Complaints Resolution Panel (CRP) and Therapeutic Goods Administration (TGA) Complaint

Use this form to submit complaints about advertisements for therapeutic goods.

The advertisement
You MUST provide a copy of the advertisement you are complaining about, and indicate clearly where it was published. If it was published in a magazine or newspaper, state in which edition it appeared, the date, and the page number. If it was published in outdoor media, state the location and the date on which the advertisement was observed, and where possible provide a photograph of the advertisement. If it was published on the internet, state the website address and the date on which the advertisement was observed. If it was broadcast, identify the television or radio station, and the date and time of the advertisement.

<table>
<thead>
<tr>
<th>Publications (screen shots appended):</th>
<th>Date/edition:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• The sponsor’s web site: <a href="https://www.souvenaid.com.au">https://www.souvenaid.com.au</a></td>
<td></td>
</tr>
<tr>
<td>• Numerous Internet pharmacies, for example</td>
<td></td>
</tr>
</tbody>
</table>

The product advertised
Identify the therapeutic goods that are promoted in the advertisement. If possible, identify the party responsible for publishing the advertisement.

**Souvenaid (no ARTG number found)**

*Nutricia Advanced Medical Nutrition*
*Talavera Corporate Centre,*
*Level 4, Building D *
*12-24 Talavera Road *
*Macquarie Park NSW 2113 *

*PO Box 1007 *
*North Ryde Business Centre *
*NSW 1670 *

*Freephone: 1800 060 051 *
*Fax: (02) 9870 7204 *

Confidentiality
You may request that your details be kept confidential; however it is recommended that you still provide contact details (below).

Please keep my personal details confidential: **No**

Details of the complaint
State clearly the nature of your complaint. It is not sufficient to highlight or quote words from the advertisement – explain what aspects of the advertisement are of concern to you, and why. If possible, identify sections of the Therapeutic Goods Advertising Code, Act, or Regulations you believe may have been breached in the advertisement. Attach a separate page if necessary.

This company is promoting a therapeutic good (which they call a “medical food”) for Alzheimer’s disease.
The following claims are made:

- “For the treatment of mild Alzheimer’s disease”,
- “A new approach in the early stages of Alzheimer’s Disease”,
- “Nutritionally supports memory function in the early stages of Alzheimer’s disease” and
- “Clinically proven”.

I submit that the claims made, the defined ingredients (see below) and the dose (1 bottle a day) satisfy the definition of a therapeutic good contained in the Therapeutic Goods Act 1989. As such the promotion of this product breaches Section 22(5); 42C(1) and 42DL(1)(c) of the Act and at least Section 4(1)(a), 5 and 6(b)(iv) of the Therapeutic Goods Advertising Code 2007.

Alternatively, these claims breach Section 7 of Standard 1.2.7 (Nutrition, Health and Related Claims) under section 92 of the Food Standards Australia New Zealand Act 1991 &/or Section 3 of Standard 1.1A.2 as amended operating as a transitional alternative standard to Standard 1.2.7.

In addition, it can be argued that the results obtained in the various studies cited (attached) are modest at best, of dubious clinical significance and not in accord with the CRP guidelines on “clinically proven”. This may breach Section: 4(1)B), 4(2)(a) and 4(2)(C) of the Therapeutic Goods Advertising Code 2007.

Furthermore, the so-called “equivalence” of the ingredients (below) with nutritious foods undermines the epidemiologically proven value of a Mediterranean diet and Australian Dietary Guidelines (2013); an alternative to “medical foods” that, if implemented correctly, may have lower costs, fewer side effects and stronger epidemiological health outcomes.

Finally, although the promotion makes much of the need for medical supervision, both the sponsor’s web site and numerous internet pharmacies promote sales online.

The ingredients (amount in one bottle of Souvenaid® (125 ml))

- EPA* (fish oil): 300mg – said to be equivalent to 4 tins of tuna/ 100g fresh tuna
- DHA** (fish oil): 1200mg – said to be equivalent to 4 tins of tuna/ 100g fresh tuna
- Phospholipids: 106mg – said to be equivalent to 4 eggs
- Choline: 400mg – said to be equivalent to 100 g of minced beef
- UMP (uridine monophosphate): 625mg - said to equal 1 kg tomatoes
- Vitamin E: 40mg – said to be equivalent to the above tuna portion
- Vitamin C: 80mg – said to be equivalent to 1 orange
- Selenium: 60µg – said to be equivalent to a handful of brazil nuts
- Vitamin B12: 3µg – said to be equivalent to 4 cans of tuna:
- Vitamin B6: 1mg – said to be equivalent to 710 g of spinach
- Folic Acid: 400 µg: – said to be equivalent to 1.2 kg broccoli.

* EPA = eicosapentaenoic acid
** DHA = docosahexaenoic acid

Because of these serious, multiple and misleading breaches of the Act and Code I submit that an immediate CRP &/or TGA assessment and retraction order is warranted, the latter to run for the same duration and with the same number of insertions as the original promotion in the media used to distribute it.

The complainant
Complaints may be made anonymously, but you are encouraged to provide your contact details so that you can be advised of the outcome of your complaint. You may request (above) that your personal details not be provided to the advertiser or included in the published determination.

<table>
<thead>
<tr>
<th>Name: Dr Ken Harvey</th>
<th>Telephone: 0419181910</th>
</tr>
</thead>
<tbody>
<tr>
<td>Email: <a href="mailto:k.harvey@medreach.com.au">k.harvey@medreach.com.au</a></td>
<td>Fax: 03 9818 1875</td>
</tr>
<tr>
<td>Postal address: 35a Mary St Hawthorn Vic 3122</td>
<td>I am associated with the therapeutic goods industry: No</td>
</tr>
<tr>
<td>Signature: Dr Ken Harvey</td>
<td>Date: May 13, 2013</td>
</tr>
</tbody>
</table>

"Souvenaid is a nutritional management for the treatment of mild Alzheimer’s disease". “Associate Professor Michael Woodward is the head of aged and residential care services at Austin Health and a paid advisor to the manufacturers of the drink. He has 50 patients currently taking Souvenaid”. "Many of them have reported very positive findings…”


“Packed into one tiny dose is the equivalent nutritional benefit of 1 kg of tomatoes, 1.2 kg broccoli, 710 g of spinach, 100 g of fresh tuna, 100 g of minced beef, 4 eggs, one orange and a handful of brazil nuts”

Welcome to the Souvenaid® website

This website contains information about Alzheimer’s disease and Souvenaid®, a Food for Special Medical Purposes for the dietary management of the early stages of Alzheimer’s disease. Souvenaid® must be used under medical supervision of a healthcare professional, such as your doctor, nurse or pharmacist.

If you are a healthcare professional, please click here

Visit website for Health Care Professionals

If you are member of the general public, please click here

- ENTER PATIENT / CARER SITE

Nutricia Advanced Medical Nutrition is a global leader in providing medical nutrition for the management of a broad spectrum of disease states. Nutricia takes a scientific approach to nutrition that encompasses extensive research, clinical studies and co-operation with healthcare professionals around the world to deliver easy-to-use products that improve the quality of life for patients and their carers.

Nutricia supplies high quality foods, systems and support services to patients and healthcare professionals. Through our Nutricia Home service, we deliver nutritional foods, enteral feeding systems and nursing care directly to patients’ homes.

Souvenaid®
A new approach in the early stages of Alzheimer’s Disease

Souvenaid® is a medical nutrition drink that nutritionally supports memory function in the early stages of Alzheimer's disease. Souvenaid® is available in a once a day, 125ml bottle in both strawberry and vanilla flavours.

Call the Souvenaid® Helpline for more information: 1800 038 314

How does Souvenaid® work?

There is a growing body of evidence showing that people with early Alzheimer's disease have low levels of certain nutrients compared to healthy individuals of the same age. These nutrients play an important role in brain health and memory function. Souvenaid® has been designed to supply these nutrients to aid the naturally occurring processes in the brain, that are involved in maintaining the brain's integrity. The combination of nutrients is unique, and they work together, so that the effect is greater than when taking them individually.

Souvenaid® contains nutrients that occur naturally in food, although the amount of these nutrients would be difficult to achieve through a normal diet. In clinical trials, Souvenaid® was shown to have a positive effect in people during the early stages of Alzheimer's disease.

Clinically Proven

Two clinical trials have been carried out to look at the effect of Souvenaid® on patients with mild Alzheimer's disease and one in mild to moderate Alzheimer's disease. Souvenir I trial, investigated the effects of Souvenaid® over a 12 week period. In Souvenir II, the study period was extended to 24 weeks.

All trials showed, in patients taking Souvenaid®, the nutrients were absorbed, as reflected in the increased measured blood samples during the trial period. In addition to the improved nutritional status, other standardised test measures were positively influenced in the patients with mild Alzheimer's disease who had been taking Souvenaid®, compared to those receiving a control product. The control product looked and tasted like Souvenaid®, but did not contain Fortasyn™ Connect.

Souvenaid® must be recommended to you by a healthcare professional such as your own physician, dietician or other qualified medical professional such as a pharmacist.

Speak to your healthcare professional about Souvenaid® to determine whether it is the right product for you.

Souvenaid® is available as a once a day 125ml drink in two tasty flavours: strawberry and vanilla. Both flavours are available online in cases of 24 x 125ml bottles (RRP $99.84), or in pharmacy, in cases of 24 x 125ml bottles (RRP $99.84) or in packs of 4 bottles (RRP $16.64).

If Souvenaid® has been recommended to you by your healthcare professional you can either:

1. Purchase Souvenaid® online, following the link below, or
2. Purchase Souvenaid® from your local pharmacy.

https://www.souvenaid.com.au/Souvenaid/Where-can-I-get-Souvenaid%C2%AE-
What is in Souvenaid®?

The picture below illustrates the quantities of food that would need to be consumed daily, on top of a normal diet, to obtain the levels of nutrients that one 125ml bottle of Souvenaid® provides. Click on the components on the left hand side of the picture to view a breakdown of the dietary equivalence of each component in Souvenaid®.

Dietary equivalents for one bottle of Souvenaid® 1-5

Select component to see the amount in Souvenaid® and dietary equivalent.

- DHA
- EPA
- UMP
- Choline
- Phospholipids
- Folic Acid
- Vitamin B6
- Vitamin B12
- Vitamin C
- Vitamin E
- Selenium

Visit website of Health Care Professional

Below you will find some important information to consider before you start taking Souvenaid®. Click the link to the right to print this page and take it with you to your healthcare professional.

Souvenaid® contains a unique combination of nutrients, called Fortasyn™ Connect, designed to meet the specific nutritional needs of people in the early stages of Alzheimer's disease. These include omega-3 polyunsaturated fatty acids (fish oil), uridine monophosphate and choline, together with phospholipids, B vitamins and other nutrients at levels difficult to achieve from normal dietary intake alone. Together, these nutrients have been shown to nutritionally support memory function in the early stages of Alzheimer's disease.

Souvenaid® is a once a day multi-nutrient drink available in a 125ml bottle in 2 flavours - vanilla and strawberry.

### Nutritional composition of 125ml bottle of Souvenaid®

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>125</td>
</tr>
<tr>
<td>Protein, g</td>
<td>3.8</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>16.5</td>
</tr>
<tr>
<td>Fat, g</td>
<td>4.9</td>
</tr>
</tbody>
</table>

Souvenaid® is not suitable as a sole source of nutrition and should only be used in addition to normal dietary intake.

