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Featured Articles

Efficacy of a medical food in mild Alzheimer's disease: A randomized, controlled trial

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Abstract

Objective: To investigate the effect of a medical food on cognitive function in people with mild Alzheimer's disease (AD).

Methods: A total of 225 drug-naïve AD patients participated in this randomized, double-blind controlled trial. Patients were randomized to active product, Souvenaid, or a control drink, taken once-daily for 12 weeks. Primary outcome measures were the delayed verbal recall task of the Wechsler Memory Scale–revised, and the 13-item modified Alzheimer's Disease Assessment Scale–cognitive subscale at week 12.

Results: At 12 weeks, significant improvement in the delayed verbal recall task was noted in the active group compared with control ($P = .021$). Modified Alzheimer's Disease Assessment Scale–cognitive subscale and other outcome scores (e.g., Clinician Interview Based Impression of Change plus Caregiver Input, 12-item Neuropsychiatric Inventory, Alzheimer's disease Co-operative Study–Activities of Daily Living, Quality of Life in Alzheimer's Disease) were unchanged. The control group neither deteriorated nor improved. Compliance was excellent (95%) and the product was well tolerated.

Conclusions: Supplementation with a medical food including phosphatide precursors and cofactors for 12 weeks improved memory (delayed verbal recall) in mild AD patients. This proof-of-concept study justifies further clinical trials.

Keywords: Alzheimer's disease; Nutritional intervention; Synapse formation; Membrane phosphatide synthesis; B vitamins; Omega-3 fatty acids; Nucleotides; Uridine; Phospholipids; Choline; Antioxidants; ADAS-cog, delayed verbal recall; Medical food; Dietary management; Randomized clinical trial; Dementia

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1. Introduction

Alzheimer's disease (AD) is the leading cause of dementia. The underlying neurodegenerative mechanism involves several interacting processes—membrane degeneration, central oxidative stress, abnormal protein processing (beta-amyloid, tau), and mitochondrial dysfunction. These result in the characteristic accumulation of beta-amyloid plaques, neurofibrillary tangles, and synaptic loss, ultimately leading to cerebral atrophy and enlargement of ventricles. Ongoing neurodegeneration, particularly synaptic loss [1,2], leads to the classic clinical features of AD—memory impairment, language deterioration, and executive and visuospatial dysfunction. Current therapies, presumed to act by modulating central cholinergic or glutaminergic neurotransmission, provide only symptomatic relief.

New approaches to prevent and treat AD are urgently needed. Because the cognitive disturbances of AD best correlate with loss of hippocampal and cortical synapses [2], a possible therapeutic strategy might involve steps to restore such synapses. Preclinical studies indicate that such an effect can be induced by co-administration of rate-limiting precursors for membrane phosphatide synthesis, such as the nucleotide uridine, omega-3 polyunsaturated fatty acids, and choline [3–5]. These nutrients synergistically increase brain levels of the phosphatide molecules that comprise the bulk of synaptic membranes, and brain levels of specific synaptic proteins, suggesting that they also increase synapse formation [3–5]. Moreover, administration of combinations of these nutrients produces major increases in hippocampal dendritic spines [6], the anatomical precursor of and surrogate marker of new synapses [7–9], and enhances cognitive function [10,11]. These combined observations raise the question as to whether these nutrients have a role in the management of AD, especially of its main symptom—memory dysfunction.

The hypothesis that combinations of certain nutrients could provide clinically relevant benefits to patients with AD formed the basis of the development of the medical food* Souvenaid, which is a multinutrient drink designed to improve synapse formation. Souvenaid contains the necessary precursor and supporting nutrients to act synergistically to enhance membrane formation and function in patients with AD. All components contained in this medical food have a history of safe use in other foods. This report presents the results of the first clinical trial evaluating the efficacy, tolerability, and safety of a medical food designed to restore synapses in brains of patients with mild AD. We designed a proof-of-concept clinical trial to investigate whether supplementation with Souvenaid could affect cognitive functions in AD. We chose a 12-week study period based on the fast-acting response seen in animal studies [3,6], and elected to study patients with (very) mild disease—a stage where intervention of this nature is likely to exert the highest effect. The coprimary outcome measures were the delayed verbal recall test of the Wechsler Memory Scale—revised (WMS-r) [13], which is seen as a sensitive measure of episodic memory [14,15], impaired in the early stage of AD [14,15]; and the 13-item modified Alzheimer's Disease Assessment Scale – cognitive subscale (ADAS-cog) [16], often seen as the ''golden standard'' assessment tool in studies of AD intervention.

2. Methods

2.1. Participants

Patients had a diagnosis of probable AD according to the criteria of the National Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association [17]; a Mini-Mental State Examination (MMSE) [18] score of 20–26, representing mild AD, and a recent magnetic resonance imaging or computed tomography scan compatible with AD. Other inclusion criteria included age ≥ 50 years; >2 years postmenopausal or surgically sterile (women); current outpatient status; Hachinski Ischemia Scale [19] score ≤ 4; and Geriatric Depression Scale (GDS) [20] score ≤ 4 on the 15-item scale. Patients needed to have a caregiver who visited them ≥ 5 days a week, and could assist the patient in taking the study products, completing diary entries, and participating in study visits.

