%) was presented by 72.1% of participants in the intervention group and 74.2% in placebo group at the end of follow-up.

3.2 | Effect of intervention on nutritional biomarkers

After 1 year, supplementation positively affected the nutritional biomarkers, providing an increase in ω-3 index (between-group differences: 2.7%, 95% confidence interval [CI] 2.3 to 3.0; *P* < .0001) and a decrease in homocysteine levels (−3.2 μmol/L, 95% CI −4.0 to −2.4; *P* < .0001) (Table 2, Figure S1A,B). From subjects with low ω-3 index at baseline (*n* = 36 intervention, *n* = 36 control), 72.2% in the active group and 2.8% in the placebo group changed to the normal range after 1 year. For those with high homocysteine (*n* = 39 intervention, *n* = 34 control), 74.4% of participants in the active group and 35.3% in the placebo group changed to normal range.

3.3 | Effect of intervention on cognitive tests

Despite the beneficial effects on nutritional biomarkers related to cognitive function, supplementation did not show an effect on the CCS or the CFI self-assessment. On the other hand, for the CFI study partner score a significant between-group difference (−0.48, 95% CI −0.95 to −0.01; *P* = .044) favoring the intervention group was found. The PROMIS T-score (weekly measured during the first month of follow-up), in contrast, provided distinct findings. It did not change among participants receiving the NB after 1 month, but significantly increased among participants in the placebo group (indicating improvement) (between-group difference: −1.17, 95% CI −2.20 to −0.14; *P* = .026) (Table 2, Figure S1C–F).

3.4 | Effect of intervention on brain imaging measures

Intervention did not have a significant effect on AD signature CT or on HV (Table 2). A trend was observed in between-group difference in the left HV (42.4 mm^3, 95% CI −0.1 to 84.8; *P* = .051), suggesting higher annual rate of atrophy in the placebo group.

3.5 | Effect of intervention according to *APOE* ε4 status and to nutritional deficits

Analyses according to *APOE* ε4 status and to presenting low ω-3 index, high homocysteine, or low 25(OH)D at baseline are shown in

TABLE 1 Baseline characteristics of participants of the Nolan Study (modified intention-to-treat population)

	Total n = 362 mean (SD)*	Nutritional blend n = 180 mean (SD)*	Placebo n = 182 mean (SD)*	P-value
Female sex	212 (58.6%)	104 (57.8%)	108 (59.3%)	.763[d]
Age (years)	78.3 (4.8)	78.4 (4.9)	78.2 (4.8)	.724[e]
Education (n = 361)				
<4 years	4 (1.1%)	2 (1.1%)	2 (1.1%)	.836[f]
4 to 7 years	28 (7.8%)	16 (8.9%)	12 (6.6%)	
8 to 9 years	61 (16.9%)	28 (15.6%)	33 (18.2%)	
≥10 years	268 (74.2%)	134 (74.4%)	134 (74.0%)	
Weight (kg)	69.5 (13.1)	69.5 (12.6)	69.4 (13.6)	.917[g]
Body mass index (kg/m^2)	26.1 (4.0)	26.1 (4.1)	26.2 (4.0)	.910[g]
APOE ε4 status (n = 341)				
ε4 carrier	83 (24.3%)	38 (22.2%)	45 (26.5%)	.361[d]
ε4 non-carrier	258 (75.7%)	133 (77.8%)	125 (73.5%)	
Biomarkers				
Plasma homocysteine (μmol/L) (n = 356)	12.3 (4.1)	12.4 (4.5)	12.2 (3.7)	.833[g,h]
Erythrocyte ω-3 index** (%) (n = 321)	6.5 (1.3)	6.5 (1.2)	6.5 (1.3)	.726[g]
25 hydroxyvitamin D (ng/mL) (n = 356)	26.2 (10.9)	26.6 (11.6)	25.9 (10.3)	.559[g]
Cognitive tests				
CFI self-assessment score (n = 342)	4.4 (2.6)	4.5 (2.6)	4.4 (2.6)	.689[g]
CFI study partner score (n = 330)	3.4 (2.7)	3.6 (2.7)	3.2 (2.7)	.067[e]
PROMIS T-score (n = 337)	50.8 (6.1)	50.9 (6.1)	50.7 (6.1)	.749[g]
Composite cognitive score*** (n = 361)	0.01 (0.70)	0.00 (0.72)	0.01 (0.69)	.936[g]
MMSE score	28.1 (1.6)	28.1 (1.7)	28.1 (1.6)	.964[e]
CDR status				
Cognitively normal (CDR = 0)	144 (39.8%)	72 (40.0%)	72 (39.6%)	.932[d]
Mild cognitive impairment (CDR = 0.5)	218 (60.2%)	108 (60.0%)	110 (60.4%)	
Brain imaging[a]				
Hippocampal volume (left) (mm^3) (n = 280)[b]	3477.7 (448.6)	3465.4 (435.9)	3490.4 (462.9)	.642[g]
Hippocampal volume (right) (mm^3) (n = 280)[b]	3595.2 (466.2)	3591.8 (439.0)	3598.7 (494.2)	.903[g]
Total hippocampal volume (mm^3) (n = 280)[b]	7072.9 (878.3)	7057.2 (836.9)	7089.0 (921.7)	.762[g]
AD signature cortical thickness**** (mm) (n = 238)[c]	2.8 (0.2)	2.8 (0.2)	2.8 (0.1)	.262[g]