Souvenaid® contains ingredients obtained from milk, fish and soy. People who have an allergy to milk, protein, fish or soy may not be able to consume Souvenaid®.

Souvenaid® is not suitable for people with Galactosemia.

Souvenaid® is gluten free. People with Coeliac disease or gluten sensitivity can consume Souvenaid®.

Souvenaid® is lactose free. People with lactose intolerance can consume Souvenaid®.

People with diabetes can consume Souvenaid®. However, Souvenaid® does contain carbohydrate and as with other foods containing carbohydrate, it is advisable for people with diabetes to monitor their blood glucose levels in consultation with their diabetes medical team.

List of the key nutrients in 125ml bottle of Souvenaid®

- EPA (fish oil) 300mg
- DHA (fish oil) 1200mg
- Phospholipids 106mg
- Choline 400mg
- UMP (uridine monophosphate) 635mg
- Vitamin E 40mg
- Vitamin C 80mg
- Selenium 60μg
- Vitamin B12 3μg
- Vitamin B6 1mg
- Folic acid 400μg

* EPA = eicosapentaenoic acid
** DHA = docosahexaenoic acid

Souvenaid Strawberry 125ml x 4

Souvenaid® is a new medical nutrition drink that nutritionally supports memory function in the early stages of Alzheimer’s disease. Taken once daily, Souvenaid® has been shown to have a positive effect in people during the early stages of the disease, by nutritionally supporting the connections in the brain, called synapses.

**Dosage:**

Drink one bottle per day, every day

**FortasynTM Connect**

Based on more than 10 years of research and development, Souvenaid® contains a unique combination of nutrients, called FortasynTM Connect, which provide the nutritional building blocks that are required by people with early Alzheimer’s disease.

**Unique Nutrition**

The unique combination of nutrients in Souvenaid® includes omega-3 polyunsaturated fatty acids, uridine monophosphate and choline, together with phospholipids, B vitamins and other nutrients, at levels difficult to achieve from dietary intake alone. Click the button below to see what you would have to eat, on top of your normal diet, to obtain the same levels of nutrients that one 125ml bottle of Souvenaid® provides.

Souvenaid® contains a unique combination of nutrients, called FortasynTM Connect, designed to meet the specific nutritional needs of people in the early stages of Alzheimer’s disease. These include omega-3 polyunsaturated fatty acids (fish oil), uridine monophosphate and choline, together with phospholipids, B vitamins and other nutrients at levels difficult to achieve from normal dietary intake alone. Together, these nutrients have been shown to nutritionally support memory function in the early stages of Alzheimer’s disease.

Souvenaid® is a once a day multi-nutrient drink available in a 125ml bottle in 2 flavours - vanilla and strawberry

Nutricia Souvenaid Strawberry 125ml X 4

Product Information

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Nutricia Souvenaid Strawberry 125ml X 4

Code: 392812

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

Philip Scheltens\textsuperscript{a,}\textsuperscript{*}, Patrick J. G. H. Kamphuis\textsuperscript{b}, Frans R. J. Verhey\textsuperscript{c}, Marcel G. M. Olde Rikkert\textsuperscript{d}, Richard J. Wurtman\textsuperscript{e}, David Wilkinson\textsuperscript{f}, Jos W. R. Twisk\textsuperscript{g}, Alexander Kurz\textsuperscript{h}

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\textsuperscript{b}Danone Research, Centre for Specialised Nutrition, Wageningen, The Netherlands
\textsuperscript{c}Alzheimer Centre Limburg, Maastricht University Medical Centre, The Netherlands
\textsuperscript{d}Alzheimer Centre Nijmegen, Radboud University Medical Centre Nijmegen, The Netherlands
\textsuperscript{e}Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA
\textsuperscript{f}Memory Assessment and Research Centre, Moorgreen Hospital, Southampton, United Kingdom
\textsuperscript{g}Department of Health Sciences, VU University Medical Center, Amsterdam, The Netherlands
\textsuperscript{h}Department of Psychiatry, Klinikum Rechts der Isar, Technische Universität München, Germany

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.

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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
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 glutamnergic 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, tranquilizers, 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 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.)
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 tetrapackage), 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) the 13-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.

### Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount per daily dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>300 mg</td>
</tr>
<tr>
<td>DHA</td>
<td>1200 mg</td>
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<tr>
<td>Phospholipids</td>
<td>106 mg</td>
</tr>
<tr>
<td>Choline</td>
<td>400 mg</td>
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<tr>
<td>UMP</td>
<td>625 mg</td>
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<tr>
<td>Vitamin E (alpha-TE)</td>
<td>40 mg</td>
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<tr>
<td>Vitamin C</td>
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<tr>
<td>Selenium</td>
<td>60 μg</td>
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<tr>
<td>Vitamin B12</td>
<td>3 μg</td>
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<tr>
<td>Vitamin B6</td>
<td>1 mg</td>
</tr>
<tr>
<td>Folic acid</td>
<td>400 μg</td>
</tr>
</tbody>
</table>

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

*Souvenaid (125 mL daily dose) contains Fortasyn Connect.
ingredients of the active product. On the basis of these stud-
ies, 80 patients (completers) per group were required to detect
a between-group difference of 1.5 units on the WMS-r de-
layed recall test and the ADAS-cog after 12-week supple-
mentation (power of 0.80; \( P < .05 \), two-sided test). With
a drop-out rate of 25%, approximately 214 patients were re-
quired.

A prespecified blinded interim analysis of safety and pri-
mary efficacy data was done after 84 patients had completed
the core 12-week study. The results were reviewed by the in-
dependent Data Monitoring Committee to check whether the
calculated sample size was adequate and that no safety con-
cerns 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 inten-
tion-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 repre-
sentated by dummies. For parameters that had a distinctly non-
normal 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 influ-
ence the estimate of intervention effect was investigated by
comparing the estimate for intervention effect in a model in-
cluding the covariate with the estimate for intervention effect
in a model excluding the covariate (confounder analyses). In
addition, the significance of the covariate-intervention inter-
action 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 pa-
tient characteristics (patients with early AD [baseline MMSE
24–26]; patients with late-onset AD; and by apolipoprotein E

<table>
<thead>
<tr>
<th>246 assessed for eligibility</th>
<th>21 excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 did not meet inclusion criteria</td>
</tr>
<tr>
<td></td>
<td>5 withdrew consent</td>
</tr>
<tr>
<td></td>
<td>14 other reasons</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>225 randomized</th>
</tr>
</thead>
<tbody>
<tr>
<td>113 allocated to active product</td>
</tr>
<tr>
<td>8 withdrew from study</td>
</tr>
<tr>
<td>1 SAE</td>
</tr>
<tr>
<td>2 AE</td>
</tr>
<tr>
<td>3 withdrew consent*</td>
</tr>
<tr>
<td>2 protocol deviations</td>
</tr>
<tr>
<td>105 completed core 12-week study</td>
</tr>
<tr>
<td>6 excluded due to site violation*</td>
</tr>
<tr>
<td>99 included in 12-week efficacy analysis</td>
</tr>
<tr>
<td>13 did not enter extension</td>
</tr>
<tr>
<td>6 to start AD drugs</td>
</tr>
<tr>
<td>7 withdrew consent</td>
</tr>
<tr>
<td>86 entered extension phase</td>
</tr>
<tr>
<td>2 withdrew from extension</td>
</tr>
<tr>
<td>1 SAE</td>
</tr>
<tr>
<td>1 protocol deviation</td>
</tr>
<tr>
<td>84 included in 24-week efficacy analysis</td>
</tr>
</tbody>
</table>

| 112 allocated to control product |
| 6 withdrew from study |
| 3 SAE |
| 1 AE |
| 1 withdrew consent |
| 1 protocol deviation |
| 106 completed core 12-week study |
| 6 excluded due to site violation* |
| 100 included in 12-week efficacy analysis |
| 17 did not enter extension |
| 12 to start AD drugs |
| 5 withdrew consent |
| 83 entered extension phase |
| 6 withdrew from extension |
| 2 SAE |
| 1 AE |
| 3 withdrew consent |
| 77 included in 24-week efficacy analysis |

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.
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 non-parametric analyses. Both noncategorical (Mann–Whitney U Wilcoxon W test) and categorical nonparametric analyses (χ² 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 χ² test).

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 χ² 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 ≤ .001 vs controls; Fig. 3A) and plasma level of vitamin E increased significantly (+19% in active group, −1% in control group; P ≤ .001). Concomitantly, plasma homocysteine level in the active group was 23% lower than at baseline, and 19% lower than in the control group (P ≤ .001; Fig. 3B). The levels of DHA and EPA in

Table 2

Baseline demographic and clinical characteristics of the intention-to-treat efficacy population (n = 212)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 106)</th>
<th>Active (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at screening, yr [range]</td>
<td>73.3 (7.8) [52–92]</td>
<td>74.1 (7.2) [54–87]</td>
</tr>
<tr>
<td>BMI at baseline, kg/m²</td>
<td>26.2 (3.5)</td>
<td>26.2 (4.8)</td>
</tr>
<tr>
<td>Years of education beyond primary school</td>
<td>6.0 (4.0)</td>
<td>5.5 (3.9)</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>138.7 (18.6)</td>
<td>139.3 (20.0)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80.7 (10.7)</td>
<td>80.4 (10.4)</td>
</tr>
<tr>
<td>Median time from AD diagnosis to baseline, d [range]</td>
<td>31.5 [0–1036]</td>
<td>30.0 [18 to 1932]</td>
</tr>
<tr>
<td>MMSE</td>
<td>24.0 (2.5)</td>
<td>23.8 (2.7)</td>
</tr>
<tr>
<td>13-Item modified ADAS-cog</td>
<td>25.5 (8.8)</td>
<td>25.9 (7.6)</td>
</tr>
<tr>
<td>Median WMS-r delayed verbal memory test [range]</td>
<td>2.0 [0–17]</td>
<td>1.0 [0–16]</td>
</tr>
<tr>
<td>Median WMS-r immediate verbal memory test [range]</td>
<td>5.0 [0–19]</td>
<td>4.0 [0–15]</td>
</tr>
<tr>
<td>ADCS-ADL</td>
<td>61.9 (10.9)</td>
<td>61.1 (10.5)</td>
</tr>
<tr>
<td>Median NPI-12</td>
<td>4.00 [0–54]</td>
<td>4.00 [0–37]</td>
</tr>
<tr>
<td>Quality of life–AD (composite score)</td>
<td>35.3 (4.7)</td>
<td>34.9 (4.0)</td>
</tr>
</tbody>
</table>

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).

1Values are means (SD), unless stated otherwise.
2The value of −18 days represents a protocol deviation. In this case, the patient was diagnosed 18 days after baseline assessment.
3The 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.
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: 4.1%; change from baseline of −4 points or greater: 11.1%), although not reaching significance (data not shown). 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  = 0.19; P < .001).