Exclusion criteria included neurological disease other than AD that could explain dementia; previous use of cholinesterase inhibitors, *N*-methyl-D-aspartate-receptor antagonists or medications with marked cholinergic/anticholinergic effects, or expected need for these within 24 weeks; use of antidepressants, tranquillizers, sleeping pills, or lipid-lowering medications unless on a stable dose for ≥ 3 months before baseline; use of antipsychotics, antiepileptics, ginkgo biloba, intake of $>200\%$ of the recommended daily intake of vitamins B, C, or E within 1 month before baseline; fatty acid supplements taken regularly within 6 months before baseline; participation in other studies involving investigational/marketed products; excessive alcohol intake or drug abuse; or investigator's uncertainty about patient's ability to comply with protocol requirements.

Participants were recruited from AD treatment centers in The Netherlands (11), Germany (11), Belgium (5), United Kingdom (1), and United States (1) between June 2006 and June 2007. Written informed consent was obtained from patients and caregivers. The institutional review board

*A medical food is in USA defined in 21 U.S.C. § 360ee(b)(3) as ''a food which is formulated to be consumed or administered enterally under the supervision of a physician and which is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognisable scientific principles, are established by medical evaluation'' [12]. A comparable definition exist in the harmonized legislation of the European Union (cf. Article 1,2(b) of Commission Directive 1999/21/EC of 25 March 1999 on dietary foods for special medical purposes.

Table 1
Nutritional composition of Fortasyn Connect

Component	Amount per daily dose*
EPA	300 mg
DHA	1200 mg
Phospholipids	106 mg
Choline	400 mg
UMP (uridine monophosphate)	625 mg
Vitamin E (alpha-TE)	40 mg
Vitamin C	80 mg
Selenium	60 μg
Vitamin B12	3 μg
Vitamin B6	1 mg
Folic acid	400 μg

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TE, tocopherol equivalents.

*Souvenaid (125 mL daily dose) contains Fortasyn Connect.

of each center approved the protocol and study documents. The study was conducted in accordance with the Declaration of Helsinki and the ICH-GCP as appropriate to nutritional products, and legislation of the country in which the research was conducted. Trial registration number is ISRCTN72254645.

2.2. Procedures

The primary objective of this double-blind, randomized, controlled, multicenter trial was to determine the effect of a medical food (Product ID 4804/4805) on cognitive function compared with a control product in patients with mild AD, after a 12-week supplementation. Secondary objectives were to assess its effects on safety, tolerability and compliance, behavior, functional abilities, quality of life, biochemical parameters, and cognitive performance after 12 and 24 weeks of supplementation.

The trial consisted of a 12-week core study followed by a 12-week similarly designed exploratory and optional extension study. At Week 12, patients who did not need to commence AD drug treatment (according to the treating physician), were invited to enter the 12-week extension study, during which they received the same product as in the core study, in a blinded manner.

Patients received the active or control product as a drink (125 mL tetrapackages), available in two flavors, to be taken each day at breakfast, and consumed within 1 hour. The active product ''Souvenaid'' contains a specific formulation of nutrients registered as Fortasyn Connect (Table 1;NV Nutricia) plus other vitamins, minerals, trace elements, and macronutrients in order to comprise a near-complete nutritional supplement (Supplementary Table 1 [online]). The control product lacked the constituents of Fortasyn Connect, but was otherwise isocaloric, isonitrogenic, similar in flavor and appearance to the active product, and presented in identical tetrapackaging.

Assessments were done at baseline and weeks 6, 12, and 24, with other visits and phone calls to encourage protocol adherence.

Patients were randomly assigned in a 1:1 ratio to treatment or control product using a computer randomization program, in blocks of four. Each study center received its own randomization list, ensuring that patients were assigned equally. All study staff and patients were blinded to the products given.

The amount of study product taken (0, [1/4], [1/2], [3/4], 1 tetrapackage) was recorded in a diary by the patient every day. Patients who did not take any of the study product on >25% of the days or who drank on average <70% of prescribed dosage were considered noncompliant.

Coprimary outcome measures were week-12 change from baseline on the (a) delayed verbal recall test of the Wechsler Memory Scale–revised (WMS-r) [13]; and (b) the13-item modified Alzheimer's Disease Assessment Scale–cognitive subscale (ADAS-cog) [16]. The rationale for using the delayed verbal recall test of the WMS-r in this very mild AD population was based on several studies that showed it to be sensitive in detecting intervention effects within a short study period in subjects with an early stage of cognitive impairment [21]. To minimize the potential learning effect with repeated use of the delayed verbal recall test of the WMS-r, two alternating stories were used at different study visits (baseline, weeks 6, 12, and 24). In the modified ADAS-cog, two validated items are added to improve sensitivity in the mild AD population—a delayed verbal recall and a digit cancellation task [16].

Secondary outcome measures included 24-week change from baseline on modified ADAS-cog and WMS-r delayed verbal recall task, and change at 12 and 24 weeks on MMSE and WMS-r immediate verbal (logical) memory task; Clinician Interview Based Impression of Change plus Caregiver Input (CIBIC-plus) [22]; 12-item Neuropsychiatric Inventory [23]; Alzheimer's disease Co-operative Study–Activities of Daily Living (ADCS-ADL) [24]; Quality of Life in Alzheimer's Disease [25]; plasma homocysteine and vitamins C and E, and erythrocyte membrane fatty acid profile.

Safety assessments included blood and laboratory tests using local laboratories and adverse events recorded at 6, 12, and 24 weeks. Nutritional parameter assessments were conducted by Danone Research.