Abbreviations: AD, Alzheimer's disease; CDR, Clinical Dementia Rating; CFI, Cognitive Function Instrument; MMSE, Mini-Mental State Examination (score varies from 0 to 30); PROMIS, Patient-Reported Outcomes Measurement Information System Applied Cognition-Abilities.

*Except where indicated otherwise. **ω-3 index was calculated as the sum of % docosahexaenoic acid and % eicosapentaenoic acid, expressed as the percentage of total erythrocyte membrane fatty acids. ***Based on four cognitive tests (free and total recall of the Free and Cued Selective Reminding test, 10 Mini-Mental State Examination orientation items, Digit Symbol Substitution Test, and Category Naming Test. ****Alzheimer's disease signature cortical thickness was composed of the mean cortical thickness of left and right hemispheres of four different regions of interest: entorhinal, inferior temporal, middle temporal, and fusiform weighted by each region's volume.

[a] Imaging cohort was not different for baseline characteristics presented in this table compared to participants who did not perform MRI, except for age (77.9 vs. 80.0 years; P = .001).

[b] n = 142 in the nutritional blend group and n = 138 in the placebo group.

[c] n = 117 in the nutritional blend group and n = 121 in the placebo group.

[d] Chi-square test.

[e] Kruskal-Wallis test.

[f] Fisher exact test.

[g] Equal variance two sample t-test.
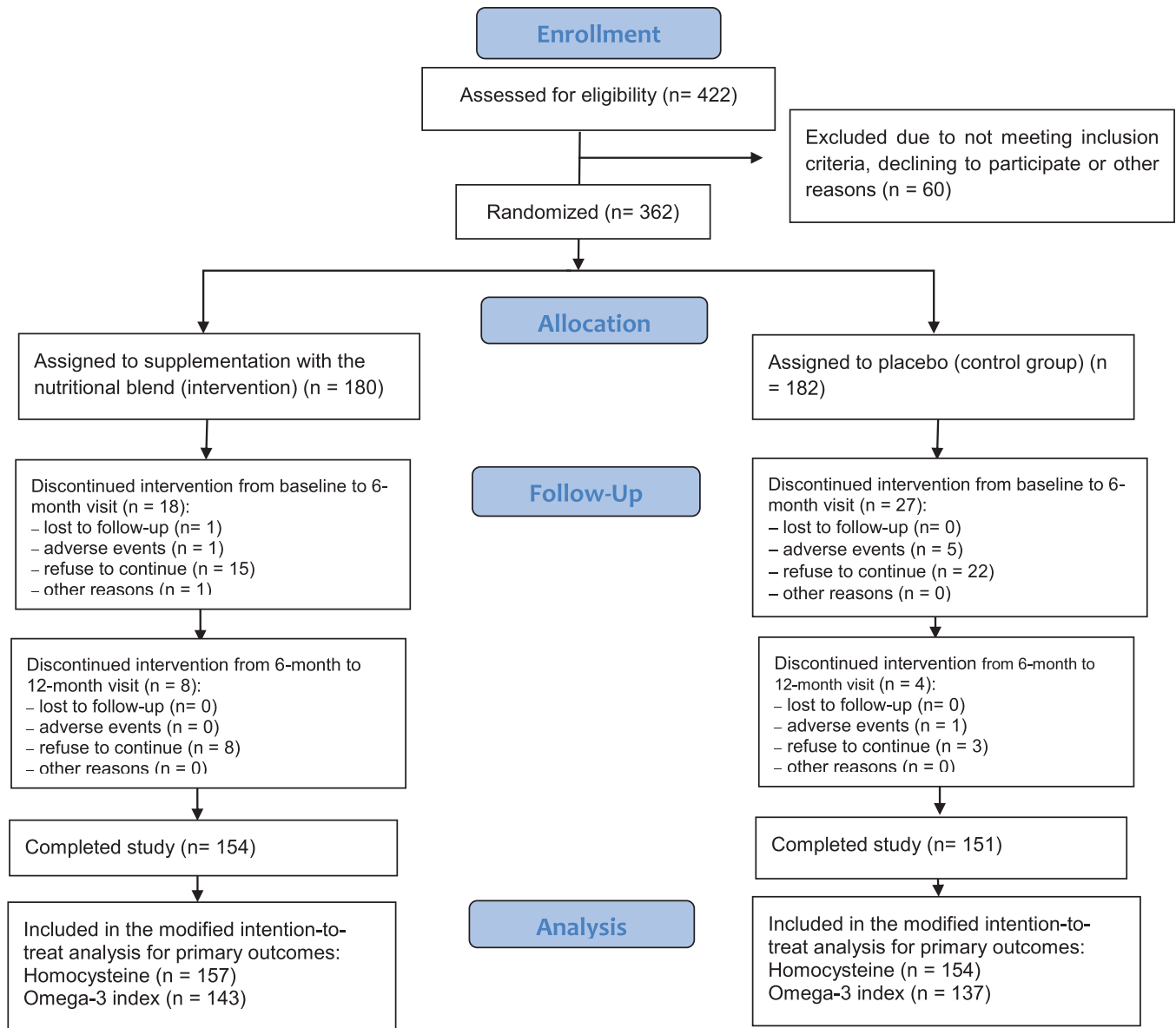
[h] P-value with log transformation.