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 ( = .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;  = .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 ( = .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.
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)

<table>
<thead>
<tr>
<th>Efficacy parameter</th>
<th>Control</th>
<th>Active</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonparametric analyses</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WMS-r delayed verbal recall test, % (co-primary outcome measure)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decline in score</td>
<td>[n = 98]</td>
<td>[n = 100]</td>
<td>.021</td>
</tr>
<tr>
<td>No change</td>
<td>34</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>42</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>WMS-r immediate verbal recall test, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decline in score</td>
<td>[n = 98]</td>
<td>[n = 100]</td>
<td>.131</td>
</tr>
<tr>
<td>No change</td>
<td>45</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>15</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>NPI-12 (frequency x severity), %</td>
<td></td>
<td></td>
<td>.728</td>
</tr>
<tr>
<td>Decline in score</td>
<td>[n = 100]</td>
<td>[n = 101]</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>48</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Improvement</td>
<td>24</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

| **Parametric analyses**<sup>1</sup> |         |        |       |
| 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.

<sup>1</sup>Nonparametric χ<sup>2</sup> test comparing three categories of difference scores compared to baseline (decline in score = difference < 0; no change and improvement = difference ≥ 0 at 12 weeks).

<sup>2</sup>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,
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 synaptic 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**

<table>
<thead>
<tr>
<th>Adverse event body system</th>
<th>Control (n = 112)</th>
<th>Active (n = 113)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total adverse events</td>
<td>49 (43.8%) 58 (51.3%)</td>
<td>55 (48.8%) 63 (55.7%)</td>
<td>.286</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>20 (17.9%) 21 (18.6%)</td>
<td>18 (16.1%) 20 (17.5%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diagnostic procedures</td>
<td>8 (7.1%) 13 (11.5%)</td>
<td>10 (8.9%) 15 (13.2%)</td>
<td>.360</td>
</tr>
<tr>
<td>Psychiatric conditions</td>
<td>11 (9.8%) 7 (6.2%)</td>
<td>12 (10.7%) 7 (6.2%)</td>
<td>.338</td>
</tr>
<tr>
<td>Infections and infestations</td>
<td>5 (4.5%) 11 (9.7%)</td>
<td>8 (7.1%) 15 (13.2%)</td>
<td>.193</td>
</tr>
<tr>
<td>Nervous system</td>
<td>9 (8.0%) 9 (8.0%)</td>
<td>10 (8.9%) 9 (8.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Locomotor/musculoskeletal, connective tissue</td>
<td>4 (3.6%) 9 (8.0%)</td>
<td>6 (5.3%) 11 (9.7%)</td>
<td>.253</td>
</tr>
<tr>
<td>Skin, subcutaneous tissue, appendages</td>
<td>7 (6.3%) 3 (2.7%)</td>
<td>5 (4.5%) 2 (1.8%)</td>
<td>.215</td>
</tr>
<tr>
<td>General, body as a whole</td>
<td>5 (4.5%) 6 (5.3%)</td>
<td>11 (9.7%) 11 (9.7%)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

*Only those reported by at least 5% of subjects in either group are shown here.
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 \( P = 0.046 \) 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 exploratory 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 \( (P = 0.009 \) 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).

Acknowledgments

Study design and planning were done in conjunction with the study sponsor, Danone Research–Centre for Specialised Nutrition (part of Groupe Danone). The sponsor provided the study products and funding. The sponsor undertook data collection. Staff of Danone Research together with an independent statistician (J.W.R.T.) undertook data analysis. All authors had full access to study data. The corresponding author had final responsibility for the decision to submit for publication.

The role of Alpha-Plus Medical Communications Limited, in assisting with developing the first draft of the manuscript and editing subsequent drafts is acknowledged.

References

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Souvenaid</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
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<td>Protein, g</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>Niacin (B3), mg NE</td>
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<td>Vitamin K, mcg</td>
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</table>

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; TE, tocopherol equivalents; NE, niacin equivalents.
EFFECTS OF A MEDICAL FOOD ON COGNITION IN AD

EFFICACY OF A MEDICAL FOOD ON COGNITION IN ALZHEIMER’S DISEASE: RESULTS FROM SECONDARY ANALYSES OF A RANDOMIZED, CONTROLLED TRIAL

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Abstract: Objective: To investigate the extent that baseline cognitive impairment and intake adherence affected the 13-item Alzheimer’s Disease Assessment Scale – cognitive subscale (ADAS-cog) intervention response of a medical food in Alzheimer’s Disease (AD) patients. Design/setting/participants /intervention/measurements: This analysis was performed on data from a proof-of-concept study, consisting of a 12-week, double-blind, randomized, controlled, multicenter trial, followed by a similarly designed 12-week extension study. Patients with mild AD (Mini-Mental State Examination [MMSE] score of 20–26) were randomized to receive active or control product as a 125 ml daily drink. One of the co-primary outcome measures was the 13-item ADAS-cog. In this analysis, the study population was divided into two subgroups: patients with ‘low’ baseline ADAS-cog scores (<25.0) and patients with ‘high’ baseline ADAS-cog scores (≥25.0). Repeated Measures Models (RMM) were used to determine the relationship between ADAS-cog score and intervention. Results: A significant treatment effect (F[1,319]=4.0, p=0.046) was shown in patients with ‘high’ baseline ADAS-cog, but not in patients with ‘low’ baseline ADAS-cog (F[1,250]=1.25, p=0.265). Overall, intake adherence was significantly correlated with ADAS-cog improvement in the active product group (correlation coefficient=0.260; p=0.019), but not the control group. Conclusion: These data indicate that baseline ADAS-cog significantly influenced the effect of Souvenaid intervention on ADAS-cog outcome. A higher intake of active study product was also associated with greater cognitive benefit. These findings highlight the potential benefits of Souvenaid in AD patients and warrant confirmation in larger, controlled studies.

Key words: Alzheimer’s disease, cognition, treatment outcome, nutrition.

Introduction

Alzheimer’s disease (AD) is a progressive, neurodegenerative disorder characterized by memory loss, cognitive deterioration, executive and visuospatial dysfunction and impaired ability to perform activities of daily living (1). Synaptic loss is thought to be a primary cause of the symptoms of AD (2, 3), particularly hippocampal and cortical synapse loss, as indicated by the nature of the cognitive dysfunction typical of the disorder (3).

Preclinical studies have indicated that the administration of nutrients involved in the synthesis of synaptic membranes increases synapse and synaptic membrane formation in the brain. These include precursors for membrane phosphatidylation such as uridine, choline and omega-3 polyunsaturated fatty acids (4–6). Reports have also indicated that combining these nutrients may improve cognition and increase hippocampal dendritic spines (7), again suggesting a positive effect on the formation of new synapses (8–10). It was therefore hypothesized that such agents may play a role in the management of AD.

This hypothesis led to the development of the medical food Souvenaid® (Nutricia N.V., Zoetermeer, The Netherlands) (11), a multinutrient drink designed to provide the precursor and supporting nutrients that may enhance synaptic membrane formation and function in patients with AD. A recent proof-of-concept study demonstrated that dietary supplementation with Souvenaid was well tolerated, and resulted in a significant improvement of memory, as measured by 12-week delayed verbal recall testing (12). The co-primary outcome measure for the study, the modified 13-item Alzheimer’s Disease Assessment Scale – cognitive subscale (ADAS-cog) (13) showed no overall intervention effect for Souvenaid, with neither the control nor active group showing any decline over 24 weeks on this outcome measure, which was attributed to a potential lack of sensitivity with the ADAS-cog measure in mild AD patients over this study period (12). ADAS-cog is widely regarded by regulatory authorities as the ‘gold standard’ outcome measure for assessing cognitive change in clinical trials, and as such it is important to further investigate factors that might influence the effect on ADAS-cog. This formed the rationale for investigating the extent that baseline cognitive impairment affected the ADAS-cog intervention response. In addition, we studied the influence of intake adherence on
ADAS-cog response.

Materials and Methods

Study design
The 24-week proof-of-concept study (12) consisted of a 12-week, double-blind, randomized, controlled, multicenter trial, followed by a similarly designed, optional 12-week extension study, to evaluate the effect of Souvenaid® on cognitive function in patients with mild AD. The methodology has been described in detail previously (12). In summary, patients ≥50 years of age with a diagnosis of probable AD and a Mini-Mental State Examination (MMSE) score of 20–26 were recruited. Patients were randomized to receive either active or control product as a 125 ml daily drink. Primary outcome measures were a delayed verbal recall task (WMS-r) (14) and ADAS-cog (13-item version, range 0–85, higher scores indicating greater cognitive deficit) (13) measures of delayed verbal memory and cognition. These parameters were measured at baseline and at Weeks 6, 12 and 24. Adherence to study product intake was measured via patient documentation of the amount of study product taken each day and verified by measuring blood plasma parameters. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use / WHO Good Clinical Practice (ICH-GCP) guidelines, as appropriate to nutritional products and legislation of the country in which the research was conducted. The clinical trial registration number is ISRCTN72254645.

Modeling analysis
The primary analysis population was the intention-to-treat (ITT) efficacy population from the proof-of-concept study, defined as all randomized patients who received at least one dose of study product and one post-baseline assessment. For the modeling analyses presented here, one patient (from the active study group) was excluded from the ITT efficacy population as he showed an extreme outlying response on the 24-week ADAS-cog outcome (Figure 1). This may be explained by a leg amputation 8 days prior to the 24-week ADAS-cog assessment, which took place in the hospital and was recorded as a serious adverse event. Based on the median as a cut-off value, the study population was divided into two subgroups: patients with ‘low’ baseline ADAS-cog scores (<25.0; lower scores indicating reduced cognitive deficit) and patients with ‘high’ baseline ADAS-cog scores (≥25.0; indicating greater cognitive deficit).

Repeated Measures Models (RMM) for each subject were used to determine the relationship between ADAS-cog score and intervention up to 24 weeks. The SAS procedure PROC MIXED (15) was used to model the covariance among the repeated measures obtained on the same individuals (16). Different structures for the means and different variance–covariance structures were tested. The structure with the best fit was selected based on the likelihood ratio test for nested models and the Akaike Information Criterion (AIC) for non-nested models.

Results
Overall, 225 patients were randomized: 112 to active product and 113 to the control product. Of these, 161 completed the 24-week study (12). Baseline characteristics for the control and active patient populations in the current analysis are reported for all subjects, together with those for the ‘high’ and ‘low’ baseline ADAS-cog subgroups (Table 1). There were no statistically significant differences in baseline characteristics between active/control groups (Table 1).

