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A Phase II Randomized Clinical Trial of a Nutritional Formulation for Cognition and Mood in Alzheimer's Disease

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Abstract.
Background: Increasing evidence points toward the efficacy of nutritional modifications in delaying cognitive decline and mood/behavioral difficulties in Alzheimer's disease (AD). Nutritional supplementation with individual agents has shown varied results suggesting the need for combinatorial intervention.
Objective: We set out to determine whether nutritional intervention could positively impact cognitive performance and behavioral difficulties for individuals diagnosed with AD.
Methods: A double-blind, multi-site, phase II study (ClinicalTrials.gov NCT01320527; Alzheimer's Association Trialmatch) was conducted in which 106 individuals with AD were randomized to a nutraceutical formulation (NF; folate, alpha-tocopherol, B12, S-adenosyl methioinine, N-acetyl cysteine, acetyl-L-carnitine) or placebo for 3 or 6 months, followed by an open-label extension where participants received NF for 6 additional months.
Results: The NF cohort improved versus the placebo cohort within 3 months (Clox-1 $p = 0.0083$, 95%CI [0.4481, 2.9343]; Dementia Rating Scale $p = 0.0266$, 95%CI [0.1722, 2.7171]). Caregivers reported non-significant improvements in Neuropsychiatric Inventory. Both cohorts improved or maintained baseline performance during open-label extensions. Activities of Daily Living did not change for either cohort.
Conclusions: These findings extend phase I studies where NF maintained or improved cognitive performance and mood/behavior.

Keywords: Acetyl-L-carnitine, Alzheimer's disease, B vitamins, behavioral difficulties, cognitive decline, N-acetyl cysteine, nutrition, supplement, S-adenosyl methionine, vitamin E

INTRODUCTION

Alzheimer's disease (AD) is characterized by cognitive decline accompanied by mood and behavioral and psychological symptoms of dementia (BPSD) [1].

It is increasingly recognized that interventions for dementia must shift toward prevention for maximal efficacy and disease modification [1–3]. By contrast, current pharmacological interventions require prior cognitive decline before administration is appropriate

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[4]. Increasing evidence points toward the efficacy of nutritional modifications in delaying cognitive decline and BPSD in dementia [4, 5].

We previously conducted phase I studies, encompassing five separate trials, in which a combinatorial nutraceutical formulation [NF; folate, alpha-tocopherol, B12, S-adenosyl methionine (SAM), N-acetyl cysteine (NAC) and acetyl-L-carnitine (ALCAR)] improved cognitive performance and BPSD with no serious adverse events [6–8]. In these studies, community-dwelling individuals with mild-moderate AD statistically improved in cognitive performance and BPSD within 6 months and maintained improvement for 28 months [6]. NF significantly delayed cognitive decline and moderately improved BPSD over 9 months for individuals with moderate to advanced AD [7]. Community-dwelling individuals with no known or suspected dementia randomized to NF displayed significantly improved cognitive performance within 3 months (the earliest time tested) versus those randomized to placebo [8]. In these studies, code was broken at this point, and the initial placebo cohort received NF for 3 months in an open-label extension, after which they displayed improvement identical to that of the initial NF group. The initial NF cohort demonstrated further improvement during this open-label extension. Participants declined to baseline following NF withdrawal for 3 months and resumed significant improvement within 3 months after NF restoration. During subsequent examination of earlier intervals, an additional cohort of adults with no known or suspected dementia randomized to NF displayed significant improvement in cognitive performance within 2 weeks versus those randomized to placebo. In a two-week open label extension, the initial placebo cohort displayed identical improvement in cognitive performance to that of the initial randomized NF cohort, and the initial NF cohort demonstrated further statistical improvement [8]. A third cohort of adults with no known or suspected dementia receiving NF under open label conditions demonstrated improvement in cognitive performance statistically identical to the above cohort receiving NF under double-blind conditions [8].

Based on the positive findings in all of our phase I investigations, we carried out a phase II with NF which included 106 individuals diagnosed with AD.

METHODS

Intervention

NF consisted of 400 μg folic acid, 6 μg B12, 30 I.U. alpha-tocopherol, 400 mg SAM (200 mg active ion), 600 mg NAC, and 500 mg ALCAR (Nutricap Labs, Farmingdale, NY; USP grade; FDA-approved, cGMP conditions) with 2 tablets/daily dose; placebo tablets consisted of the identically appearing inert ingredients distinguishable only by lot number [6–8]. Participants were randomized to NF or placebo for 3–6 months, with subsequent open-label extensions as described in detail below.

Trial registration

This study was registered with ClinicalTrials.gov (NCT01320527) and the Alzheimer's Association's TrialMatch (http://alz.org/Trialmatch).

Inclusion/exclusion criteria

Inclusion criteria consisted of these findings: dementia (e.g., probable AD and/or senile dementia of the Alzheimer type), personal physicians' approval to participate, ability to swallow pills, availability of a personal or professional caregiver, and signed consent from the participant or health-care proxy. Exclusion criteria consisted of inability to swallow pills and known or suspected bipolar disorder (for which SAM is contraindicated) [9].