Enrollment

Assessed for eligibility (n= 422)

Excluded due to not meeting inclusion criteria, declining to participate or other reasons (n = 60)

Randomized (n= 362)

Allocation

Assigned to supplementation with the nutritional blend (intervention) (n = 180)

Assigned to placebo (control group) (n = 182)

Follow-Up

Discontinued intervention from baseline to 6-month visit (n = 18):
– lost to follow-up (n= 1)
– adverse events (n = 1)
– refuse to continue (n = 15)
– other reasons (n = 1)

Discontinued intervention from baseline to 6-month visit (n = 27):
– lost to follow-up (n= 0)
– adverse events (n = 5)
– refuse to continue (n = 22)
– other reasons (n = 0)

Discontinued intervention from 6-month to 12-month visit (n = 8):
– lost to follow-up (n= 0)
– adverse events (n = 0)
– refuse to continue (n = 8)
– other reasons (n = 0)

Discontinued intervention from 6-month to 12-month visit (n = 4):
– lost to follow-up (n= 0)
– adverse events (n = 1)
– refuse to continue (n = 3)
– other reasons (n = 0)

Completed study (n= 154)

Completed study (n= 151)

Analysis

Included in the modified intention-to-treat analysis for primary outcomes:
Homocysteine (n = 157)
Omega-3 index (n = 143)

Included in the modified intention-to-treat analysis for primary outcomes:
Homocysteine (n = 154)
Omega-3 index (n = 137)

FIGURE 1 Flow diagram describing the Nolan Study population

Tables 3–5 and Table S2, respectively. The effect of intervention within individuals with and without combined nutritional deficits are reported in Table S3. In general, the positive effect on biomarkers was similar to the main analyses, and no effect was observed for the CCS and the CFI self-assessment, regardless of the *APOE ε*4 status or the presence of deficits. The positive effect of intervention on the CFI study partner was observed only among those with high homocysteine (Table 5). The unexpected finding with the PROMIS T-score in the main analysis was observed only among *APOE ε*4 non-carriers (Table 3), those with normal 25(OH)D (Table S2), and those with one or no nutritional deficit (Table S3). No difference was observed in the effect of treatment between the subgroups.

4 | DISCUSSION

This study assessed the efficacy of a 1-year NB supplementation on erythrocyte ω-3 index and homocysteine levels, as well as on cognitive outcomes among community-dwelling older adults, and successfully showed a positive effect of intervention in increasing ω-3 index and decreasing homocysteine concentrations. Some effects were observed in secondary outcomes, but intervention was not able to prevent CD as measured by neuropsychological tests.