Table 1
Patient characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Randomized study group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control product</td>
</tr>
<tr>
<td>Total patient population</td>
<td>(n = 106)</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>52 (49)</td>
</tr>
<tr>
<td>Age ± SD, yr</td>
<td>73.3 ± 7.8</td>
</tr>
<tr>
<td>13-item ADAS-cog, mean ± SD</td>
<td>25.5 ± 8.8</td>
</tr>
<tr>
<td>MMSE, mean ± SD</td>
<td>24.0 ± 2.5</td>
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<tr>
<td>‘Low’ baseline ADAS-cog group</td>
<td>(n = 43)</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>25 (58)</td>
</tr>
<tr>
<td>Age ± SD, yr</td>
<td>71.4 ± 8.4</td>
</tr>
<tr>
<td>13-item ADAS-cog, mean ± SD</td>
<td>17.6 ± 5.4</td>
</tr>
<tr>
<td>MMSE, mean ± SD</td>
<td>25.3 ± 2.1</td>
</tr>
<tr>
<td>‘High’ baseline ADAS-cog group</td>
<td>(n = 63)</td>
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<td>Men, n (%)</td>
<td>27 (43)</td>
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<td>Age ± SD, yr</td>
<td>74.6 ± 7.0</td>
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<tr>
<td>13-item ADAS-cog, mean ± SD</td>
<td>30.9 ± 6.3</td>
</tr>
<tr>
<td>MMSE, mean ± SD</td>
<td>23.1 ± 2.4</td>
</tr>
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</table>

All patients included in the modeling analysis: intention-to-treat efficacy population (12) minus one outlier; ADAS-cog = Alzheimer’s Disease Assessment Scale – cognitive subscale (0–85; higher scores indicate greater cognitive dysfunction); MMSE = Mini-Mental State Examination (0–30; lower scores indicate greater cognitive dysfunction).
**Subgroup of patients with ‘high’ baseline ADAS-cog**

Raw mean ADAS-cog scores for patients with ‘high’ baseline ADAS-cog are summarized in Table 2 (at baseline and Weeks 6, 12 and 24). Within this subgroup the ADAS-cog data were substantially skewed and a transformation was performed to adjust for this.

**Table 2**

<table>
<thead>
<tr>
<th></th>
<th>‘Low’ baseline ADAS-cog group</th>
<th>‘High’ baseline ADAS-cog group</th>
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<td></td>
<td>n Control</td>
<td>n Active</td>
</tr>
<tr>
<td>Baseline</td>
<td>43</td>
<td>52</td>
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<tr>
<td>Week 6</td>
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<td>51</td>
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<tr>
<td>Week 12</td>
<td>39</td>
<td>51</td>
</tr>
<tr>
<td>Week 24</td>
<td>34</td>
<td>44</td>
</tr>
</tbody>
</table>

All patients included in the modeling analysis: intention-to-treat efficacy population (12) minus one outlier; ADAS-cog = Alzheimer’s Disease Assessment Scale – cognitive subscale (0–85; higher scores indicate greater cognitive dysfunction).

RMM slope analysis of the transformed ADAS-cog, using ADAS-cog at baseline, 6, 12 and 24 weeks as the dependent variable, showed a significant treatment effect ($F_{[1,319]} = 4.0$, $p = 0.046$). Sensitivity analyses showed a strong indication (all $p$-values ranging from 0.029–0.067) that this effect is independent of: (a) the type of structure for the means used (modeling each visit separately using dummy variables instead of modeling the means as a straight-line and evaluating slopes); (b) the type of variance–covariance model used (compound symmetry instead of heterogeneous compound symmetry); and (c) the type of ADAS-cog transformation used (logarithm instead of square-root). Figure 2 shows the results from the RMM analyses (estimated means) for ADAS-cog over 24 weeks for patients with ‘high’ baseline ADAS-cog scores. The clear upward slope (representing a decrease in ADAS-cog score and indicating cognitive improvement) for the active group contrasts with the almost unchanged level of the control group. These data indicate that Souvenaid significantly improved cognitive performance versus the control product in patients with ‘high’ baseline ADAS-cog.

**Subgroup of patients with ‘low’ baseline ADAS-cog**

Raw mean ADAS-cog scores for patients with ‘low’ baseline ADAS-cog are summarized in Table 2 (at baseline and Weeks 6, 12 and 24). Within this subgroup there was no suggestion of an intervention effect, indicated by a non-significant intervention*time parameter ($F_{[1,250]} = 1.25$, $p = 0.265$).

**Baseline ADAS-cog value as a predictor of ADAS-cog change from baseline**

In order to determine whether the two ADAS-cog subgroups significantly differed from each other, they were combined into a single model. A patients’ membership to either subgroup was found to be a significant predictor of ADAS-cog intervention response (RMM: $F_{[1,657]} = 3.94$, $p = 0.048$ for the subgroup*slope coefficient, using untransformed ADAS-cog and allowing for different heterogeneous compound symmetry variance–covariance matrices for subgroups).

**Figure 2**

Estimated marginal mean ADAS-cog scores over 24 weeks for patients receiving active or control product who recorded a ‘high’ (≥median) ADAS-cog score at baseline (back-transformed data; transformed [square-root] data were squared).

**Impact of intake adherence on intervention response**

The relationship between intake adherence (represented as a percentage of the total study product consumed by the patient) and 24-week ADAS-cog change is shown in Figure 3. The active group showed a significant correlation between intake adherence and ADAS-cog improvement ($r = -0.260$, $p = 0.019$), but this correlation was not observed in the control group ($r = 0.108$, $p = 0.343$). This difference in correlation coefficients between the active and control group was statistically significant (Fisher’s Z transformation, $Z = 2.32$, $p = 0.020$). Effect modifier analyses to determine the relationship between intake adherence and ADAS-cog response showed a significant interaction ($F_{[1,546]} = 5.88$, $p = 0.016$; RMM model using untransformed...
ADAS-cog and including variable “intake adherence”).

In summary, together with the incidence of adverse events (12), baseline ADAS-cog and intake adherence appear to be important effect modifiers that can influence the 24-week ADAS-cog intervention effect.

Discussion

This post-hoc analysis indicates that baseline ADAS-cog score significantly influenced the effect of Souvenaid intervention on this outcome measure. Within the group of patients with higher ADAS-cog scores at baseline, Souvenaid significantly improved ADAS-cog scores compared with the control group. These observations are in line with recent publications that bring into question the sensitivity of ADAS-cog in mild cognitive impairment and mild AD (17-19). This may be due to the poor psychometric properties of the ADAS-cog measure, such as inadequate assessment of cognitive domains such as attention, working memory and executive function (19, 20) and the presence of floor effects (17). Furthermore, several recent studies have reported slower rates of placebo decline in AD patients than traditionally assumed by older models and clinical trials (12, 21-23); they have also shown that baseline ADAS-cog significantly affects the rate of AD progression (23, 24).

Most AD intervention studies report on a mild–moderate dementia population. Only a few prospective intervention studies have been performed in an exclusively mild AD population using ADAS-cog as an outcome measure (25-28). Of these, only the study reported by Seltzer et al. (25) reported a significant benefit on ADAS-cog.

In the study reported here, an absolute difference in ADAS-cog score between study groups of 2 points was demonstrated in favor of the active intervention group, for patients with higher ADAS-cog at baseline. This subgroup represents patients at a more advanced stage within the mild AD study population. This effect was observed despite the small sample size of this subgroup. However, it should also be noted that the statistical phenomenon of linear regression to the mean may have contributed to the apparent treatment effect.

The clinical importance of ADAS-cog change has been reviewed in several recent publications (22, 29). Vellas et al. reported that a 2-point effect on ADAS-cog outcome at 18 months may be considered clinically relevant, but greater differences (3–4 points) for clinical relevance have also been proposed (30). Taking these suggestions into account, the 2-point ADAS-cog intervention difference (13-item scale, range 0–85) may be considered a relevant finding that warrants further investigation in patients at a more advanced stage of AD.

Within the active study group a significant correlation between intake adherence and ADAS-cog improvement was observed. This indicates that a higher intake of Souvenaid (up to and including the prescribed dosage) provides greater cognitive benefit in AD patients up to 24 weeks. As expected, this relationship was not observed in the control group. In the study, excellent intake adherence was also demonstrated: the average 24-week compliance was 94% (percentage product intake versus prescribed dosage). These results, combined with the finding that intake adherence appears positively correlated to ADAS-cog improvement, highlight the potential of Souvenaid in AD.

Thus, although ADAS-cog is still considered the ‘gold standard’ measure of cognitive function in clinical trials for AD and other dementias, in modern studies it may be unable to detect subtle changes in patients with milder stages of the disease (18). To account for this issue, an ongoing study to investigate the efficacy of Souvenaid in AD with ADAS-cog as the primary outcome measure (S-CONNECT; NTR1683) includes patients with more moderate cognitive dysfunction (MMSE 14–24) than the original study (MMSE 20–26). Certainly, in the current analysis when the subgroup of AD patients with ‘high’ ADAS-cog scores at baseline was analyzed using RMM, the data showed that Souvenaid provided beneficial effects compared with control for up to 24 weeks. In addition, the results of this analysis indicate that adverse events, baseline cognitive severity and intake adherence should be taken into account when designing, and interpreting the results of, future studies.

In conclusion, the results of a controlled, 24-week, proof-of-concept study demonstrated that dietary supplementation with Souvenaid yields improvements in the memory of patients with mild and very mild AD (12). The analysis presented here also suggests that Souvenaid may provide cognitive benefits to patients with more moderate stages of the disease. These hypothesis-generating results warrant confirmation in larger scale, controlled studies.

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References

EFFECTS OF A MEDICAL FOOD ON COGNITION IN AD


Efficacy of Souvenaid in Mild Alzheimer’s Disease: Results from a Randomized, Controlled Trial


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Abstract. Souvenaid aims to improve synapse formation and function. An earlier study in patients with Alzheimer’s disease (AD) showed that Souvenaid increased memory performance after 12 weeks in drug-naïve patients with mild AD. The Souvenir II study was a 24-week, randomized, controlled, double-blind, parallel-group, multi-country trial to confirm and extend previous findings in drug-naïve patients with mild AD. Patients were randomized 1:1 to receive Souvenaid or an iso-caloric control product once daily for 24 weeks. The primary outcome was the memory function domain Z-score of the Neuropsychological Test Battery (NTB) over 24 weeks. Electroencephalography (EEG) measures served as secondary outcomes as marker for synaptic connectivity. Assessments were done at baseline, 12, and 24 weeks. The NTB memory domain Z-score was significantly increased in the active versus the control group over the 24-week intervention period (p = 0.023; Cohen’s d = 0.21; 95% confidence interval [−0.06]–[0.49]). A trend for an effect was observed on the NTB total composite z-score (p = 0.053). EEG measures of functional connectivity in the delta band were significantly different between study groups during 24 weeks in favor of the active group.

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INTRODUCTION

The main pathological hallmarks of Alzheimer’s disease (AD) are known to include the accumulation of amyloid-β plaques and neurofibrillary tangles due to abnormal protein processing. From the very start of the disease process, there is synaptic loss and reduced synaptic activity/connectivity in specific brain areas [1, 2]. In addition, synaptic loss is considered to be the most direct structural correlate of cognitive performance in AD [3], compared with the number of plaques or tangles, or degree of neuronal loss [2]. Synapses consist principally of neuronal membranes, and the neuronal and synaptic loss observed in AD has been linked to the degeneration of these membranes [4, 5]. This degeneration may account for the disturbed organization of brain networks, and support for this has been found in human studies showing impaired functional brain connectivity in patients with AD compared with controls [6, 7]. As such, synaptic loss and membrane-related pathology provide potentially useful targets for intervention in AD.

Administration of nutrients that are precursors for the abundant phosphatides in neuronal membranes can, in experimental animals, increase the formation and cellular levels of brain phosphatides [8]. In vitro and in vivo studies have demonstrated that supplementation with docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA), uridine (as uridine monophosphate [UMP]), and choline not only increase phosphatide synthesis [8], but also increase neurite outgrowth, levels of specific pre- or post-synaptic proteins, and the number of dendritic spines, all prerequisites for new synapse formation [9, 10]. Furthermore, B vitamins as co-factors enhance endogenous precursor synthesis via the regeneration of methyl groups, therefore affecting the availability of precursors [11], and potentially synaptogenesis. The administration of combinations of phospholipid synthesis-promoting nutrients has also been shown to enhance cognitive function and neurotransmitter release in animal models [12, 13].