Participant demographics

Individuals diagnosed with AD ($n = 106$; aged 77.8 ± 8.4 years; 13.6 ± 2.3 years education) were recruited from 6 sites (nursing homes, assisted living facilities, senior centers, and private clinics) along with community-dwelling residents. All participants completed the Mini-Mental State Examination (MMSE) at their baseline visit (Table 1). Participants were randomized to NF or placebo and tested between March 2010 and April 2012. Participant numbers were restricted by amount and duration of funding.

Table 1
Baseline participant demographics of total participant population and NF and placebo cohorts following randomization

	n	Age*	Education (y)*	MMSE*
Total participant population	106	77.8 ± 9.3	13.6 ± 2.1	22.2 ± 5.1
Randomized to NF	62	78.7 ± 7.9	14.0 ± 3.1	22.2 ± 5.5
Randomized to placebo	44	79.7 ± 8.6	13.6 ± 2.7	22.2 ± 6.0

*values represent the mean ± standard deviation.

Outcome measures

Primary outcome was defined as cognitive performance, ascertained by participant performance on Clox-1 and the age- and education-adjusted (AEMSS) Dementia Rating Scale (DRS) [10, 11]. Secondary outcomes were defined as BPSD and daily function, ascertained by caregiver completion of the Neuropsychiatric Inventory (NPI) and the Alzheimer's Disease Cooperative Study – Activities of Daily Living (ADL) [2, 13]. Tests were completed at baseline and at 3-month intervals until 12 months. Primary analysis was Intention-To-Treat; all enrolled participants were included in analysis, subject to attrition. All instruments have established reliability and validity and were utilized in our phase I studies [6, 7]. RR communicated with all coordinators at all sites regarding administration of test instruments.

Randomization and masking

NF and placebo were distributed to individual sites under blind conditions. Participants selected a random sealed bag containing NF or placebo from a group of bags while respective site coordinators recorded the lot number. Following randomization, the NF and placebo cohorts displayed an identical range of age, education, and baseline MMSE (Table 1). Participants were tested from March 2010 through April 2012. Participant test results were communicated to RR as two cohorts without revealing their distribution code. The protocol was approved by the New England IRB (Newton, MA) and by respective institutional review boards.

Study endpoints

Our phase I studies previously demonstrated that NF statistically improved cognitive performance and BPSD within 3 months [6–8]. Based on this prior efficacy, if NF was indeed effective, we anticipated statistical improvement within 3–6 months [6–8]. Our protocol specified that code would be broken after 6 months, and all participants (including placebo) would receive NF under open-label conditions for the duration of the study. Our protocol further specified that, should comparison of performance of NF versus placebo cohorts following this 6-month code break, it would reveal that statistical improvement had actually been achieved within the first 3 months, randomization would be halted at 3 months, and open-label extensions would be initiated for all subsequent participants. Comparison of performance of the first participants ($n = 20$) to reach 6 months revealed statistical improvements in primary outcome for participants receiving NF within the first 3 months (delta of 1.7 ± 0.6; $p = 0.03$). Randomization was therefore halted at 3 months for all subsequent participants and open-label extensions were initiated as specified in our protocol. This resulted in initial cohorts randomized for 6 months, and subsequent cohorts randomized for 3 months, both of which were followed by respective open-label extensions. Initial placebo cohorts are referred to as "delayed start" cohorts during these open-label extensions [8]. Results are presented separately for each cohort, as well as pooled for the entire participant pool at baseline and 3 months. Team members administering Clox-1 and DRS were blinded to treatment groups prior to code break and caregivers completing NPI and ADL were also blinded to treatment groups prior to code break.

Based on prior efficacy, and efficacy observed during the course of the present study, a company expressed interest in marketing NF toward the end of this study. To maximize NF availability to the public, we elected to close the study early to avoid any potential conflict of interest on the part of the sponsoring institution (UMass Lowell) and lead investigator (TBS), who share a financial interest in NF. Notably, closure at that time prevented only a single participant from completing 3 months of randomization. Furthermore, the majority of participants completed all or portions of their respective open-label extensions (Fig. 1). Study closure preceded licensing agreements between UMass Lowell and the company.

Statistical analyses

Data were independently analyzed by statisticians (CM and RP) not involved in data acquisition. Methods included paired Student's *t*-tests (2-tailed) of individual participant performance versus baseline, unpaired 2-tailed *t*-tests of NF versus placebo cohorts with confidence intervals, and Cohen's effect size (calculated as [(cohort mean at treatment time) - (cohort mean at baseline)]/standard deviation at baseline of the entire participant pool; p values <0.05 and effect sizes >0.2 were considered significant. Based on previous effect sizes >0.8 [6, 7], our total participant size (106) exceeds the 90 participants required to detect statistical significance ($p < 0.05$ and power of 0.8) [14]. Participants/caregivers completing each test vary both among tests and sampling intervals due to attrition (see figure legends for respective participant numbers).