The NB provided to participants was composed of several nutrients known to be linked to cognitive function through multiple mechanisms. DHA and EPA have been associated with a multitude of

TABLE 2 Mixed-effects linear regression analysis for change from baseline in primary, secondary, and exploratory outcome measures according to randomized intervention groups among the modified intention-to-treat population of non-demented, community-dwelling older adults

	Estimated mean within-group change from baseline[a] (95% CI); *P*-value		Between-group difference over time (95% CI); *P*-value
	Nutritional blend	Placebo	Nutritional blend vs. placebo
Biomarkers			
Homocysteine (μmol/L)	−2.24 (−2.81, −1.68; <**0.0001**	0.96 (0.38, 1.53); **.001**	−3.20 (−4.01, −2.39); <**.0001**
ω-3 index[b] (%)	1.53 (1.25, 1.80); <**.0001**	−1.13 (−1.41, −0.85); <**.0001**	2.66 (2.27, 3.05); <**.0001**
Cognitive tests			
PROMIS T-score (8 items)*	−0.03 (−0.75, 0.70); .945	1.15 (0.41, 1.88); **.002**	−1.17 (−2.20, −0.14); **.026**
Composite cognitive score[c]	−0.10 (−0.18, −0.02); **.016**	−0.10 (−0.18, −0.01); **.022**	0.00 (−0.12, 0.11); .953
CFI self-assessment score	−0.49 (−0.79, −0.19); **.001**	−0.38 (−0.69, −0.07); **.015**	−0.11 (−0.54, 0.31); .606
CFI study partner total score	−0.34 (−0.80, 0.12); .134	0.14 (−0.34, 0.62); .544	−0.48 (−0.95, −0.01); **.044**
Brain imaging[d]			
Hippocampal volume left (mm^3)	−36.02 (−65.98, −6.06); **.019**	−78.39 (−108.31, −48.46); <**.0001**	42.37 (−0.10, 84.83); .051
Hippocampal volume right (mm^3)	−40.93 (−71.86, −10.01); **.010**	−55.71 (−86.63, −24.79); **.001**	14.78 (−29.04, 58.60); .507
Total hippocampal volume (mm^3)	−75.47 (−126.45, −24.49); **.004**	−135.00 (−185.95, −84.04); <**.0001**	59.53 (−12.72, 131.78); .106
AD signature CT[e] (mm)	−0.01 (−0.02, 0.01); .357	−0.03 (−0.04, −0.01); **0.001**	0.02 (0.00, 0.04); .088

Abbreviations: AD, Alzheimer's disease; CFI, Cognitive Function Instrument; CT, cortical thickness; PROMIS, Patient-Reported Outcomes Measurement Information System Applied Cognition-Abilities.

*Change after 1 month, and all other outcomes after 12 months of follow-up.

[a]Estimated with the mean at baseline.

[b]ω-3 index was calculated as the sum of % docosahexaenoic acid and % eicosapentaenoic acid, expressed as the percentage of total erythrocyte membrane fatty acids.

[c]Based on four cognitive tests (free and total recall of the Free and Cued Selective Reminding test, ten Mini-Mental State Examination orientation items, Digit Symbol Substitution Test and Category Naming Test.

[d]Magnetic resonance imaging was performed in a subsample (in the present analysis, *n* = 177 for hippocampal volume and *n* = 145 for AD signature cortical thickness). Analyses on hippocampal volume and AD signature CT were adjusted for baseline total intracranial volume.

[e]Alzheimer's disease signature cortical thickness was composed by the mean cortical thickness of left and right hemispheres of four different regions of interest: entorhinal, inferior temporal, middle temporal, and fusiform weighted by each region's volume.

neurobiological effects related to brain aging, including the modulation of neuronal membrane fluidity, stimulation of neuroplasticity, anti-neuroinflammatory effects, and the reduction of brain oxidative stress.[9] B vitamins (specifically B6, B12, and folate),[4] as well as choline[10] have shown an ability to decrease homocysteine concentrations. Antioxidants such as vitamin C,[11] vitamin E,[12] and selenium[13] were shown to protect against oxidative damage and inflammation in brain. Vitamin D deficiency, in turn, is associated with all-cause dementia.[14] Finally, the non-essential amino acid citrulline (a nitric oxide precursor) has shown beneficial effects on the cardiovascular system, specifically in terms of improving blood flow, endothelial function, and blood pressure,[26] and might also be involved in the biological mechanisms of neurodegeneration.[27] Altogether, the aforementioned nutrients particularly benefited the biomarkers after 1 year. Notwithstanding, and in accordance with our findings, some meta-analyses have concluded that solely lowering homocysteine does not guarantee preventing CD or dementia.[4,6] On the other hand, a more recent meta-analysis has identified homocysteine-lowering treatments (based on folic acid, vitamin B12, and vitamin B6 supplementation) as promising interventions for AD prevention.[28]

Similarly, benefits of ω-3 PUFA are not warranted by a simple increase in its concentrations. In this sense, a systematic review and meta-analysis evaluating trials of ω-3 supplementation was not able to observe a positive effect on global cognitive function, despite some studies showing positive effects in raising erythrocyte or plasma ω-3.[29] Thus a sufficient decrease in homocysteine and increase in ω-3 index that would lead to identifiable positive effects in cognitive function still remains to be defined.