These observations suggested that administering specific nutrients could increase synapse formation and synaptic function, and could potentially ameliorate cognitive disturbances of patients with AD [14]. This hypothesis formed the basis for the development of Souvenaid® (Nutricia N.V., Zoetermeer, The Netherlands), a product intended as a medical food* for oral consumption under medical supervision with the purpose of addressing disease-specific nutrient requirements. Souvenaid contains the nutritional combination Fortasyn™ Connect, which includes precursors and other specific nutrients required to enhance neuronal membrane formation (Table 1).

Synaptic dysfunction is a pathological process involved in the early stages of AD [1, 2]. Targeting synaptic loss and membrane-related pathology might therefore be most efficacious at this stage. Furthermore, it is commonly accepted in the field that interventions must be administered as early as possible. Therefore, the efficacy and tolerability of Souvenaid was first tested in a double-blind, controlled, multi-country, proof-of-concept study (Souvenir I study, Dutch Trial Register #ISRCTN72254645) involving 225 drug-naïve patients with mild AD (Mini-Mental State Examination [MMSE] scores, 20–26). In that study, Souvenaid was well tolerated and improved 12-week memory performance as measured by delayed verbal recall testing, the co-primary endpoint of the study. The other co-primary outcome (Alzheimer’s Disease Assessment Scale-cognitive

* A medical food is (in the 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 recognized scientific principles, are established by medical evaluation.” A comparable definition exists in the harmonized legislation of the European Union (cf. Article 1.2(b) of Commission Directive 1999/02/EC of 25 March 1999 on dietary foods for special medical purposes.

Table 1: Nutritional composition of Fortasyn Connect, the nutrient combination in Souvenaid

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount per daily dose*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eicosapentaenoic acid, mg</td>
<td>300</td>
</tr>
<tr>
<td>Docosahexaenoic acid, mg</td>
<td>1200</td>
</tr>
<tr>
<td>Phospholipids, mg</td>
<td>106</td>
</tr>
<tr>
<td>Choline, mg</td>
<td>400</td>
</tr>
<tr>
<td>Uridine monophosphate, mg</td>
<td>625</td>
</tr>
<tr>
<td>Vitamin E (alpha-tocopherol equivalents), mg</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>80</td>
</tr>
<tr>
<td>Selenium, µg</td>
<td>60</td>
</tr>
<tr>
<td>Vitamin B12, µg</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin B6, mg</td>
<td>1</td>
</tr>
<tr>
<td>Folic acid, µg</td>
<td>400</td>
</tr>
</tbody>
</table>

*Souvenaid (125 mL [125 kcal] daily dose) contains Fortasyn Connect. Souvenaid is a registered trademark of Nutricia N.V. Fortasyn is a trademark of Nutricia N.V.

subscale [ADAS-cog] remained unchanged [15]. The results of the Souvenir I study provided a first indication to support the hypothesis above; however, no biomarkers of synaptic activity and connectivity were measured to further validate this hypothesis.

Although it is not possible to quantify synaptic density directly in humans, electrical brain activity can be measured directly at the skull with electroencephalography (EEG). The resulting time series are a compound of synaptic activity in the cerebral cortex underneath the EEG electrodes. Newer techniques for the analysis of EEG signals allow us to study whether changes in synaptic activity can be detected as changes in functional connectivity [16].

It has been reported previously that the ADAS-cog as a tool for measuring cognition may not be adequate to detect changes in patients with milder stages of AD [17], something which is in line with the observations of the Souvenir I study [15, 18]. The use of a Neuropsychological Test Battery (NTB) is increasingly seen as a promising method to detect changes in cognition in early AD [17]. In addition, early AD is characterized by deficits in episodic memory [19] and the NTB has shown to be able to detect changes in memory performance [20].

Taking into account all of the above, the ‘Souvenir II’ study was designed to evaluate the effect of Souvenaid on memory in drug-naïve patients with mild AD (MMSE ≥ 20), using an intervention period of 24 weeks and the NTB memory domain score as the primary outcome measure. EEG was included as a secondary parameter to substantiate the biological effect on synaptic function.

MATERIALS AND METHODS

Patients

The Souvenir II study was a 24-week, randomized, controlled, double-blind, parallel-group, multi-country, trial to assess the efficacy and tolerability of Souvenaid in drug-naïve patients with mild AD. Patients aged ≥ 50 years with a diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) criteria [21], an MMSE score of ≥ 20, and a responsible caregiver were eligible for inclusion if a recent magnetic resonance imaging (MRI) or computed tomography (CT) scan had shown no evidence of any other potential causes of dementia.

Exclusion criteria included diagnosis of a significant neurological disease other than AD; a Geriatric Depression Scale (15-item) score of >6; use of a cholinesterase inhibitor or NMDA-receptor antagonist within 3 months prior to baseline; use of omega-3 fatty acid containing supplements or regular consumption of oily fish (>twice/week) within 2 months prior to baseline; use of atropine, scopolamine, tolterodine, hyoscyamine, biperiden, benztropine, trihexyphenidyl, oxybutynin, antipsychotics, vitamins B, C, and/or E (>200% of the recommended daily intake), consumption of high energy and/or high protein nutritional supplements, a change in dose of lipid-lowering medications, antidepressants, antihypertensives, or the use of other investigational products within 1 month prior to baseline; excessive alcohol intake or drug abuse; nursing home institutionalization; or investigator uncertainty regarding the willingness or ability of the patient to comply with the protocol.

Procedures

Participants were recruited from 27 AD centers: The Netherlands (9 centers), Germany (5 centers), Belgium (4 centers), Spain (3 centers), Italy (3 centers), and France (3 centers). Written informed consent was obtained from patients and caregivers. The Ethics Committees of each participating center reviewed and approved the protocol. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation guidelines for Good Clinical Practice as appropriate for nutritional products, and local legislation of the country in which the research was conducted. The Dutch Trial Registration number for this study is NTR1975.
Upon entry into the study, patients were randomized 1:1 to receive the active product Souvenaid, containing the specific nutrient combination Fortasyn Connect (DHA, EPA, phospholipids, choline, UMP vitamin B12, B6, and folate, vitamins C and E, and selenium [Table 1]), together with other vitamins, minerals, trace elements and macronutrients, or an iso-caloric control product that lacked Fortasyn Connect, but that was otherwise identical to the active product. Both study products were available as a 125 mL drink with an identical taste profile and appearance, taken once daily for 24 weeks. Allocation to the study groups was performed using a central randomization procedure in the Electronic Data Capture system, using four different randomization codes (A, B, C, and D). The investigator, study-site staff, Danone Research staff, study staff of the Clinical Research Organisation, patients, and caregivers were all blinded to the study products. The randomization code was not broken until initial statistical modeling of the primary outcome was complete. Major study parameters were assessed at baseline, 12, and 24 weeks. Adverse events (AEs) and changes in concomitant medication and nutritional supplements were recorded every three weeks.

Assessments

Primary outcome measure

The primary outcome measure to assess the effect of Souvenaid on memory performance over 24 weeks was the memory function domain score (z-score) based on the NTB [22]. This domain includes Rey Auditory Verbal Learning Test immediate recall, delayed recall and recognition performance, and Wechsler Memory Scale-revised (WMS-r) verbal paired associates immediate and delayed recall.

Secondary outcome measures

Secondary outcome measures included the executive function domain score (z-score) based on the WMS-r Digit Span, Trail Making Tests parts A and B (Delis Kaplan Executive Function System™ condition 2 and condition 4, respectively), Category Fluency, and the Controlled Oral Word Association Test. Study staff performing the assessments received appropriate training on outcome assessments prior to the start of the study.

Other secondary outcome measures included the total NTB composite score (based on all 12 NTB components), individual item scores from the NTB, assessments of functional ability (Disability Assessment for Dementia scale [DAD]) [23], nutritional blood parameters (plasma vitamin E, erythrocyte DHA and EPA, and homocysteine), and EEG to assess eyes-closed resting-state oscillatory brain activity and functional brain connectivity.

EEG data were available for a subset of subjects, as not all study sites were able to collect high quality EEG data. Relative and absolute power in alpha, beta, theta, and delta frequency bands and peak frequency (i.e., the dominant frequency of the power spectrum [24]) were used to assess oscillatory brain activity. The Phase Lag Index (PLI) in the frequency bands was used as the biomarker of functional connectivity, as it is not sensitive to volume conduction [25]; the higher the mean PLI value, the more brain regions are functionally connected. Extensive EEG protocol and analysis methods are described in the Supplementary data (available online: http://www.j-alz.com/issues/31/vol31-1.html#supplementarydata06).

Safety assessments

Safety assessments included the examination of patient medical history, recording of adverse events, and the monitoring of vital signs and additional laboratory parameters.

Statistical analyses

Sample size was based on repeated measurement analysis. Using an estimated effect size between active and control groups of 0.4 over 24 weeks, a significance level (α) of 0.05 and power (β) of 80%, and assuming a 15% drop-out rate, a sample size of 226 randomized patients was calculated. A pre-specified, blinded, re-estimation of the nuisance parameters was conducted to assess whether the calculated sample size was adequate. The results of this analysis and blind interim safety data were reviewed by an independent Data Monitoring Committee, which recommended continuation of the trial without modification.

Efficacy analyses were performed on the intent-to-treat population, defined as all patients randomized. Safety analyses were performed on the all-subjects-treated population, defined as all randomized patients who received at least one dose of study product. NTB domain z-scores were calculated by averaging the individual NTB item z-scores, which were calculated using the following equation: (individual NTB item score - baseline mean of study group)/baseline standard deviation.
Changes from baseline in outcome measures were analyzed using a mixed model for repeated measures (MMRM) with SAS® software (SAS Enterprise Guide 4.3 for Windows, SAS Institute Inc., Cary, NC, USA). In a three-level model with random site-specific intercepts and random subject-specific intercepts, group (treatment arm), time (treatment duration), and the group*time interaction were tested and adjusted for baseline values (baseline was included as covariate). The two degrees of freedom (df) contrast describing the difference in trajectories over time between active and control groups was taken as the primary indication of treatment effect during the intervention period. In addition, endpoint contrasts were reported. Sensitivity analyses were performed to check sources of variability and uncertainty of the data.

EEG data (relative and absolute power, PLI in alpha, beta, theta, and delta frequency bands and peak frequency) were analyzed using MMRM (2 df contrast) with post-baseline measurements as an outcome. All analyses included baseline and sample frequency as covariates. For the EEG power and PLI data, the modeling approach first examined whether an interaction between treatment and time could be considered constant over six brain areas (left/right fronto-central, left/right temporal, left/right parieto-occipital). Non-parametric alternatives (e.g., the Mann-Whitney U test) were used for non-normal distributions that were not sufficiently improved by transformations.

RESULTS

In total, 259 patients were randomized to intervention between November 2009 and May 2011 (Fig. 1). Twenty-one patients (8.1%) discontinued the study. Reasons for discontinuation included withdrawal of consent (n = 11), (serious) AEs (SAEs) (n = 5, 3 in the active group and 2 in the control group), patient lost to follow-up (n = 1), major protocol deviation (n = 1), and ‘other’ (n = 3). Baseline characteristics are summarized in Table 2. The study groups were well matched with regard to all characteristics. The mean baseline MMSE score for the overall study group was 25.0, suggestive of a population with very mild AD.