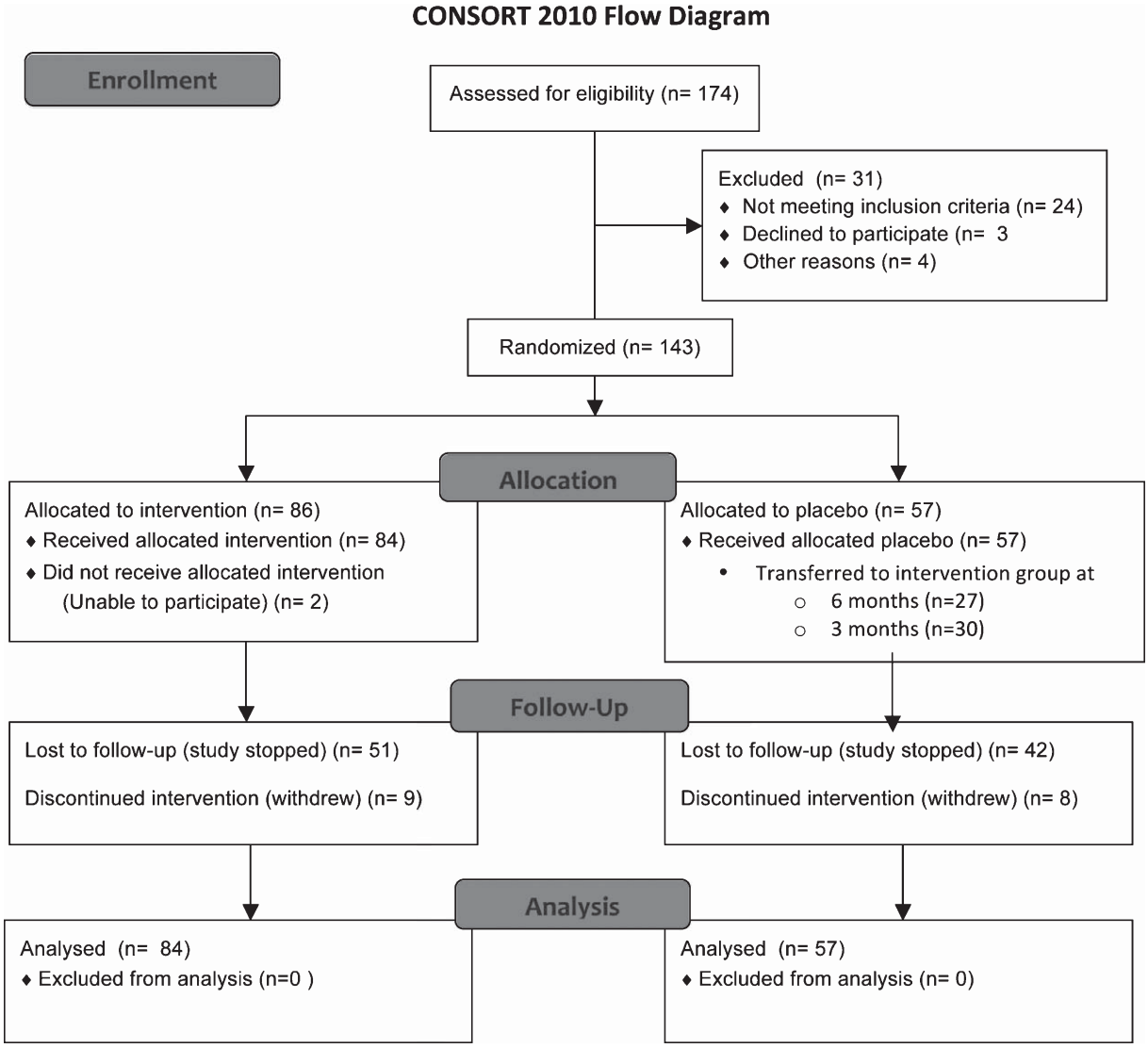

Fig. 1. Participant Flow Chart. The first 3 months of randomization and open-label protocols are considered "Allocation"; the open-label extensions of the randomization protocol are considered as "follow-up." Participant withdrawal is indicated; 14 participants (13% of those initially enrolled) withdrew during the course of the study; none of which were due to adverse reactions with NF. Participants lost due to study closure are also indicated. *Five participants were excluded due to baseline test compromise; 3 due to anxiety resulting from travel to an unfamiliar test location and 2 who, unbeknownst to the test administrator, were admitted to a nursing home 2 days prior to testing.