Although the present trial was able to impact its primary outcomes, results should be interpreted in the context of the study design. The duration of follow-up may have been insufficient to verify a positive effect of nutrients in cognitive tests and brain imaging outcomes. Similarly, no overall cognitive improvement was found after a 6-month intervention with a supplement (composed of ω-3 PUFA, vitamin D, resveratrol, and whey protein) among Irish older adults.[30] Indeed, a meta-analysis of animal studies specifically evaluating ω-3 supplementation on cognitive function, brain Aβ deposition, and hippocampal neuron loss suggested that exposure to nutrient supplementation in intervention studies with humans may be too short (around 0.5% to 1.8% of the lifespan of participants).[31] However, matching the

TABLE 3 Mixed-effects linear regression analysis for change from baseline in outcome measures according to randomized intervention groups and APOE ε4 status among the modified intention-to-treat population of non-demented, community-dwelling older adults

| | Estimated mean within-group change from baseline[a] (95% CI); P-value | | | | Between-group difference over time (95% CI); P-value | | Difference in between-group differences over time for APOE ε4 carrier vs. non-carrier |
| | Nutritional blend | | Placebo | | Nutritional blend vs. placebo | | |
	APOE ε4 non-carrier	APOE ε4 carrier[b]	APOE ε4 non-carrier	APOE ε4 carrier[b]	APOE ε4 non-carrier	APOE ε4 carrier[b]	
Biomarkers							
Plasma homocysteine (μmol/L)	−2.40 (−3.05, −1.75); <.0001	−2.01 (−3.23, −0.79); .001	1.11 (0.43, 1.79); .001	0.15 (−0.97, 1.28); .789	−3.51 (−4.45, −2.57); <.0001	−2.16 (−3.82, −0.50); .011	1.35 (−0.56, 3.26); .164
ω-3 index[c] (%)	1.51 (1.20, 1.83); <.0001	1.87 (1.25, 2.49); <.0001	−1.15 (−1.47, −0.82); <.0001	−1.04 (−1.60, −0.48); .0003	2.66 (2.21, 3.11); <.0001	2.91 (2.08, 3.75); <.0001	0.25 (−0.70, 1.20); 0.601
Cognitive tests							
PROMIS T-score[d] (8 items)	0.13 (−0.73, 0.98); .770	−0.26 (−1.76, 1.25); .737	1.37 (0.48, 2.26); .003	0.78 (−0.66, 2.21); .287	−1.25 (−2.48, −0.01); .048	−1.03 (−3.11, 1.04); .328	0.21 (−2.20, 2.63); .861
Composite cognitive score[e]	−0.09 (−0.19, 0.01); .069	−0.15 (−0.33, 0.04); .114	−0.11 (−0.21, −0.01); .035	−0.10 (−0.27, 0.07); .234	0.02 (−0.12, 0.16); .782	−0.05 (−0.29, 0.20); .719	−0.07 (−0.35, 0.22); .653
CFI self-assessment score	−0.58 (−0.92, −0.24); .001	−0.25 (−0.90, 0.40); .449	−0.35 (−0.71, 0.01); .055	−0.61 (−1.24, 0.02); .056	−0.23 (−0.72, 0.27); .369	0.36 (−0.54, 1.27); .430	0.59 (−0.44, 1.62); .262
CFI study partner score	−0.42 (−0.79, −0.04); .030	−0.77 (−1.49, −0.04); .038	0.10 (−0.30, 0.50); .624	−0.28 (−0.98, 0.42); .431	−0.52 (−1.07, 0.03); .066	−0.49 (−1.49, 0.52); .343	0.03 (−1.11, 1.18); .959

Abbreviations: CFI, Cognitive Function Instrument; PROMIS, Patient-Reported Outcomes Measurement Information System.

[a] Estimated with the mean at baseline.

[b] Defined as presenting at least one ε4 allele.

[c] Calculated as the sum of % docosahexaenoic acid and % eicosapentaenoic acid, expressed as the percentage of total erythrocyte membrane fatty acids.

[d] After 1 month, and all other outcomes after 12 months.

[e] Based on four cognitive tests (free and total recall of the Free and Cued Selective Reminding test, 10 Mini-Mental State Examination orientation items, Digit Symbol Substitution Test and Category Naming Test.