Primary outcome parameter

The NTB memory domain data are presented in Fig. 2 and Table 3. The memory domain z-score showed significant differences between the active and control groups in the trajectory over time during the 24-week intervention period (MMRM, 2 df contrast, \( p = 0.023 \), Cohen’s \( d \) on 24-week change from baseline scores: \( d = 0.21 \), 95% confidence interval \([-0.06, -0.49]\)). The significance of the difference between the active and control groups in their trajectories over time was primarily driven by the changes...
Fig. 2. Mean change from baseline in the Neuropsychological Test Battery (NTB) memory composite score. Error bars represent standard errors. The difference in trajectories over time between active and control groups during the 24-week intervention period: \( p = 0.023 \) (mixed model for repeated measures, 2 degrees of freedom contrast).

from Week 12 to Week 24 (MMRM, 1 df contrast, \( p = 0.006 \)).

Additional sensitivity analyses confirmed the robustness of the significant intervention effect. For example, the effect remained significant in statistical models with different options for the site variance, including site as fixed effect factor, or including an additional random site*treatment interaction effect. In addition, to evaluate the effect of missing NTB items, multiple imputation was used for the calculation of the NTB composite scores in the case of one or two missing NTB items. This confirmed the significant effect on the memory domain score over the 24-week intervention period (MMRM, 2 df contrast, \( p = 0.032 \)).

Secondary outcome parameters

There was a trend (MMRM, 2 df contrast, \( p = 0.053 \)) for an effect on NTB total composite score during the 24-week study period (see Fig. 3 and Table 3). No significant intervention effect on the NTB executive function domain (MMRM, 2 df contrast, \( p = 0.686 \), Fig. 3 and Table 3) was observed over 24 weeks. The results of all NTB individual items are summarized in supplementary Table 1.

For the DAD scores (ranging from 0% [most severe disability] to 100% [no disability]), a large proportion (26%) of patients achieved a maximum score at baseline (median = 91.2% [range 27.5-100%], overall study group), resulting in a far from normal distribution. Non-parametric testing of DAD scores did not reveal any significant difference between groups at study endpoint (Mann-Whitney U test, \( p = 0.361 \)).

EEG data were available for a subset of 179 subjects (86 and 93 from active and control groups, respectively). Parameters on EEG recording and analysis are presented in the Supplementary data, as are baseline characteristics for the subset of subjects for whom EEG data were available (see supplementary Tables 2 and 3). The predefined EEG power and PLI analyses were conducted over all brain areas since no significant interaction effects with brain area were observed. No significant differences between the groups were identified in the relative and absolute power of the different frequency bands. As expected in progressive AD, peak frequency slowed in the control group, which is indicative of cognitive deterioration [24]. The change in peak frequency over

Table 3

Descriptive statistics for NTB composite scores (intent-to-treat population)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 129)</th>
<th>Active (n = 130)</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTB memory domain z-score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.078 (0.884) [118]</td>
<td>-0.021 (0.812) [116]</td>
<td></td>
</tr>
<tr>
<td>Change baseline – Week 12</td>
<td>0.143 (0.429) [100]</td>
<td>0.069 (0.384) [107]</td>
<td></td>
</tr>
<tr>
<td>Change baseline – Week 24</td>
<td>0.111 (0.463) [103]</td>
<td>0.262 (0.395) [103]</td>
<td>( p = 0.090 )</td>
</tr>
<tr>
<td>24-week trajectory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTB executive function domain z-score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.043 (0.779) [113]</td>
<td>0.067 (0.734) [113]</td>
<td></td>
</tr>
<tr>
<td>Change baseline – Week 12</td>
<td>0.014 (0.351) [97]</td>
<td>0.028 (0.337) [100]</td>
<td></td>
</tr>
<tr>
<td>Change baseline – Week 24</td>
<td>0.006 (0.325) [99]</td>
<td>0.048 (0.333) [93]</td>
<td>( p = 0.386 )</td>
</tr>
<tr>
<td>24-week trajectory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTB total composite z-score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.115 (0.719) [105]</td>
<td>0.029 (0.695) [102]</td>
<td></td>
</tr>
<tr>
<td>Change baseline – Week 12</td>
<td>0.075 (0.262) [84]</td>
<td>0.065 (0.284) [85]</td>
<td></td>
</tr>
<tr>
<td>Change baseline – Week 24</td>
<td>0.035 (0.286) [89]</td>
<td>0.120 (0.278) [83]</td>
<td>( p = 0.035 )</td>
</tr>
<tr>
<td>24-week trajectory</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NTB, Neuropsychological Test Battery. Data are presented as mean (standard deviation) [N], unless stated otherwise.†Mixed model for repeated measures with change from baseline as outcome, baseline as covariate.
Fig. 3. Mean change from baseline in the (A) Neuropsychological Test Battery (NTB) total composite score and (B) NTB executive function domain score. Error bars represent standard errors; \( p \)-values represent the difference in trajectories over time between active and control groups during the 24-week intervention period; A) \( p = 0.053 \) (mixed model for repeated measures, 2 degrees of freedom contrast); B) \( p = 0.686 \) (mixed model for repeated measures, 2 degrees of freedom contrast).

Functional connectivity analysis (PLI) for the delta band revealed a significant difference in trajectory over 24 weeks between study groups in favor of the active group (MMRM, 2 df contrast, \( p = 0.011 \)) (see Fig. 4). This effect was not observed for the other frequency bands.

Significant biochemical changes of increased erythrocyte DHA and EPA (Mann-Whitney U test, \( p < 0.001 \)), plasma vitamin E, and decreased plasma homocysteine (Mann-Whitney U test, \( p < 0.001 \)) were detected in the active group (Fig. 5), indicative of a high adherence to intervention. Indeed, the calculated patient reported compliance during 24 weeks was very high (96.6% in the control group and 97.1% in the active group), with no difference between study groups (\( t \)-test, \( p = 0.536 \)).

Safety and tolerability parameters

In total, 145 patients (56.2%) reported at least one AE during the study. Of these, 67 patients (51.9%; 154 AEs in total) were in the active group, and 78 patients (60.5%; 185 AEs in total) were in the control group (\( p = 0.209 \); Fisher’s exact test). The majority of reported AEs were assessed as being ‘not related’ or ‘unlikely to be related’ to the intervention (84.1% overall; 82.5% active group; 85.4% control group), with no significant differences between study groups. The number and proportion of patients experiencing one or more AEs are summarized by body system in Table 4.

Eighteen SAEs occurred in 16 patients during the study: 11 SAEs in 10 patients in the active group and 7 SAEs in 6 patients in the control group. No SAEs were considered to be related to the study product by the investigator. Four SAEs resulted in premature discontinuation of the study (influenza-like symptoms, cerebrovascular disorder, depression, malaise). No deaths were reported.

No clinically relevant differences between study groups in vital signs and in blood parameters were observed.

DISCUSSION

The results of this multi-country, multi-center, clinical trial confirm the earlier finding that Souvenaid improved memory performance in drug-naïve patients with mild AD. Similar to previous observations, this
Fig. 5. Mean (A) docosahexaenoic acid (DPA) percentage of fatty acids in erythrocyte membrane, (B) eicosapentaenoic acid (EPA) percentage of fatty acids in erythrocyte membrane, (C) plasma vitamin E levels, and (D) plasma homocysteine. Error bars represent standard errors \( p < 0.001 \) (Mann-Whitney U test).

24-week study showed a positive safety profile and confirmed that Souvenaid is well tolerated. The EEG outcomes in this study demonstrate that there is a significant biological effect that could be interpreted in terms of changes in functional connectivity, supporting the hypothesis that the intervention enhances synapse formation and function. Thus, data from this study suggest that Souvenaid has a beneficial effect on cognitive function in mild AD, most probably by influencing functional connectivity.

Synapse loss is a principal cause of cognitive decline, thus enhancing synapse formation is a compelling and novel interventional target. The ingredients of Souvenaid act together to promote the synthesis of synaptic membranes and, consequently, synaptogenesis [8]. Synapse formation and elimination occurs throughout life and individual brain synapses are presently thought to be permanently remodeled in the adult brain [26]. Souvenaid was specifically designed and intended as a medical food with the action to feed a normal metabolic process by supplying membrane precursors obtained from dietary sources, and nutritional cofactors that enhance the membrane precursor availability. The intention is to address the specific nutritional requirement that patients with AD may have due to their synapse loss. This nutritional approach has been studied extensively in preclinical studies, and is reviewed elsewhere [8].

AD-related synaptic dysfunction is a pathological process thought to be involved early in the disease process, before the emergence of clinical symptoms [27]. In early AD, (episodic) memory dysfunction is one of the key manifestations, expected to be the most sensitive measure of cognition [19] and thought to be associated with reduction of synaptic contacts [28]. This is in line with our earlier findings in the proof-of-concept Souvenir I study, in which Souvenaid was shown to increase the co-primary outcome of memory performance after 12 weeks in mild AD (MMSE 20–26) and in a pre-specified subgroup of very mild AD (MMSE 24–26) as measured by the single WMS-r delayed verbal memory task [15]. The Souvenir II study design was based on these findings and is assessing memory in an early AD population (MMSE ≥ 20).
using the a priori defined NTB memory domain composite score, derived from five neuropsychological memory tasks.

Due to the different nature of the memory tests in the two studies with Souvenaid, including paragraph recall, word learning, and paired word association, the Souvenir II study extends our understanding of the effect that Souvenaid has on memory performance in patients with mild AD. The use of the NTB memory domain composite score and clustering of its raw memory test scores decreases variation associated with individual tests and helps to improve the robustness of the underlying cognitive constructs [29, 30].

The evidence for the degree of cognitive change as measured by the NTB is somewhat limited, which makes it more difficult to relate the memory effects reported here in terms of clinical effectiveness. A previously reported study showed that the mean 12-month NTB memory domain change from baseline for mild-to-moderate AD patients receiving placebo was −0.17 [20], while the average 12-month NTB change from baseline for patients with mild AD was −0.21 [22]. Another study by Salloway et al. [31] has reported similar NTB changes from baseline during 78 weeks. Even though the NTB composition employed in the Souvenir II study varied slightly from previous studies, the 24-week change from baseline of the NTB memory domain composite score of 0.20 in the active group is in the same order of magnitude, but in the positive direction compared to the decline observed in the placebo groups of these longitudinal studies.

The 24-week Souvenir II open-label extension study (Dutch Trial Registration number NTR2571) will provide more data on the NTB memory domain, and further studies are needed to investigate the long-term outcome of Souvenaid intervention.

The EEG signal reflects synchronous activity of many synapses and is therefore a derivative of underlying synaptic function [32]. The increasingly sophisticated tools available for the analysis of EEG signals provide opportunities to study small longitudinal changes, and as such EEG is increasingly valuable as an outcome measure in studies on AD and other dementias [33–36]. Quantitative frequency analysis and analysis of functional connectivity have shown slowing of the peak frequency and decreased functional connectivity between brain regions in patients with AD compared with controls [37]. The present findings of a significant difference in peak frequency and functional connectivity in the delta band over the 24-week intervention period between the active and control groups suggest preserved and even increased synaptic function in the active group. AD-related synaptic dysfunction is a pathological biomarker believed to rise before functional abnormalities manifest [27]. To our knowledge this study is the first to provide evidence for the hypothesis that supporting synaptic function in the mild stage of AD by using a nutritional intervention may be related to improved memory performance. This provides the impetus for further investigations using even more sophisticated techniques to study connectivity, such as magnetoencephalography.