Monitors from the Clinical Research Organization and the sponsor visited investigators regularly to conduct quality control checks to ensure the validity and accuracy of recording and overall adherence to study protocol. Data were entered by double entry and computerized checks were performed to ensure consistency of data.

2.3. Statistical analysis

To determine sample size, we drew upon studies of the effect of citicoline supplementation on ADAS-cog [26] and the WMS-r delayed verbal recall test [21]. Following consumption, citicoline is metabolized to choline and uridine,

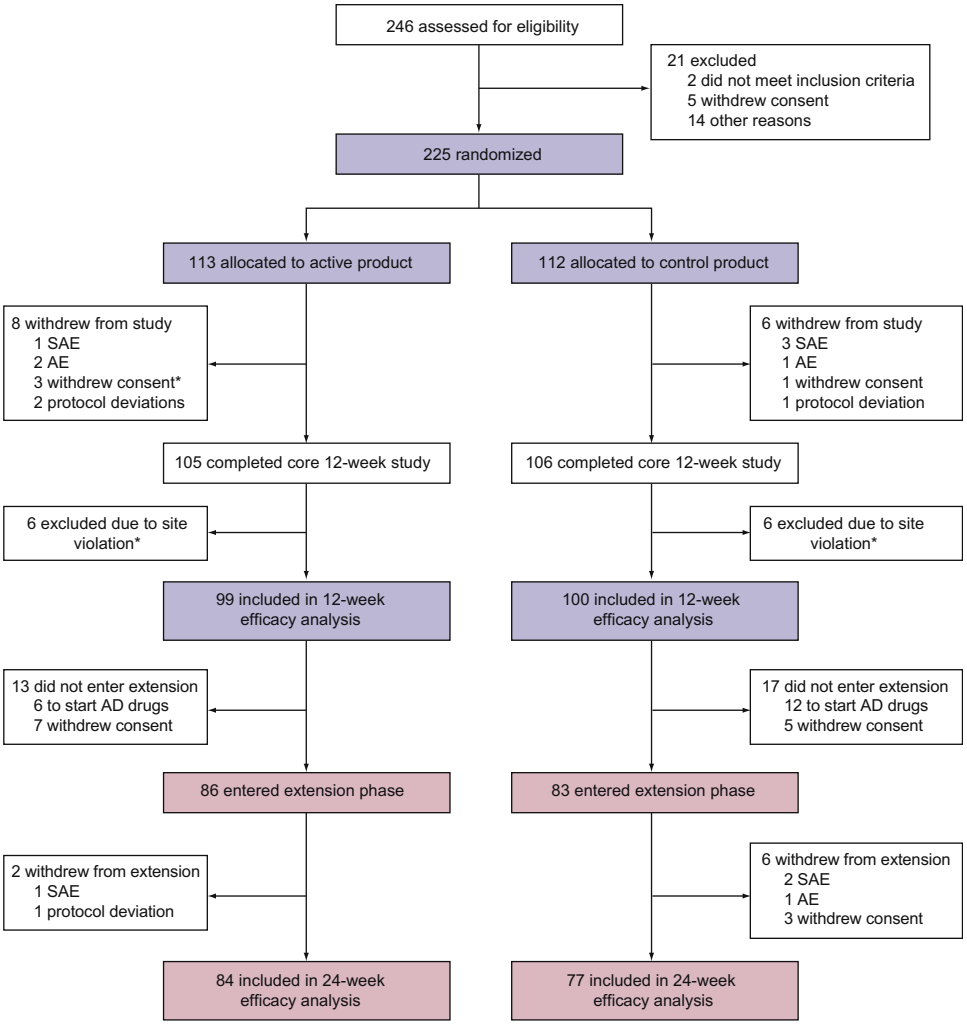

Fig. 1. Trial profile. *13 patients were excluded from the efficacy analysis due to a site violation—7 patients from the active group, 1 of whom withdrew consent before receiving active product, and 6 patients from the control group.

ingredients of the active product. On the basis of these studies, 80 patients (completers) per group were required to detect a between-group difference of 1.5 units on the WMS-r delayed recall test and the ADAS-cog after 12-week supplementation (power of 0.80; $P = .05$, two-sided test). With a drop-out rate of 25%, approximately 214 patients were required.

A prespecified blinded interim analysis of safety and primary efficacy data was done after 84 patients had completed the core 12-week study. The results were reviewed by the independent Data Monitoring Committee to check whether the calculated sample size was adequate and that no safety concerns had arisen.

All randomized participants were included in the safety analysis. Those who had at least one dose of study product and one assessment post-baseline were included in the intention-to-treat (ITT) efficacy analysis. Frequency distributions for each outcome measure were examined. Where possible, data were analyzed using a repeated-measures mixed model in which time was treated as a categorical variable and represented by dummies. For parameters that had a distinctly nonnormal distribution, nonparametric analyses were applied. All tests were conducted at $P < .05$. Statistical analyses were performed in SPSS 15.0 for Windows. Confounder and effect modifier analyses were performed. The potential of the covariates (e.g., number of adverse events per patient, use of concomitant medication, intake adherence) to influence the estimate of intervention effect was investigated by comparing the estimate for intervention effect in a model including the covariate with the estimate for intervention effect in a model excluding the covariate (confounder analyses). In addition, the significance of the covariate-intervention interaction parameter (e.g., the extent that the covariate affects the intervention effect) was evaluated (moderator analyses). Treatment effects were further examined in pre-specified subgroup analyses to determine the influence of baseline patient characteristics (patients with early AD [baseline MMSE 24–26]; patients with late-onset AD; and by apolipoprotein E

[*APOE*] genotype). Center was included as a separate level in the models.