RESULTS

Impact of NF on cognitive performance for participants diagnosed with AD

Participants receiving NF ($n=48$) improved statistically versus placebo ($n=34$) within 3 months in cognitive performance ascertained by Clox-1 ($p=0.0083$; difference in means = 1.69, 95%CI = [0.4481, 2.9343]; 2-tailed unpaired t-test) and the DRS AEMSS ($p=0.0266$; difference in means = 1.44, 95%CI = [0.1722, 2.7171] 2-tailed unpaired t-test). Participants receiving NF for 3 months also demonstrated statistically ($p=0.0002$, 2-tailed paired t-test; 95%CI [0.8727, 2.6273]) and clinically (0.24) significant improvement in Clox-1 versus their baseline performance, and statistically ($p<0.0001$; 95%CI [1.2368, 3.2283]) and clinically (0.24) significant improvement in DRS AEMSS scores versus baseline. Those receiving placebo did not

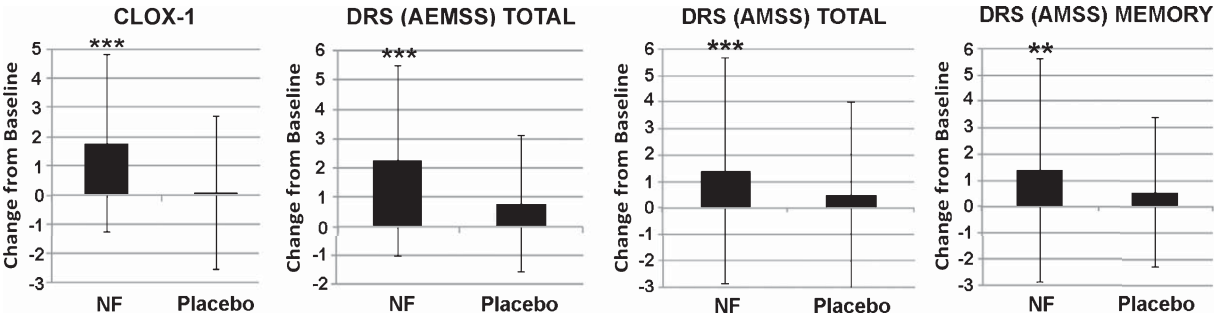

Fig. 2. Impact of NF on cognitive performance and BPSD for participants with AD. Performance on Clox-1 and the DRS (AEMSS) as indicated of the entire AD participant pool 3 months after randomization to NF ($n = 48$ for Clox-1, 43 for AEMSS) or placebo ($n = 34$ for Clox-1, 33 for AEMSS). Values present the mean change (±standard deviation) in performance at 3 months versus baseline; p values were derived from 2-tailed paired t-tests. Performance on the individual domains of the DRS is also presented. Three or two asterisks indicate a significant difference between a respective cohort at 3 months versus baseline ($p < 0.01$ and $p < 0.02$, respectively).

improve in either test (Fig. 2). Participants receiving NF for 3 months demonstrated statistical ($p = 0.0131$) and clinical (0.50) improvement versus baseline in the memory domain, and in total score ($p = 0.0026$) of the age-adjusted DRS. Participants receiving placebo did not display any change in these DRS domains (Fig. 2). Neither NF nor placebo differed from baseline in other DRS domains and values are, therefore, not presented.

Impact of NF on BPSD for participants diagnosed with AD

Neither NF ($n = 49$) nor placebo ($n = 34$) cohorts displayed statistical or clinical change from baseline in total NPI, nor did they differ statistically from each other. Participants receiving NF displayed a 1.86-fold improvement in total NPI versus those receiving placebo at 3 months; however, caregiver reports for both cohorts displayed considerable variance as reflected by large standard errors (-1.5 ± 8 versus -0.8 ± 1, NF and placebo, respectively; mean ± standard deviation). The NF cohort displayed a trend toward statistical ($p = 0.0585$) improvement at 3 months versus baseline in the NPI Depression domain (-0.1 ± 1 versus -0.02 ± 0.6, NF and placebo, respectively, mean ± standard deviation). The placebo cohort displayed a trend ($p = 0.0585$) toward statistical improvement in the motor domain (-0.2 ± 0.6 versus -0.1 ± 0.1, placebo and NF, respectively, mean ± standard deviation). Fewer appetite abnormalities were reported for the NF versus the placebo cohort (-0.02 ± 1.1 versus -0.4 ± 1.5, NF and placebo, respectively; mean ± standard deviation. Participants did not display statistical or clinical change in other domains of the NPI or in ADL. These values are, therefore, not presented.

To examine the relative impact of NF on participants with varying stages of AD, we classified our participants according to their baseline AEMSS performance as cognitively intact or having mild, moderate, or severe dementia according to the DRS manual [15]. We then examined the performance of participants receiving NF versus placebo of each of these categories. NF had a similar impact on performance for participants classified as cognitively intact or having mild and moderate dementia, but markedly less impact for those with severe dementia (Fig. 3). Similarly, the improvement in the NPI depression domain for participants receiving NF was confined to those participants classified according to their baseline AEMSS as cognitively intact or having mild dementia (-0.9 ± 3.5 and -0.8 ± 2.4, respectively; mean ± standard deviation); participants classified as having moderate or severe dementia did not display improvement (0.0 ± 0.0 and 0.3 ± 1.7, respectively; mean ± standard deviation). This differential efficacy is consistent with our phase I studies, in which individuals with mild-moderate dementia receiving NF statistically improved versus placebo (and versus their own baseline performance) [6], while individuals with moderate-severe dementia receiving NF did not statistically improve, but nevertheless maintained their baseline performance while placebo participants declined [7].