TABLE 4 Mixed-effects linear regression analysis for change from baseline in outcome measures according to randomized intervention groups and omega-3 status among the modified intention-to-treat population of non-demented, community-dwelling older adults

	Estimated mean within-group change from baseline[a] (95% CI); P-value				Between-group difference over time (95% CI); P-value		Difference in between-group differences over time for low vs. normal ω-3
	Nutritional blend		Placebo		Nutritional blend vs. placebo		
	Normal ω-3 index	Low ω-3 index[b]	Normal ω-3 index	Low ω-3 index[b]	Normal ω-3 index	Low ω-3 index[b]	low vs. normal ω-3
Biomarkers							
Plasma homocysteine (μmol/L)	−2.34 (−3.03, −1.66); <**.0001**	−1.65 (−2.82, −0.48); **.006**	0.68 (−0.03, 1.39); .060	1.93 (0.79, 3.07); **.001**	−3.03 (−4.01, −2.04); <**.0001**	−3.58 (−5.21, −1.94); <**.0001**	−0.55 (−2.46, 1.36); .572
ω-3 index[c] (%)	1.47 (1.14, 1.80); <**.0001**	1.69 (1.10, 2.29); <**.0001**	−1.04 (−1.38, −0.70); <**.0001**	−1.37 (−1.96, −0.78); <**.0001**	2.51 (2.06, 2.96); <**.0001**	3.06 (2.32, 3.81); <**.0001**	0.56 (−0.32, 1.43); .213
Cognitive tests							
PROMIS T-score[d] (8 items)	−0.30 (−1.19, 0.59); .509	0.81 (−0.73, 2.36); .301	0.97 (0.04, 1.89); **.040**	1.65 (0.09, 3.21); **.038**	−1.27 (−2.55, 0.02); .053	−0.84 (−3.03, 1.36); .453	0.43 (−2.11, 2.97); .740
Composite cognitive score[e]	−0.06 (−0.16, 0.04); .235	−0.18 (−0.35, −0.01); **.041**	−0.11 (−0.21, 0.00); **.041**	−0.04 (−0.20, 0.13); .653	0.05 (−0.10, 0.19); .513	−0.14 (−0.38, 0.10); .248	−0.19 (−0.47, 0.09); .184
CFI self-assessment score	−0.54 (−0.90, −0.18); **.003**	−0.47 (−1.09, 0.15); .136	−0.34 (−0.72, 0.04); .075	−0.43 (−1.03, 0.18); .168	−0.20 (−0.72, 0.32); .456	−0.05 (−0.91, 0.82); .916	0.15 (−0.86, 1.16); .770
CFI study partner score	−0.48 (−0.89, −0.07); **.021**	−0.66 (−1.34, 0.02); .058	0.00 (−0.43, 0.43); .998	−0.20 (−0.88, 0.48); .566	−0.48 (−1.07, 0.11); .112	−0.46 (−1.42, 0.50); .349	0.02 (−1.11, 1.15); .970

Abbreviations: CFI, Cognitive Function Instrument; PROMIS, Patient-Reported Outcomes Measurement Information System.

[a] Estimated with the mean at baseline.

[b] ω-3 index ≤5.69%.

[c] Calculated as the sum of % docosahexaenoic acid and % eicosapentaenoic acid, expressed as the percentage of total erythrocyte membrane fatty acids.

[d] After 1 month, and all other outcomes after 12 months.

[e] Based on four cognitive tests (free and total recall of the Free and Cued Selective Reminding test, 10 Mini-Mental State Examination orientation items, Digit Symbol Substitution Test and Category Naming Test.

TABLE 5 Mixed-effects linear regression analysis for change from baseline in outcome measures according to randomized intervention groups and homocysteine status among the modified intention-to-treat population of non-demented, community-dwelling older adults