3. Results

3.1. 12-Week primary study–efficacy results

On average, each center contributed 7.8 patients (SD: 5.8; range: 1–24). In total, 225 patients were randomized to active or control product (Fig. 1). After the blinded interim analysis, the Data Monitoring Committee recommended continuation of the trial without modification. After a blinded data validation phase and subsequent quality control audit, it was determined that one study site failed to comply with ICH-GCP guidelines. A recommendation to exclude the relevant patient data (n = 13) from the efficacy analysis but include it in the safety analysis was endorsed by the Data Monitoring and Steering Committees. Consequently, safety data on all 225 randomized patients and ITT efficacy data on 212 patients are reported.

Baseline characteristics of the efficacy population (n = 212) are presented in Table 2. The study groups were well-matched, with no statistically significant differences noted. In the overall population, mean MMSE score was 23.9; mean age 73.7 years; 50% were men; and the mean level of education beyond primary school was 5.8 years. On average, the duration of primary school education was 6 years, in accordance with the education systems of the participating countries. Approximately 90% of patients reported one or more previous or current medical conditions, most commonly vascular, e.g., hypertension (105/225; 47%); metabolic, e.g., hypercholesterolemia (79/225; 35%); and locomotor, e.g., osteoporosis (61/225; 27%).

Of the 212 patients included in the ITT efficacy analysis, 199 patients (94%) completed the 12-week study. Compliance was excellent, with 96% and 95% of the active and control groups, respectively, classified as compliant.

At baseline, approximately 40% of patients scored 0 [lowest score] on the WMS-r delayed verbal recall scale of 0–25. Given this skewed distribution, it was necessary to substitute the planned mixed-model analysis of 12-week data with nonparametric analyses. Both noncategorical (Mann–Whitney *U* Wilcoxon W test) and categorical nonparametric analyses (χ^2 test) gave similar results. Improvement in a patient was defined as change from baseline >0 points; no change as 0 points; decline as change from baseline <0 points. A statistically significant improvement in WMS-r delayed verbal recall was observed in the active group but not in the control group (Z = –2.23, P = .026; Wilcoxon testing), with the more accessible categorical analyses presented in Fig. 2 and Table 3. Thus, at 12 weeks, 40% of patients in the active group showed an improvement in WMS-r delayed recall compared with 24% in the control group; the mean change in WMS-r delayed recall was comparable between active and control groups. During this period, however, the modified ADAS-cog scores did not change in either group (Table 3). No differences in secondary outcome measures were observed between groups (Table 3), including CIBIC-plus 7-category scores (P = .905; Pearson χ^2 test).

Table 2
Baseline demographic and clinical characteristics of the intention-to-treat efficacy population (n = 212)[†]

Variable	Control (n = 106)	Active (n = 106)
Men	52 (49%)	54 (51%)
Age at screening, yr [range]	73.3 (7.8) [52–92]	74.1 (7.2) [54–87]
BMI at baseline, kg/m^2	26.2 (3.5)	26.2 (4.8)
Years of education beyond primary school	6.0 (4.0)	5.5 (3.9)
Blood pressure, mm Hg		
Systolic	138.7 (18.6)	139.3 (20.0)
Diastolic	80.7 (10.7)	80.4 (10.4)
Median time from AD diagnosis to baseline, d [range]	31.5 [0–1036]	30.0 [–18 to 1932][‡]
MMSE	24.0 (2.5)	23.8 (2.7)
13-Item modified ADAS-cog	25.5 (8.8)	25.9 (7.6)
Median WMS-r delayed verbal memory test [range]	2.0 [0–17]	1.0 [0–16]
Median WMS-r immediate verbal memory test [range]	5.0 [0–19]	4.0 [0–15]
ADCS-ADL	61.9 (10.9)	61.1 (10.5)
Median NPI-12	4.00 [0–54]	4.00 [0–37]
Quality of life–AD (composite score)[§]	35.3 (4.7)	34.9 (4.0)

Abbreviations: BMI, body mass index; MMSE, Mini-Mental State Examination (0–30; with lower scores indicating more severe cognitive deficit); ADAS-cog, Alzheimer's disease Assessment Scale—cognitive subscale (0–85; with higher scores indicating more severe cognitive deficit); WMS-r, Wechsler Memory Scale—revised (0–25; with lower scores indicating more severe memory impairment); ADCS-ADL, Alzheimer's disease Co-operative Study—Activities of Daily Living (0–78; with higher scores indicating better functioning); NPI-12, Neuropsychiatric Inventory (0–144; with higher scores indicating more behavioral problems).

[†]Values are means (SD), unless stated otherwise.

[‡]The value of –18 days represents a protocol deviation. In this case, the patient was diagnosed 18 days after baseline assessment.

[§]The quality of life–AD (composite score) is calculated by multiplying the patient score by 2, adding the caregiver score, and dividing the sum by 3, thus weighting the patient's score. Scores range from 13 to 52, with higher scores indicating greater quality of life.