Open-label extensions

Following initial randomization, code was broken after either 3 or 6 months and all participants received NF under open label conditions for the remainder of the study (Tables 2 and 3); placebo cohorts are referred to as "Delayed Start."

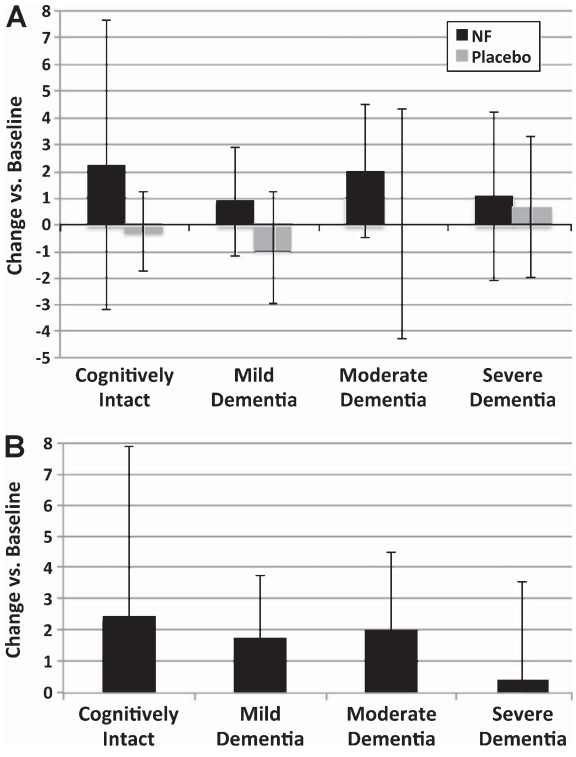

Fig. 3. Relative impact of NF on cognitive performance for participants with varying stages of dementia. Participants were classified according to their baseline AEMSS scores as Cognitively Intact (AEMSS 18–9), Mild Dementia (AEMSS 8–6), Moderate Dementia (AEMSS 5–4), and Severe Dementia (AEMSS 3–0) as defined in the DRS manual [15]. Participant distribution following this classification was as follows: NF, $n = 9$, 7, 15, and 19 for Cognitively intact, Mild, Moderate, and Severe Dementia, respectively; placebo, $n = 4$, 9, 4, and 17, respectively. A) Values represent the mean change in Clox (±standard deviation) for NF versus placebo participants after 3 months. Performance for the total population of NF participants differed significantly from performance of placebo participants ($p = 0.012$; ANOVA). B) Values represent the delta of the change in Clox (±standard deviation) for NF versus placebo participants after 3 months calculated as the (change from baseline for participants randomized to NF) – (change from baseline for participants randomized to placebo).

In the cohort for whom code was broken at 3 months (the "3-month cohort"), participants receiving NF displayed continued improvement in Clox1 and AEMSS over 9 months. Cognitive performance of the Delayed Start cohort improved during the open-label extension to an identical extent as the initial NF group by 9 months. Caregivers reported improvement in NPI for participants receiving NF, which achieved clinical significance versus baseline by 9 months. Caregivers for the Delayed Start cohort reported a decline in NPI over the first 3 months (i.e., while they received placebo). However, following transfer to NF in the open-label extension, they improved within 3 months to the extent that they matched the performance of the original NF group, and maintained this improvement for the duration of the study. Caregivers reported that both cohorts displayed performance identical to baseline in ADL over 9 months (Table 2).

In the cohort for whom code was broken at 6 months (the "6-month cohort"), participants receiving NF improved statistically in Clox-1 within 3 months, which they maintained until 12 months; clinical significance was obtained within 3 months and maintained until 9 months. The Delayed Start cohort displayed no change over 12 months. Participants receiving NF did not change significantly in the AEMSS versus their respective baseline over 12 months. The Delayed Start cohort maintained identical performance to their respective baseline for the first 3 months, declined slightly between 3-6 months, but during the open-label extension improved to the extent that they displayed clinical significance versus baseline and maintained this level until 12 months. Caregivers reported that both the NF and Delayed Start cohorts displayed clinically significant improvement in NPI within 3 months and retained this improvement over 12 months. Caregivers reported that participants receiving NF maintained their baseline performance in ADL over 12 months. Caregivers reported that the Delayed Start cohort maintained their baseline performance in ADL for 6 months, displayed a transient clinically significant improvement in the 3 months of the open-label extension, and returned to basal levels by 12 months (Table 3).