The favorable safety profile of the nutritional intervention confirmed the hypothesis that the intervention would be well tolerated. AEs were consistent with those expected in an elderly population with mild AD. There were no differences between study groups in discontinuations due to (S)AEs, and none of the SAEs were considered to be related to the use of the study

<table>
<thead>
<tr>
<th>Body system</th>
<th>Control (n = 129)</th>
<th>Active (n = 129)</th>
<th>p-value/ Fisher's exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Example adverse events</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body as a whole</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue, asthenic-like symptoms</td>
<td>20 (15.5%)</td>
<td>11 (8.5%)</td>
<td>0.125</td>
</tr>
<tr>
<td>Central and peripheral nervous system disorders</td>
<td>18 (14.0%)</td>
<td>11 (8.5%)</td>
<td>0.237</td>
</tr>
<tr>
<td><strong>Disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastro-intestinal system disorders</td>
<td>30 (23.3%)</td>
<td>22 (17.1%)</td>
<td>0.277</td>
</tr>
<tr>
<td>Congenital,development, flatulence, nausea</td>
<td>9 (7.0%)</td>
<td>13 (10.1%)</td>
<td>0.505</td>
</tr>
<tr>
<td>Metabolic and nutritional disorders</td>
<td>9 (7.0%)</td>
<td>10 (7.8%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Musculo-skeletal system disorders</td>
<td>9 (7.0%)</td>
<td>10 (7.8%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Arthralgia, neuralgia</td>
<td>16 (12.4%)</td>
<td>15 (11.6%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>15 (11.6%)</td>
<td>10 (7.8%)</td>
<td>0.400</td>
</tr>
<tr>
<td>Respiratory system disorders</td>
<td>18 (14.0%)</td>
<td>21 (16.6%)</td>
<td>0.520</td>
</tr>
<tr>
<td>Pharyngitis, bronchitis</td>
<td>10 (7.8%)</td>
<td>4 (3.1%)</td>
<td>0.168</td>
</tr>
<tr>
<td>Skin and appendages disorders</td>
<td>8 (6.2%)</td>
<td>8 (6.2%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Rash, skin dry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fall, surgical intervention</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Only those reported by at least 5% of patients in either group are shown. Adverse events occurring in less than 5% of patients were: cardiovascular disorders, hearing and vestibular disorders, respiratory system disorders, metabolic and nutritional disorders, gastro-intestinal system disorders, musculo-skeletal system disorders, psychiatric disorders, skin and appendages disorders, body system disorders, psychiatric disorders, musculo-skeletal system disorders, dermatologic disorders, renal and urinary disorders, vascular disorders, central and peripheral nervous system disorders, and general disorders. **Examples adverse events are shown. **Table 4—Number(%) of patients experiencing one or more adverse events, by body system (all-subjects-treated population)*
product. Safety data were in line with those reported for the previous proof-of-concept study with Souvenaid [15]. The favorable safety profile and risk-benefit ratio, and mechanism of action make Souvenaid a promising candidate for further clinical investigation in the earlier stages of the AD spectrum, especially considering the disappointing results of single-nutrient intervention in many clinical studies [38].

There are some limitations to the study that should be considered when interpreting the results. Notably, the study was performed in an early AD population, and while the probable AD diagnosis was made according to the NINCDS-ADRDA criteria, no biomarker evidence was utilized for diagnosis. Also, the study population was moderate in size when compared to other AD clinical trials. Despite this, significant intervention effects were clearly identified and larger studies with more frequent assessments may reveal additional intervention effects. Secondly, an apparent placebo effect was observed after 12 weeks (the first assessments after baseline) for the overall NTB memory domain and various other NTB parameters. This may be a genuine placebo effect, or partly due to other factors such as learning/familiarity with the tests themselves, which again given the mildly affected state of the patients may have happened. In future studies, using two baseline scores and parallel versions of tests may overcome this [39]. Third, although no effect was observed on the overall executive function domain, dissociation between cognitive domains linked to the mechanism of action is not uncommon, and has been reported in previous studies utilizing the NTB as an outcome measure [20, 40]. Similarly, no effect was shown on the DAD, which may be due to the high percentage of patients showing a maximum DAD score at baseline, which in turn may be due to the very mild nature of AD in this study population. In addition, it is less conventional in an AD clinical trial to take the response profile from a MMRM analysis as the primary approach to identify an intervention effect. Nevertheless, this approach is particularly beneficial when the functional form of the response profiles is difficult to anticipate, and linear time trends may not adequately describe the response. Therefore, we pre-defined “trajectory of change” as our primary indicator of treatment effect. Finally, while a 24-week double-blind study has been typical for clinical trials in AD, and appropriate in view of ethical considerations in a drug-naïve population, it may be short given that AD is a chronic disease. Further studies are therefore warranted to investigate the longer-term outcome of Souvenaid intervention and to broaden our understanding of how the changes in functional connectivity over time for the delta band and mean peak frequency are related to clinical outcomes and other characteristics of brain network organization. These studies should be performed both with and without concomitant AD medications to reflect real-life clinical practice.

In conclusion, this study confirms that Souvenaid is well tolerated and improves memory performance. The testing of the underlying hypothesis has now made significant advances, offering hope towards a future management strategy directed at one of the major pathological manifestations or early AD. Our results warrant further investigation of the clinical potential of Souvenaid in preclinical or clinical conditions characterized by synaptic loss, in particular AD.

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CONTRIBUTORS


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REFERENCES


‘Brainbooster’ to be sold for Alzheimer’s

Niamh Mullen  all articles by this author
1 comment

A BRAIN-boosting nutraceutical drink said to improve memory in mild Alzheimer’s disease (AD) will go on sale in Australia from May.

Souvenaid is a 125ml drink containing omega-3 fatty acids, uridine, choline, phospholipids and B vitamins, which manufacturer Nutricia claims support synapse formation and memory function.

Two randomised controlled trials found it improved memory over 24 weeks. The Souvenir II study of 259 drug-naive patients found it improved memory in mild AD.

Electroencephalography (EEG) measures suggested it had an effect on brain functional connectivity, supporting the underlying hypothesis of changed synaptic activity.

Another trial of 527 people showed its effects were less pronounced in people with a mini-mental state exam score of 10—19.

An additional 24-month trial is underway in people with prodromal AD.

President of the Australian and New Zealand Society for Geriatric Medicine, Dr Robert Prowse, who has been approached by the company to join its advisory board, said he was initially sceptical but became impressed by the evidence. He said he wouldn’t be “pushing this out to the world” and cautioned it was not yet an established treatment.

A consultant in geriatric medicine at Ballarat Health Services, Associate Professor Mark Yates, who is on the advisory board, said it was something GPs could consider. “It would be surprising if it was a panacea, but it does change synaptic growth,” he said.

Professor Henry Brodaty, director of the Dementia Collaborative Research Centre at the University of New South Wales, also on the advisory board, said there was no evidence yet that it would improve function, or whether
any difference would be noticeable to carers or family.

Associate Professor Michael Woodward, also on the advisory board, said data showed 60% of people with AD were using a complementary medicine, many of which were not evidence based.

“I think it would be fair to say we have a lot more data for Souvenaid than we have for any other nutrient or medicinal food.”

A spokesperson for Alzheimer’s Australia, which receives funding from Nutricia, said it was essentially a sophisticated vitamin supplement packaged in a way that would be difficult for an individual to put together.

“For people in the early stages of [AD] there is reasonably solid looking evidence that it can help,” said national research manager Dr Chris Hatherly (PhD).

**Key points**

- Ingredients: Omega-3 polyunsaturated fatty acids, uridine, choline, phospholipids, B vitamins
- 125ml drink
- To be taken once daily
- Will be available from pharmacies from 1 May
- Does not require a prescription
- Costs about $4.16 per day
- Comes in strawberry and vanilla flavour
- Gluten and lactose free

**Tags:** Alzheimer’s disease, Souvenaid, Nutricia, memory, synapse, Medical News
Use of medical foods and nutritional approaches in the treatment of Alzheimer’s disease

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SUMMARY

Alzheimer’s disease, the most common cause of dementia, has a high global economic impact. To date, there is no curative treatment; therefore, many efforts are directed not only at novel potential disease-modifying treatments and interventions, but also to develop alternative symptomatic and supportive treatments. Examples of these efforts include the medical foods. There are three medical foods that claim to offer symptomatic benefits: Axona®, Souvenaid® and CerefolinNAC®. Axona supplies ketone bodies as alternative energy source to neurons. Souvenaid provides precursors thought to enhance synaptic function. CerefolinNAC addresses the role of oxidative stress related to memory loss. The current scientific evidence on these medical foods is reviewed in this article. Furthermore, we also review the concept and evidence supporting use of the Mediterranean diet, a possible alternative to medical foods that, if implemented correctly, may have lower costs, fewer side effects and stronger epidemiological health outcomes.

Alzheimer’s disease (AD) is the most common cause of dementia, affecting over 5 million North Americans and 14 million individuals worldwide [1]. In its early stages, AD affects predominantly short-term memory and language ability, with progressive changes in cognition, function, mood and behavior, resulting in increased caregiver burden [2]. In 2010, the global economic impact of dementia was estimated to be US$604 billion. This figure dwarfs the costs of cancer or heart disease [3]. Based on demographics, the Alzheimer’s Disease International (ADI) foresees an 85% increase in cost by 2030, with the developing countries bearing an increasing share of the disease burden [3]. At present, only symptomatic treatment is available for AD in the USA, comprised of four acetylcholinesterase inhibitors (tacrine, donepezil, rivastigmine and galantamine) and one glutamate receptor antagonist (memantine). While these medications offer modest benefit, they are not curative [4]. Thus, there is active research to develop novel approaches to AD treatment with the hopes of either modifying disease pathophysiology or enhancing cognitive function with alternative approaches. One such approach currently available is the use of medical foods and dietary modifications.

Medical foods were defined in 1988 as a special category of products intended for the specific dietary management of a disease or condition that has distinctive nutritional...
requirements, established by medical evaluation and based on recognized scientific principle [101]. The US FDA criteria for a medical food are [5]:

- A specially formulated and processed product for the partial or exclusive feeding of a patient by means of oral intake or enteral feeding by tube;
- Intended for the dietary management of a patient who, because of therapeutic or chronic medical needs, has limited or impaired capacity to ingest, digest, absorb or metabolize ordinary foodstuffs or certain nutrients, or who has other special medically determined nutrient requirements, the dietary management of which cannot be achieved by the modification of the normal diet alone;
- Provides nutritional support specifically modified for the management of the unique nutrient needs that result from the specific disease or condition, as determined by medical evaluation;
- Intended to be used under medical supervision.