In the prespecified subgroup analysis of patients with very mild AD (baseline MMSE: 24–26; n = 120), the active group showed a significant improvement in WMS-r delayed verbal recall compared with controls (Z = –2.53, P = .011, Wilcoxon testing). This was paralleled by an improvement in WMS-r immediate verbal recall score versus controls (Z = –1.42, P = .157, Wilcoxon testing; P = .033 for χ^2 testing). No significant effects were observed in the other predefined subgroups.

In the overall active group, a significant uptake of DHA (docosahexaenoic) and EPA into erythrocyte membranes was observed ($P \leq .001$ vs controls; Fig. 3A) and plasma level of vitamin E increased significantly (+19% in active group, –1% in control group; $P \leq .001$). Concomitantly, plasma homocysteine level in the active group was 23% lower than at baseline, and 19% lower than in the control group ($P \leq .001$; Fig. 3B). The levels of DHA and EPA in

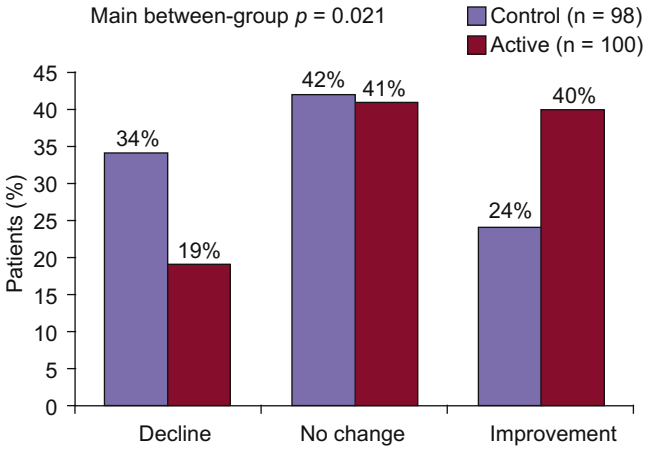

Fig. 2. Change in Wechsler Memory Scale–revised (WMS-r) delayed verbal recall score (coprimary outcome measure) after 12 weeks of supplementation with active or control product in the intention-to-treat efficacy population (n = 212). Because 40% of patients scored 0 on the week-12 WMS-r delayed recall task (lowest score), it was necessary to substitute the planned multilevel modeling with the χ^2 test comparing three categories of difference scores compared with baseline—decline in score, no change, and improved score at 12 weeks. Abbreviations: WMS-r, Wechsler Memory Scale—revised (0–25, with lower scores indicating more severe deficit).

erythrocyte membranes and plasma vitamin E and homocysteine in the control group remained unchanged throughout the study. The results of plasma vitamin C analyses varied greatly, preventing meaningful interpretation (data not shown).

3.2. 12-Week primary study–post hoc analysis of efficacy results

As there was no decline in either study group with regard to mean ADAS-cog score during the 12-week core study period, we undertook an analysis to further explore response on ADAS-cog. In terms of individual response, an improvement is generally defined as –7 or –4 points [27]. The percentage of patients defined as responders was higher in the active group (change from baseline of–7 points or greater: 8.9 %; change from baseline of–4 points or greater: 17.8 %) as compared to the control group (change from baseline of–7 points or greater: 5.1%; change from baseline of–4 points or greater: 11.1 %), although not reaching significance ($P = .215$ for change from baseline of–7 points or greater; $P = .126$ for change from baseline of–4 points or greater).

Defining treatment response in AD is acknowledged to be challenging [27]; it is difficult to find a scale that accurately captures all aspects of the disease, such as changes in cognition, function, and behavior. Consequently, response on at least two scales is regarded as a more reliable indicator of change. Therefore, we expanded our post hoc analysis to further examine response to intervention. Scores on the modified ADAS-cog scale (cognition), ADCS-ADL scale (function), and CIBIC-plus (behavior) were combined to define ''response.'' A patient was classified as a responder when they fulfilled at least two of the following three criteria relative to baseline scores:

- Modified ADAS-cog $\geq$4 point decline (clinical improvement)
- ADCS-ADL total score $\geq$4 point increase
- CIBIC-plus ''improvement''

After 12 weeks, the percentage of patients defined as responders was significantly greater in the active group versus control group (18.2% vs 7.2%; $P = .031$, Fisher exact test).

3.3. Safety and tolerability results (primary and extension study)

As shown in Table 4, there was no significant difference in the incidence of adverse events between groups over 24 weeks ($P = .286$ for between-group difference). Most adverse events were classified as unrelated to study products; gastrointestinal adverse events ranked highest in both groups. A total of 27 serious adverse events were reported, 14 in the control group (occurring in 11 patients), and 13 in the active group (occurring in 7 patients). None were considered to be related to study product except one serious adverse event (panic attack/hyperventilation), which was classified as possibly related to control product. No clinically relevant changes in blood pressure measurements or liver and kidney function were observed.

3.4. Exploratory extension study efficacy results

Of the 199 patients in the 12-week efficacy population, 169 (85%) continued in the 12-week extension study. Some patients had to commence AD medication and were therefore ineligible for entry (6 in the active group, 12 in the control group; $P = .217$). Patients were not re-randomized and continued to receive the same study product in a blinded manner. Of this cohort, 161 of 169 patients (95%) completed 24-week supplementation (Fig. 1). Although no significant differences in either of the primary outcome measures were observed at 24 weeks, a significant improvement in WMS-r immediate verbal recall score was observed in the active group ($P = .046$ vs controls, Wilcoxon testing). There was no evidence of an intervention effect on any other outcome measure. Biochemical parameter measurements were consistent with those of week 12, confirming excellent compliance.