| | Estimated mean within-group change from baseline[a] (95% CI); P-value | | | | Between-group difference over time (95% CI); P-value | | Difference in between-group differences over time |
| | Nutritional blend | | Placebo | | Nutritional blend vs. placebo | | for high vs. normal homocysteine |
	Normal homocysteine	High homocysteine[b]	Normal homocysteine	High homocysteine[b]	Normal homocysteine	High homocysteine[b]	
Biomarkers							
Plasma homocysteine (μmol/L)	−2.37 (−3.06, −1.68); **<.0001**	−1.82 (−3.18, −0.47); **.008**	0.44 (−0.24, 1.13); .207	2.64 (1.24, 4.03); **.0002**	−2.82 (−3.73, −1.90); **<.0001**	−4.46 (−6.10, −2.83); **<.0001**	−1.65 (−3.52, 0.23); .085
Omega-3 index[c] (%)	1.47 (1.16, 1.78); **<.0001**	1.71 (1.14, 2.29); **<.0001**	−1.11 (−1.42, −0.79); **<.0001**	−1.21 (−1.80, −0.62); **<.0001**	2.58 (2.14, 3.02); **<.0001**	2.92 (2.10, 3.75); **<.0001**	0.34 (−0.60, 1.28); .474
Cognitive tests							
PROMIS T-score[d] (8 items)	0.05 (−0.78, 0.89); .897	−0.42 (−1.95, 1.12); .591	1.09 (0.25, 1.93); **.011**	1.31 (−0.28, 2.91); .106	−1.03 (−2.22, 0.15); .086	−1.73 (−3.95, 0.48); .125	−0.70 (−3.21, 1.81); .584
Composite cognitive score[e]	−0.04 (−0.13, 0.06); .437	−0.30 (−0.45, −0.14); **.0003**	−0.06 (−0.15, 0.04); .245	−0.23 (−0.40, −0.06); **.009**	0.02 (−0.12, 0.15); .790	−0.06 (−0.30, 0.17); .587	−0.08 (−0.35, 0.19); .545
CFI self-assessment score	−0.59 (−0.93, −0.25); **.001**	−0.26 (−0.85, 0.33); .385	−0.58 (−0.92, −0.24); **.001**	0.42 (−0.24, 1.07); .210	−0.01 (−0.49, 0.47); .970	−0.68 (−1.56, 0.20); .131	−0.67 (−1.67, 0.33); .190
CFI study partner score	−0.37 (−0.76, 0.01); .057	−0.81 (−1.46, −0.16); **.014**	−0.10 (−0.49, 0.29); .612	0.34 (−0.40, 1.09); .362	−0.27 (−0.82, 0.27); .328	−1.16 (−2.15, −0.17); **.022**	−0.88 (−2.01, 0.25); .125

Abbreviations: CFI, Cognitive Function Instrument; PROMIS, Patient-Reported Outcomes Measurement Information System.

[a] Estimated with the mean at baseline.

[b] Homocysteine concentrations >14 μmol/L.

[c] Calculated as the sum of % docosahexaenoic acid and % eicosapentaenoic acid, expressed as the percentage of total erythrocyte membrane fatty acids.

[d] After 1 month, and all other outcomes after 12 months.

[e] Based on four cognitive tests (free and total recall of the Free and Cued Selective Reminding test, 10 Mini-Mental State Examination orientation items, Digit Symbol Substitution Test and Category Naming Test.

proportional duration of effective supplementation given to rodents (10%–50% of their lifespan) would be expensive and complex.[31] It is possible that the characteristics of our participants may also have exalted this relatively short-time condition: the population was composed of globally healthy individuals, for whom a 1-year time frame may not have been sufficient to detect important declines in cognitive function and brain imaging outcomes. Third, the composition of the daily dose may also be mentioned as another potential factor influencing the results. Despite promising findings from previous tests in animals,[19,20] it is possible that for some nutrients a higher dose would be needed to detect a effects among older adults. This may be the case of DHA and citrulline. A recent trial demonstrated a 28% increase in cerebrospinal fluid (CSF) DHA levels and a 200% increase in plasma DHA after a 6-month DHA supplementation of 2152 mg/day among cognitively unimpaired individuals at risk of dementia, and suggested that lower DHA doses (≤1 g/day) would less likely lead to meaningful DHA brain delivery.[32] Although no study to date has investigated the effect of citrulline supplementation on cognitive function, trials targeting physical function among adults ≥50 years of age showed an efficacy with doses varying from 5 g to 10 g/day, mainly among obese, dynapenic-obese, hypertensive, or malnourished individuals[33] (for comparison, Nolan's blend provided 770 mg/day of DHA and 3 g/day of citrulline).

Despite no effect on the CFI self-assessment or on the CCS, a positive effect of intervention on the exploratory CFI study partner was observed. Despite the modest, non-clinically relevant difference between groups, we might speculate whether such changes may precede objective cognitive changes, in a hypothesis that demands further exploring. About the unexpected finding with the PROMIS questionnaire at 1 month, it should be considered that, unlike the other questionnaires, the PROMIS was individually self-answered in the absence of a clinician or interviewer. Unfortunately, other outcomes were not measured at 1 month, preventing the investigation of whether this small but statistically significant result would translate into other measurable cognitive changes. Taken together, the findings from the cognitive tests indicate that the potential effect of NB remains to be explored in other human populations.