NF was overall more effective for participants in the 3-month cohort versus the 6-month cohort, and in particular for the Delayed-Start participants. In efforts to determine the reason behind this differential efficacy, we compared the demographics of the 3- and 6-month cohorts. We noted that the 6-month cohort displayed significant increased age, significantly less education, and a significantly lower baseline MMSE versus the 3-month cohort (Table 4). Moreover, 48% of the 6-month cohort had a baseline MMSE <20 (encompassing moderate-severe AD) [16] versus only 28% of the 3-month cohort (Table 4). As with the entire participant pool, NF was more effective for those individuals in the 6-month cohort that were classified according to baseline AEMSS as cognitively intact or having mild dementia versus those with moderate or severe dementia (Fig. 4). These findings are consistent with reduced efficacy of NF with more severe AD in our phase I studies [6, 7]. Our initial randomization generated statistically identical NF and placebo participant pools (Table 1). However, as described in Methods, our protocol initially established code

Table 2
Randomization for 3 months followed by an open-label extension. Participants were randomized to NF or placebo for 3 months, after which all participants received NF under open label conditions (shaded in the table) as described in methods. The initial placebo cohort is therefore referred to as "Delayed Start." Values represent the mean (±standard error of the mean) total scores of participants on Clox-1 and the DRS (AEMSS), and corresponding caregiver evaluations for the NPI and ADL for each cohort as indicated. Since a higher score signifies improvement on the NPI, the y axis is inverted. The 12-month timepoint is not presented since only a single participant receiving NF, and no participants in the Delayed start cohort, completed this timepoint

			Randomized		Open-Label Extension	
			Baseline	3 months	6 months	9 months
Clox-1	NF	Total Score	9.5 ± 3.8	11.3 ± 2.8	11.9 ± 3.2	12.6 ± 4.1
		Effect Size	–	0.26	0.32	0.36
		p value	–	0.002	0.003	0.017
	Delayed Start	Total Score	9.8 ± 3.7	10.6 ± 3.3	10.1 ± 5.1	12.8 ± 3.3
		Effect Size	–	0.110	0.040	0.390
		p value	–	0.450	0.680	0.051
DRS (AEMSS)	NF	Total Score	3.8 ± 2.9	7.3 ± 4.7	8.6 ± 4.3	9.2 ± 5.6
		Effect Size	–	0.43	0.54	0.52
		p value	–	0.0001	0.0001	0.0302
	Delayed Start	Total Score	3.6 ± 2.9	5.2 ± 3.8	7.9 ± 4.8	9.2 ± 6.3
		Effect Size	–	0.19	0.46	0.48
		p value	–	0.0427	0.0001	0.0001
NPI	NF	Total Score	11.5 ± 9.1	9.9 ± 9.2	9.5 ± 8.4	9.6 ± 5.3
		p value	–	0.098	0.0204	0.3736
	Delayed Start	Total Score	10.8 ± 12.4	12 ± 9.2	9.3 ± 12.3	9.8 ± 8.4
		p value	–	0.7596	0.0228	0.872
ADL	NF	Total Score	58 ± 16.4	59.4 ± 15.2	60.3 ± 13.9	59.2 ± 13.8
	Delayed Start	Total Score	61.9 ± 18.8	61 ± 19.4	58.3 ± 23.6	61 ± 27

Note that *p* values and effect sizes are included only if ≥1 value achieved statistical ($p < 0.05$) and/or clinical (>0.2) significance (derived from comparison testing the difference of each time point from baseline).

Table 3
Randomization for 6 months followed by an open-label extension. Participants were randomized to NF or placebo for 6 months, after which all participants received NF under open label conditions (shaded in the table) as described in methods and in the legend for Table 2

			Randomized			Open-Label Extension	
			Baseline	3 months	6 months	9 months	12 months
Clox-1	NF	Total Score	7.6 ± 4.2	10.6 ± 4.6	10.2 ± 3.2	11.2 ± 3.8	9.4 ± 5.7
		Effect Size	–	0.32	0.3	0.42	0.18
		p value	–	0.0003	0.0005	0.00004	0.014
	Delayed Start	Total Score	8.9 ± 4.9	8.6 ± 4.8	9 ± 4.2	9.3 ± 3.7	9.3 ± 4.5
DRS (AEMSS)	NF	Total Score	5.1 ± 3.6	5.5 ± 3.7	5.1 ± 4.4	5.8 ± 3.7	6.4 ± 5.6
	Delayed Start	Total Score	4.7 ± 4.5	5.1 ± 4.7	4.3 ± 4.1	6.3 ± 6.1	6 ± 4.5
		Effect Size	–	0.15	−0.15	0.38	0.38
NPI	NF	Total Score	17.1 ± 28.3	7.9 ± 9.3	9.6 ± 12	8.5 ± 11	7.6 ± 13.7
		Effect Size	–	0.21	0.17	0.19	0.2
	Delayed Start	Total Score	17.9 ± 28	11.8 ± 12.6	4.4 ± 3.7	9.3 ± 2.7	7.3 ± 6.4
		Effect Size	–	0.47	0.8	0.6	0.7
ADL	NF	Total Score	57.5 ± 16.4	54.1 ± 18.2	54.5 ± 15	54.8 ± 15.5	52 ± 19.8
	Delayed Start	Total Score	48.9 ± 19.0	49.1 ± 23.4	48.3 ± 21.2	54.7 ± 15.5	49.8 ± 22.9
		Effect Size	–	0.02	−0.04	0.46	−0.04