Adjustment for potential confounders in all analyses (12-week primary study and exploratory extension study) did not change the results. In order to determine which factors influenced treatment effect, models were re-run with covariates as possible effect-modifiers. Several effect-modifiers were observed, most important of which was the effect of adverse events on modified ADAS-cog at Week 24 (Fig. 4; Cohen's $d = 0.19$; $P < .001$).

Table 3
Results of efficacy parameters analyzed either by nonparametric or parametric testing following 12 weeks of supplementation with active or control product in the intention-to-treat efficacy population (n = 212)

Efficacy parameter	Control	Active	*P*
Nonparametric analyses[†]			
WMS-r delayed verbal recall test, % (co-primary outcome measure)	[n = 98]	[n = 100]	.021
Decline in score	34	19	
No change	42	41	
Improvement	24	40	
WMS-r immediate verbal recall test, %	[n = 98]	[n = 100]	.131
Decline in score	45	31	
No change	15	19	
Improvement	40	50	
NPI-12 (frequency x severity), %	[n = 100]	[n = 101]	.728
Decline in score	48	49	
No change	24	20	
Improvement	28	32	
Parametric analyses[‡]			
13-item modified ADAS-cog score, mean (SD) (coprimary outcome measure)			.826
Baseline	25.5 (8.8) [n = 106]	25.9 (7.6) [n = 106]	
Week 12	25.8 (7.8) [n = 99]	25.9 (7.7) [n = 101]	
MMSE score, mean (SD)			.528
Baseline	24.0 (2.5) [n = 105]	23.8 (2.7) [n = 105]	
Week 12	24.0 (3.4) [n = 96]	24.1 (3.5) [n = 99]	
ADCS-ADL score, mean (SD)			.313
Baseline	61.9 (10.9) [n = 106]	61.1 (10.5) [n = 106]	
Week 12	62.6 (11.4) [n = 99]	62.3 (10.7) [n = 101]	
Quality of life–AD (composite score), mean (SD)			.305
Baseline	35.3 (4.7) [n = 106]	34.9 (4.0) [n = 105]	
Week 12	35.6 (4.3) [n = 99]	34.8 (4.2) [n = 101]	

Abbreviations: WMS-r, Wechsler Memory Scale—revised (0–25, with lower scores indicating more memory deficit); NPI-12, Neuropsychiatric Inventory (0–144, with higher scores indicating more behavioral problems); ADAS-cog, Alzheimer's Disease Assessment Scale—cognitive subscale (0–85, with higher scores indicating more severe cognitive deficit); MMSE, Mini-Mental State Examination (0–30, with lower scores indicating more severe cognitive deficit); ADCS-ADL, Alzheimer's disease Co-operative Study—Activities of Daily Living (0–78, with higher scores indicating better functioning); The Quality of Life—AD (composite score) is calculated by multiplying the patient score by 2, adding the caregiver score, and dividing the sum by 3, thus weighting the patient's score. Scores range from 13 to 52, with higher scores indicating greater quality of life.

[†]Nonparametric χ^2 test comparing three categories of difference scores compared to baseline (decline in score = difference <0; no change and improvement = difference >0 at 12 weeks).

[‡]Repeated-measures mixed model, in which time was treated as a categorical variable and represented by dummies.

4. Discussion

In this randomized, double-blind, controlled multicenter trial we demonstrated that patients with mild AD who consumed the medical food Souvenaid for 12 weeks experienced a statistically significant improvement in WMS-r–delayed verbal recall score versus controls ($P = .026$). Furthermore, in a prespecified subgroup analysis of patients with very mild AD, an improvement in delayed as well as immediate verbal recall was observed at 12 weeks in those supplemented with active product versus controls. Excellent compliance (>95%) was confirmed by markedly increased DHA and EPA levels in erythrocyte membranes, elevated plasma vitamin E, and concomitant reduction in plasma homocysteine. No differences were observed between active and control groups either in biochemical safety markers or in the incidence of adverse events or severe adverse events. With patients from 29 AD treatment centers from 5 countries, the results of this study are robust.

To our knowledge, this is the first multicenter, controlled clinical trial to show that a treatment designed to restore synapses through nutritional supplementation [3] can provide significant benefits to patients with mild AD. Although several preclinical studies have demonstrated the potential of various nutrients (single or in combination) to positively affect the pathophysiology and symptoms of AD, clinical evidence is scant. Moreover, none of these earlier studies has chosen nutrients based on their ability, jointly, to promote synaptogenesis. Previous studies on nutrients and cognition have, in general, used epidemiological methods to assess potential relations between diet or specific nutrients and the risk of developing AD or dementia, or developed trials based on hypothesis derived from these epidemiological studies. A few small studies have reported the benefits of certain nutrients in people with confirmed dementia or AD, e.g., B vitamins [28], EPA [29], and omega-3 fatty acids [30]. Although the results of these studies suggest, at best, a moderate effect of single nutrients, the findings of our study demonstrate that a specific combination of nutrients with known neurochemical effects has the potential to provide clinically significant benefits to patients with AD. The observed effectiveness of the combined nutrients in our novel medical food,