Of interest, analyses of imaging outcomes showed a trend in the left HV, suggesting a potential benefit of the blend in slowing hippocampal atrophy. This finding is consistent with the notion that brain changes precede changes in cognitive function. Despite the lack of studies evaluating HV changes after nutritional supplementation among cognitively normal older adults, some studies were performed with subjects presenting mild cognitive impairment (MCI).[34–36] The RCT Homocysteine and B Vitamins in Cognitive Impairment (VITACOG), providing B-vitamin supplementation to elderly subjects with MCI, observed a positive effect of treatment in bilateral hippocampal gray matter volume after 2 years, what authors credited to lowering mean plasma homocysteine concentrations by 29% after performing a voxel-based analysis.[34] Notably, after dividing participants according to their overall baseline median homocysteine, authors further showed that treatment was significantly beneficial (for whole gray matter) only for participants with higher homocysteine levels.[34] This group also

presented slower CD over time.[35] In another study, positive findings in both cognitive function and HV were seen after 1-year of DHA supplementation in older adults with MCI.[36]

The effect of intervention was also tested in an exploratory manner according to the presence of nutritional deficits (low ω-3 index, high homocysteine, low 25(OH)D), to investigate if individuals with one or multiple nutritional deficits should be specific targets for nutritional interventions. It is also possible that the minimum duration of effective nutritional interventions may vary according to the number and type of nutritional deficits, which could be explored in future investigations. This idea is reinforced by the findings of the VITACOG trial, which observed that higher concentrations of DHA significantly enhanced the effects of B-vitamin supplementation in cognitive outcomes and brain atrophy rates among older adults with MCI, whereas treatment had no effect on individuals with low ω-3 PUFA.[17,37] In addition, a trial with patients with AD found that ω-3 supplementation improved cognitive performance (measured by MMSE) only among participants with lower homocysteine concentrations, suggesting that adequate B-vitamin status is required to obtain beneficial effects of ω-3 PUFA on cognition.[38] These important results highlight the need for fighting nutritional deficits for optimizing possible interventions. In this context, the multitarget nutritional intervention is a key difference of our trial compared to previous studies.

The originality of this trial using a combined approach targeting nutritional biomarkers primarily, neuropsychological tests, cognitive patient-reported outcomes, and brain imaging outcomes among older adults should be noted as a strength of this study, and also its randomized, controlled design. Moreover, many other secondary and exploratory outcomes of cognitive function (biomarkers, clinical tests, subjective tests, and brain imaging) were assessed and will support further analyses to help build evidence concerning the nutritional aspects associated with cognitive aging. However, some limitations must be noted. As mentioned previously, the 1-year duration of intervention was not adequate to evidence clinically meaningful results. Given that our sample size calculation was based on primary outcomes (biomarkers), it is possible that it did not achieve adequate statistical power for identifying changes in cognitive tests. As an intrinsic limitation of RCT, adherence decreased with time. The MRI was only performed in a subgroup of participants. Finally, as expected in a preventive trial, education level was particularly high, implicating a high level of cognitive reserve that might have possibly contributed to a slower CD in this sample.

5 | CONCLUSIONS

This 1-year trial found positive effects of a daily NB supplementation on nutritional biomarkers, but was not able to show improvements in cognitive function among dementia-free, community-dwelling older adults. Given the evidence on the ability of high homocysteine and low ω-3 index in predicting impairments in cognitive function, enhancing both biomarkers in a 1-year range can be possibly considered an initial step on protecting cognition over aging. Findings may help to

set nutritional strategies for optimizing brain health and preventing or slowing CD, as also identifying individuals to whom specific nutrient supplementation may be more effective. Additional investigations are needed, especially trials with longer follow-up periods that target subjects who are *APOE ε4* carriers and with nutritional deficiencies.

6 | Nolan/DSA Study group

6.1 | Nolan Study group

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CONFLICT OF INTEREST
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AUTHOR CONTRIBUTIONS
Sandrine Andrieu, Bruno Vellas, and Jeroen A. J. Schmitt conceived the Nolan Study and designed the research; Duygu Tosun conducted the research; Christelle Cantet analyzed the data; Kelly V. Giudici wrote the paper; Kelly V. Giudici and Christelle Cantet had primary responsibility for the final content. Kelly V. Giudici, Sophie Guyonnet, Christelle Cantet, Philipe de Souto Barreto, Michael W. Weiner, Duygu Tosun, Corina Boschat, Julie Hudry, Sandrine Andrieu, Bruno Vellas,

and Jeroen A. J. Schmitt interpreted the data and revised the draft of the paper critically for intellectual content. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.