Note that *p* values and effect sizes are included only if ≥1 value achieved statistical ($p < 0.05$) and/or clinical (>0.2) significance (derived from comparison testing the difference of each time point from baseline).

break at 6 months, which was subsequently accelerated to 3 months following the observation that the first individuals reaching their 6-month point displayed statistical improvement within their first 3 months of receiving NF. Resultant separation of participants into 6- and 3-month cohorts by chance generated distinct populations with respect to the above demographics. These findings underscore the theory that nutritional intervention should be initiated as early as possible for maximal efficacy [3–5].

Adverse events for participants initially randomized to NF included 1 individual with gastrointestinal

Table 4
Baseline participant demographics of total participant population and NF and placebo cohorts following separation into cohorts for differential code break

Cohort	n	Age*	Education (y)*	MMSE*	% participants with MMSE ≥20
3-month code-break	73	77.5 ± 7.6	13.8 ± 1.9	22.9 ± 5.4	28%
6-month code-break	31	82.2 ± 9.4	12.8 ± 2.4	20.8 ± 6.3	48%
p value**		0.05	0.02	0.05	

*values represent the mean ± standard deviation. **comparison of participant pools in the 3-month versus 6-month cohorts via one tailed Student's *t*-test.

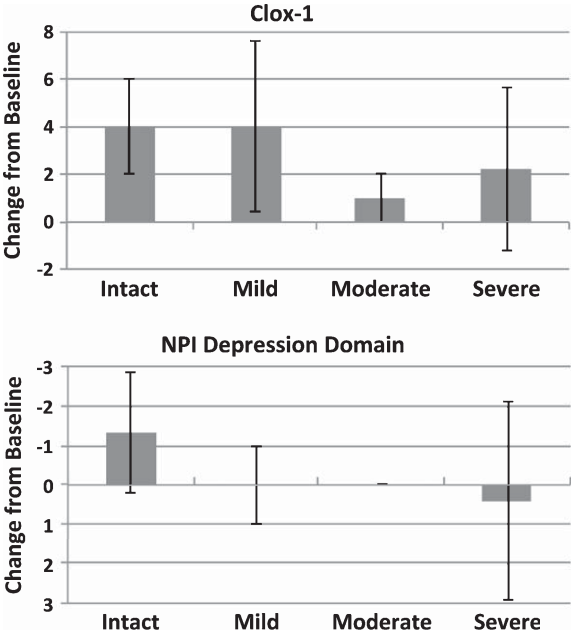

Fig. 4. NF was more effective for participants that were cognitively intact or had only mild dementia at baseline. Values represent the mean change (±standard deviation) in Clox-1 and the NPI depression domain for individuals in the 6 month cohort after receiving NF for 3 months. Participants were classified according to their baseline AEMSS scores as described in the legend to Figure 3. Participant $n = 3, 3, 3$, and 5 for Cognitively intact, Mild, Moderate, and Severe Dementia, respectively. There was no statistically-significant difference among these values, likely due to the small number of participants.

upset (which resolved following consuming NF with food), 1 individual who lost the ability swallow pills, 1 withdrawal in accord with family request, and transfer of 2 individuals to long-term care facilities. Adverse events for participants initially randomized to placebo included 1 participant death shortly after baseline testing, inability of 1 participant to continue to participate due to advancing dementia, and withdrawal of 1 participant due to diarrhea. No serious adverse events (i.e., related to consumption of NF) were reported for any participants.

DISCUSSION

These findings extend prior studies on NF efficacy [6–8]. NF was well-tolerated: >300 participants consumed NF daily for periods ranging from 2 weeks to 2.4 years in the present plus prior studies with no serious adverse events. Of these participants, 280 consumed NF daily for 3 months, 180 consumed NF daily for 6 months, 73 consumed NF daily for 9 months, and 25 consumed NF daily for ≥1 year, respectively.