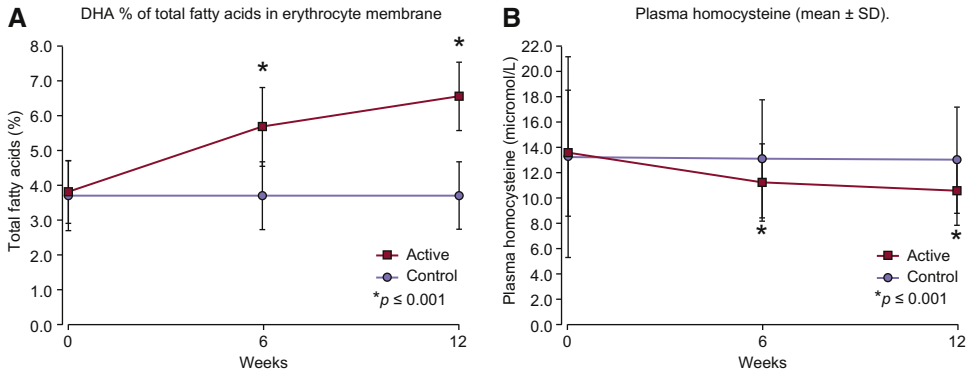

Fig. 3. DHA percentage of (**A**) fatty acids in erythrocyte membrane and (**B**) plasma homocysteine levels at baseline, 6 and 12 weeks, given as means (SD). Footnote: *P* values calculated using *t*-test. Abbreviations: DHA, docosahexaenoic acid.

utilizing the body's normal metabolic pathways, is in line with synergistic actions demonstrated in preclinical studies [3,4,10,31].

The underlying hypothesis tested in the present study was based on observations that (1) cognitive decline in AD correlates with loss of synapses [1,2]; (2) patients with AD appear to be subclinically deficient in certain nutrients, some of which are required for synaptic membrane synthesis [32]; and (3) preclinical studies show that combined administration of specific nutrients increases brain levels of synaptic membrane [3] and enhances cognitive functions [10,11]. Uridine monophosphate, DHA, and choline act synergistically to increase brain phosphatides and synaptic protein levels, and those of hippocampal dendritic spines [6], at least 96% of which are thought to become new synapses by attaching to a terminal bouton of a presynaptic neuron [7]. The medical food used in this study provides phosphatide precursors, as well as B vitamins (for endogenous choline synthesis), vitamins C and E, selenium, and phospholipids, which further enhance membrane formation, integrity, and function. The latest preclinical findings show that combined administration of this specific mixture of nutrients is more effective than single nutrients at improving membrane-bound cholinergic receptor functioning [33], and at reducing beta-amyloid production, plaque burden, and neurodegeneration in the APP/PS1 mouse model of AD pathology [31]. In terms of a human model to support biological plausibility, it has been proposed that patients with AD may have specific nutrient needs that could be a consequence of the disease process itself, or reflect a low intake or reduced bioavailability of specific nutrients needed for synapse synthesis and function [32]. An emerging nutritional deficiency may accelerate the disease process. Altogether, there is a compelling body of evidence in support of the proposition that administration of phosphatide precursors in combination with cofactors stimulates synapse formation and mitigates pathological processes in AD [32].

Although we observed significant between-group differences in delayed verbal recall, there was no suggestion of an intervention effect on any secondary efficacy parameter and no differences were observed between treatment groups on the modified ADAS-cog. However, it is also important to note that no decline was seen on mean ADAS-cog scores at 12 weeks, in either group. The absence of a between-group treatment effect on the modified ADAS-cog despite significant effects on delayed verbal recall may relate to the

Table 4
Number (%) of patients experiencing one or more adverse events per class over 24 weeks of supplementation with control or active product*

Adverse event body system	Control (n = 112)	Active (n = 113)	*P*
Total adverse events	49 (43.8%)	58 (51.3%)	.286
Gastrointestinal	20 (17.9%)	21 (18.6%)	1.000
Diagnostic procedures	8 (7.1%)	13 (11.5%)	.360
Psychiatric conditions	11 (9.8%)	7 (6.2%)	.338
Infections and infestations	5 (4.5%)	11 (9.7%)	.193
Nervous system	9 (8.0%)	9 (8.0%)	1.000
Locomotor/musculoskeletal, connective tissue	4 (3.6%)	9 (8.0%)	.253
Skin, subcutaneous tissue, appendages	7 (6.3%)	3 (2.7%)	.215
General, body as a whole	5 (4.5%)	6 (5.3%)	1.000

*Only those reported by at least 5% of subjects in either group are shown here.

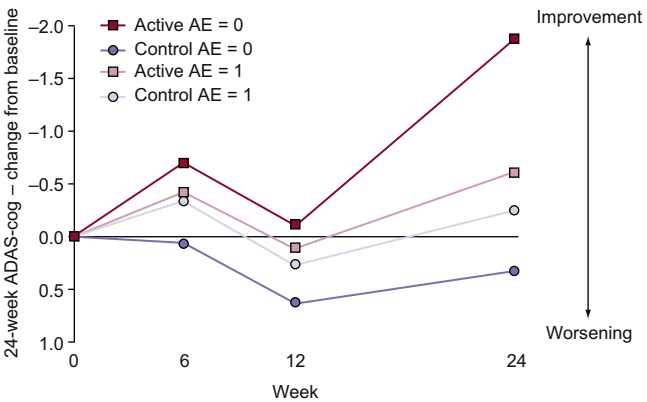

Fig. 4. Moderator analysis–effect of adverse events (AE) rate per patient on week-24; 13-item modified ADAS-cog score change from baseline. Abbreviations: ADAS-cog, Alzheimer's Disease Assessment Scale—cognitive subscale (0–85, with higher scores indicating more severe cognitive deficit).

ADAS-cog's lack of sensitivity in this population. Recently, it has been suggested that the ADAS-cog, widely used to assess cognitive outcome in trials of AD, may not be sensitive enough for trials in mild AD [34].

The improvement in memory seen in the active group versus the control group at 12 weeks was not observed in the exploratory extension study (although a significant improvement in WMS-r immediate verbal recall score was observed in the active group at 24 weeks [$P = .046$ vs controls]). A possible explanation for this may be that even though changes were detected at week 12 with a population of 199 patients, there was insufficient power to detect changes at week 24 with a population of 161 patients. In addition, during the 24-week explanatory study an increase in change of raters of the WMS-r was observed (possibly due to change of staff at various sites), compared with the 12-week primary study; this may have influenced the results. Furthermore, no decline in the placebo group was detected between weeks 12 and 24, a phenomenon that has been described recently; patients in placebo groups of recent randomized controlled trials have declined only slightly compared to those in historical studies [34,35]. Finally, there was a clear floor effect on the WMS-r delayed verbal recall scale (at baseline 40% of the patients scored 0), so decline over time in the placebo group was difficult to detect. In addition, the optional nature of the extension study invalidates the principle of randomization, as illustrated by the significant difference in mean WMS-r delayed verbal recall scores seen in the two groups as they entered the extension ($P = .009$ for variance).

This study has limitations, notably, the lack of improvement in ADAS-cog as discussed. However, treatment effects in mild disease will by definition be small, and longer studies to show maintenance of improvement or reduced rates of decline are needed. Twelve weeks is the minimum period for a trial of AD intervention, and therefore the use of a more global measure such as CIBIC-plus or the Clinical Dementia Rating scale as a primary outcome measure is not justified. More sensitive measures for episodic memory, such as word-list recall tasks are needed to detect treatment effects in mild AD. These points will be addressed in future studies.

We found the baseline scores of several parameters to be distributed in a non-normal manner, including WMS-r delayed recall, WMS-r immediate recall, and 12-item Neuropsychiatric Inventory, necessitating analysis by nonparametric modeling. In addition, significant effect-modifiers were identified, most notably the effect of the per-patient adverse event rate on the magnitude of the 24-week ADAS-cog score. This indicates that adverse events need to be meticulously assessed at each visit in order to assess the reliability of the scoring.

In conclusion, this proof-of-concept study showed that supplementation with the multi-nutrient drink Souvenaid for 12 weeks is well-tolerated and results in an improvement in memory in patients with mild AD. Further clinical trials with this product in patients with AD are justified, with Souvenaid given both as add-on therapy in patients with mild to moderate AD receiving approved anti-AD medication, and in drug-naïve patients in order to confirm and extend the results of the current study. Future clinical trials aim to measure relevant biomarkers, in support of the hypothesis that Souvenaid can improve synapse formation. Measurement of cerebrospinal fluid biomarkers to show brain penetration and exposure as well as brain imaging (magnetic resonance imaging) will be addressed in the LipiDiDiet Study (NTR5433), and electroencephalogram and magnetoencephalogram in the Souvenir II Study (NTR1975). These trials have started in Europe, as well as in the United States (S-Connect Study, NTR1683).

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Supplementary Table 1. Nutritional composition of 125 mL Souvenaid and 125 mL control product

Component	Souvenaid	Control
Macronutrients		
Energy, kcal	125	125
Protein, g	3.8	3.8
Carbohydrate, g	16.5	16.5
Fat, g	4.9	4.9
Fortasyn Connect		
EPA, mg	300	0
DHA, mg	1200	0
Phospholipids, mg	106	0
Choline, mg	400	0
UMP (uridine monophosphate), mg	625	0
Vitamin E (alpha-TE), mg	40	0
Vitamin C, mg	80	0
Selenium, mcg	60	0
Vitamin B12, mcg	3	0
Vitamin B6, mg	1	0
Folic acid, mcg	400	0
Minerals		
Sodium, mg	125	125
Potassium, mg	187.5	187.5
Cloride, mg	156.3	156.3
Calcium, mg	100	100
Phosphorus, mg	87.5	87.5
Magnesium, mg	25.0	25.0
Other trace elements		
Iron, mg	2	2
Zinc, mg	1.5	1.5
Iodine, mcg	16.3	16.3
Manganese, mg	0.41	0.41
Copper, mcg	225	225
Molybdenum, mcg	12.5	12.5
Chromium, mcg	8.4	8.4
Other vitamins		
Vitamin A, mcg	200	200
Thiamin (B1), mg	0.19	0.19
Riboflavin (B2), mg	0.20	0.20
Niacin (B3), mg NE	2.25	2.25
Pantothenic acid (B5), mg	0.66	0.66
Vitamin D, mcg	0.88	0.88
Biotin, mcg	5.0	5.0
Vitamin K, mcg	6.6	6.6

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TE, tocopherol equivalents; NE, niacin equivalents.