In these phase II studies, participants receiving NF improved within 3 months in cognitive performance as ascertained by Clox-1 and the AEMSS, with specific improvement in the AEMSS memory domain. These improvements are likely related since compromises in memory can impair completion of a complex task such as clock drawing. Caregiver reports displayed a larger variance than did that of participants on cognitive tests. While caregivers reported an extent of improvement in total NPI for participants receiving NF for 3 months that approached that displayed by participants in Clox-1 and total DRS, this large variation may have contributed to the lack of statistical or clinical significance. In this regard, caregiver reports are subject to variance both in participant BPSD as well as the caregiver's own burden [17]. Indeed, completion of the Geriatric Depression Scale for the caregivers themselves has been utilized to qualify their NPI reports [17]. No change from baseline in ADL was reported by caregivers for NF or placebo cohorts within 3 months. This correlates with prior studies, where caregivers reported improvement within 3 months for NPI, but not in ADL [6, 7]. In this regard, decline in domains quantified by the NPI can be more pervasive and manifest earlier than functional impairment quantified by ADL [18]. This may be expected since NPI encompasses parameters of mood and behavior while ADL encompasses psychomotor function. Mood/behavioral disorders quantified by NPI may be more amenable to therapeutic intervention than those quantified by ADL, as previously observed following administration of donepezil [19]. Importantly, neither the NPI

nor ADL declined over the course of the present study, which was consistent with a lack of any decline in NPI or ADL over 28 months for participants receiving NF in our prior studies [6]. In a recent study, vitamin E administered in isolation slowed, but did not prevent, decline in ADL [20].

Participants initially randomized to NF displayed continued improvement or maintenance of cognitive performance and BPSD over 12 months. Once receiving NF, delayed-start participants demonstrated improvement or maintenance in cognitive performance and BPSD, which was equal to that of participants initially randomized to NF. Consistent with our phase I studies, NF was more effective for individuals at earlier stages of AD than for those who initiated consumption at later stages of AD [6, 7]. This highlights the importance of early administration of nutritional interventions [3–5]. It should be recognized that maintenance of baseline levels for 12 months following consumption of NF contrasts with routine prior demonstrations of statistically significant decline in cognitive performance and BPSD for individuals with AD that were maintained on placebo for 12 months, using the same test instruments utilized herein (e.g., [20–26]). The long-term impact of NF would likely have been further highlighted had we maintained randomization herein for 12 months. However, we considered this unethical since, as in prior studies, we observed statistical improvement for participants receiving NF within 3 months [6–8]. While the number of participants exceed that required for statistical power during the initial 3-month randomized study, segregation of participants into cohorts where code was broken at 3 and 6 months, coupled with attrition, reduced the respective number of participants below that necessary to obtain statistical power. Accordingly, while receiving NF, participants improved or maintained their baseline performance for up to 12 months during open-label extensions but these latter findings should be interpreted with caution.

A limitation of our analyses is that participants were not categorized according to prior supplement/vitamin consumption or nutritional criteria. Nutritional studies should ideally segregate participants previously consuming supplements versus those that had not, and then randomize them into separate treatment and placebo cohorts [5]. Additional criteria could include segregating participants with low/inadequate versus adequate/optimal baseline nutritional status. However, recruiting sufficient participants for all such categories can be prohibitive [5]. While nutritional intervention may be more effective for individuals experiencing prior chronic vitamin/nutrient insufficiency, such individuals may harbor latent damage that precludes any beneficial effect [4, 5]. It is noteworthy that improvement was attainable despite these caveats. A further limitation is that our cohorts from whom code was broken at 6 months by chance were statistically older, had statistically less education, and a statistically lower baseline MMSE versus those for whom code was broken at 3 months; however, since the individuals in these cohorts had already been receiving either NF or placebo, we could not carry out any additional randomization. Nevertheless, the reduced efficacy of NF for the 6-month cohort underscores the necessity of initiating nutritional interventions as early as possible.

Clinical analyses were confined to symptomatic efficacy of NF. However, preclinical studies demonstrate that components of NF support general nervous system health (e.g., reduced oxidative damage and homocysteine levels, increased glutathione levels and bioavailability, increased acetylcholine levels) and aspects of neuronal health directly related to dementia (reduction of PS-1 expression, secretase activity, intracellular and extracellular amyloid-β deposits, and phospho-tau) [27–33]. NF may therefore harbor disease-modifying properties if administered early enough as part of a preventative approach. The general beneficial effects of NF in preclinical studies may have alleviated AD-related neuropathology. For example, oxidative stress is an early event in AD neuropathology, homocysteine augments amyloid-β-induced oxidative damage and is correlated with impaired executive function, reduced levels of glutathione potentiates neuronal oxidative damage, and reduced acetylcholine levels correlate with impaired cognitive performance [32, 34–37]. Maintenance of normal acetylcholine levels in mice suggests that NF may augment cholinesterase inhibitor therapy [32]. The novelty of NF precluded biochemical and postmortem histological analyses of clinical participants. This information should be included in future clinical studies.

Nutritional supplementation will likely benefit from a participant's consumption of a Mediterranean diet. Many older adults do not obtain sufficient vitamins from food, rendering supplementation a necessity. A number of studies highlight the importance of social interaction and cognitive stimulation in maintaining cognitive ability; lifestyle modifications, including but not limited to nutritional supplementation, should be undertaken at the onset of, or prior to, cognitive decline to maximize efficacy [3–5]. Imaging abnormal amyloid levels at the first indication of impaired cognitive performance opens a window of opportunity for initiation of such lifestyle changes, including

supplementation with formulations mentioned above [1, 38].

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