The characteristic of a medical food is that it is expected to be used under the regular care of a physician, so patients who are interested in using this type of material should discuss it carefully with their own physician and make sure that they, the physician and the family understand the appropriate use, realistic treatment expectations and potential adverse effects. Before discussing the currently available products, it is important to note that the FDA does not require the same high level of testing for approval of medical foods as it does for prescription medications. This may be particularly true when considering supplements that are often confused with medical foods, leaving patients and their families potentially vulnerable to unproven claims. Supplements are not regulated by the FDA, are meant for healthy individuals and are not intended to diagnose, treat or manage disease or disease symptoms [102]. Medical foods and to a lesser extent supplements are not replacements for prescription medications; however, used alongside pharmacological approaches under a physician’s supervision, both can enhance patient care.

There are three medical foods that claim to have benefits for use in dementia patients and are available in the USA and/or Europe: Axona® (AC-1202, Accera, Inc., CO, USA) [6], Souvenaid® (Danone Research, France) [7] and CerefolinNAC® (LA, USA) [103]. The goal of this article is to review the current scientific evidence of these medical foods in treating AD (Table 1). We also discuss the evidence available for the Mediterranean diet, a nonmedical food dietary approach that may alleviate the symptomatic burden of AD.

**Axona**

Known by the brand name ‘Axona’, this medical food composed of a proprietary formulation of caprylic acid proposes to target metabolic deficiencies associated with AD [6]. The product was launched by Accera in 2009. In AD, there is a characteristic cerebral hypometabolism on fluorodeoxyglucose PET scans that can be used to assist in diagnosis [8]. Cerebral perfusion is progressively decreased owing to aging and vascular risk factors and is significantly greater in AD, particularly in the temporal–parietal region [9]. Cerebral hypoperfusion may induce brain capillary degeneration and suboptimal delivery of energy substrates, such as glucose, to neural tissue. This may compromise neural stability and metabolic cascades, for example, mitochondrial dysfunction, oxidative stress and neurotransmission failure, leading to progressive cognitive decline characteristic of AD, as well as synaptic loss, senile plaques, neurofibrillary tangles, tissue atrophy and neurodegeneration [9]. This decrease in ability to utilize glucose contributes to the clinical and pathological course of disease [10]. Axona is a medium-chain triglyceride product composed of glycerin and caprylic acid, which is metabolized to the ketone body β-hydroxybutyrate (BHB) in the liver, providing neurons with an alternative energy source to
Caprylic acid contains 12 carbons and is entirely metabolized by the liver to BHB without associated changes in serum cholesterol or triglyceride levels [6]. The ketone body BHB crosses the blood–brain barrier and enters the neurons, localizing in mitochondria. Inside the mitochondria, BHB is metabolized into acetyl coenzyme A by a simple three-step process. Acetyl coenzyme A then enters the citric acid cycle, leading to production of ATP. BHB metabolism also generates nicotinamide adenine dinucleotide and succinate substrates for complexes of the mitochondrial electron transport [104]. Axona is administered orally once a day, and is supplied as a powder to be mixed with water or other foods/liquids for immediate consumption [6].

As described above, AD patients have a progressive, region-specific decline in the cerebral metabolic rate of glucose [12], especially in posterior cingulate, parietal, temporal and prefrontal cortices, which occurs early in the course of AD even before the demonstration of cell loss [13]. Normally, glucose is the main energy substrate for the brain, but under certain circumstances, such as the fasting period, ketone bodies from the liver can serve as alternative sources of energy [14]. Results from a mouse model suggest that induced ketosis may be beneficial in AD [15]. Another small study of 23 older adults with mild cognitive impairment who received either a high carbohydrate or very low carbohydrate (ketogenic) diet for 6 weeks showed improvement on verbal memory performance in the ketogenic group. This study also demonstrated that ketone body levels were positively correlated with memory performance [16]. These preliminary studies support the idea that ketone bodies may provide symptomatic benefit as an alternative source of energy to AD patients.

At the Alzheimer’s Association 2007 International Conference on Prevention of Dementia, the company presented findings from a Phase II double-blind, randomized, multicenter, placebo-controlled trial of 152 patients with probable mild-to-moderate AD. Results of the trial showed those taking Axona had significant improvement in the Alzheimer’s Disease Assessment Scale-cognitive subscale (ADAS-cog) at day 45 (p = 0.024), which was maintained through day 90, although the difference was no longer significant at that point (p = 0.0767). The difference in ADAS-cog was significant at both day 45 (p < 0.0005) and day 90 (p = 0.015) in a subset of APOE ε4-negative patients [17].

Adverse event discontinuation rates were 23% in the treatment group and 6% in the placebo group [17]. The most common adverse events were diarrhea, flatulence and dyspepsia. No significant interactions were seen with commonly prescribed AD drugs, including donepezil and/or N-methyl-D-aspartic acid-receptor agonists such as memantine.

The product contains caseinate, whey and lecithin, but is lactose free. The company recommends that it should be used with caution in patients with known hypersensitivity to palm or coconut oil, those at risk for ketoacidosis, or those with a history of gastrointestinal inflammation, metabolic syndrome and/or a history of renal dysfunction [6]. Axona should be consumed after a full meal.

Souvenaid

Souvenaid is a medical food that may soon be available in the US market. The product, a 125-ml (125-kcal) once-daily drink, combines a variety of substrates including uridine monophosphate, phospholipid, choline and omega-3 fatty acids, vitamins and antioxidants, which are thought to be essential for formation of synaptic membranes [105]. Although all of Souvenaid’s constituents can be found in food, none of the uridine contained in the average adult dietary sources is bioavailable [18]. Souvenaid contains the necessary precursor and supporting nutrients thought to act synergistically to enhance membrane formation and function in patients with AD (Figure 2) [19]. The brains of Alzheimer’s patients show evidence of synaptic failure [20,21]. Deficiency in synapses is both one of the
earliest manifestations of AD as well as the probable cause of cognitive disturbance observed in the early course of disease [21]. Souvenaid’s constituents may be involved in generation of new synaptic connections such as dendritic spine growth [22]. Uridine may help to promote neurite branching, neurite protein synthesis and stimulate neuritogenesis by activation of brain P2Y receptors that control neuronal differentiation [23]. Phosphatide molecules plus synaptic proteins comprise the bulk of synaptic membranes and can be increased by co-administration of rate-limiting precursors via the Kennedy pathway [19].

The multicentered trial, including both European and US sites, was published in 2010 [7]. This trial included 225 drug-naive patients with baseline Mini-Mental State Examination scores of 20–26. Patients were randomized to receive Souvenaid or a placebo drink once daily for 12 weeks. The study’s primary outcome measures were the delayed verbal recall task on the Wechsler Memory Scale-Revised and the 13-item modified ADAS-cog scale. At 12 weeks, the investigators found significant improvement in Wechsler Memory Scale-Revised delayed verbal recall scores in the Souvenaid group compared with the placebo group (p = 0.021). However, there was no change in ADAS-cog scores in either group [7]. The compound was well tolerated and there was no significant difference in the incidence of adverse events between the study groups. Furthermore, most of the adverse events were deemed unrelated to the study products. The adverse event discontinuation rate was 2.7% in the treatment group versus 3.6% in the placebo group. Adverse events were reported in 51% of the treatment group and 44% of the placebo group. Gastrointestinal events were the most commonly reported adverse events [5–7].

This was followed by a 3-month extension period during which patients could choose to continue with the double-blind regimen or begin taking open-label Souvenaid. Approximately 85% of subjects chose to continue with the double-blind regimen, providing the investigators with 6 months of data. Results from the extension study, published recently [24,25], indicated that a higher treatment effect (p = 0.046) was shown in patients with high-baseline ADAS-cog, but not in patients with low-baseline ADAS-cog. Overall, intake adherence was significantly correlated with ADAS-cog improvement in the Souvenaid group, but not in the control group [24]. The extension study also included BMI and the 23-item Alzheimer’s Disease Cooperative Study-Activities of Daily Living scale as secondary outcomes. Results suggested an increased BMI in the Souvenaid group versus the control group at week 24, but no treatment effect on Alzheimer’s Disease Cooperative Study-Activities of Daily Living was observed. Furthermore, BMI was found to be a significant treatment effect modifier and an increase in Alzheimer’s Disease Cooperative Study-Activities of Daily Living was observed in week 12 in patients with low-baseline BMI [25].

The second clinical trial of Souvenaid was presented at the 4th International Conference on Clinical Trials in Alzheimer’s Disease, CA, USA, in November 2011. The study included 259 patients with early AD (Mini-Mental State Examination score average of 25), who received Souvenaid or placebo daily for 24 weeks. As much as 91% of patients finished the study. Memory was tested at the beginning, at 12 weeks and at 24 weeks. The composite score was taken from the Rey Auditory Verbal Test, which examines instant recall, delayed memory and recognition. The Wechsler Scale was also carried out to test verbal association. Results showed the total scores from the Souvenaid group were significantly higher than the control group (p = 0.025) and no differences between frequency of adverse events were observed between the two groups [106].

**CerefolinNAC**

CerefolinNAC ingredients consist of methylcobalamine 2 mg (vitamin B12), l-methylfolate 5.6 mg and N-acetylcysteine 600 mg; it can be taken with or without food [103]. It is
approved by the FDA for the treatment or prevention of vitamin deficiencies associated with memory loss. The package insert also claims that CerefolinNAC addresses metabolic and genetic nutritional impairments associated with memory loss that can lead to AD. This is somewhat different from Axona and Souvenaid, which target a proposed mechanism of action relevant to those who are diagnosed with AD [107]. There is a body of literature suggesting that the reduction of blood levels of homocysteine may reduce risks of vascular disease such as heart attacks and stroke [26–28]. This may be accomplished with supplementation of B vitamins involved in homocysteine metabolism [108]. However, two meta-analyses of effects of folic acid, with or without other B vitamins, on the prevention of cognitive decline in memory-impairment patients both did not find any benefit of folic acid with or without vitamin B over placebo [29,30]. In addition, an Alzheimer Disease Cooperative Study of folate and B12 for the symptomatic benefit of AD failed to reach primary end points [31]. One possible advantage of using CerefolinNAC over other B-vitamin supplements is that it contains formulations of folic acid and vitamin B12, which are thought to be ‘active’, suggesting that they are ready to use by the body without the need for conversion [103]. This is further supported by the recent VITACOG clinical trial, which supplied high levels of folate, B12 and B6 to 271 elderly subjects with MCI, to evaluate ability of homocysteine-lowering B vitamins to arrest brain atrophy. The investigators found that only patients with the highest quartile of baseline homocysteine had benefit on brain atrophy by 53% with vitamin B treatment for 24 months. No effect was found in those with the lower quartile on baseline homocysteine [32]. Thus the level of homocysteine reduction may be critical to demonstration of clinical benefits.

CerefolinNAC’s therapeutic approach is addressing homocysteine and oxidative stress linked to progressive memory loss (Figure 3). Although the etiology of AD is evolving, some studies have demonstrated that oxidative stress plays a role in disease pathophysiology [33,34] including the fact that amyloid deposition is able to induce and be induced by oxidative stress [35]. Over time, oxidative damage is likely to be toxic to neurons. Several byproducts of protein, lipid and glucose oxidation appear to be elevated in the brains of AD patients compared with healthy controls; this may be a result of the burden of free radicals accumulating proportionally to the duration of the disease [36,37]. The brain’s unique characteristics, including its high rate of metabolism and its long-living cells, make it more susceptible to oxidative damage compared with other organs [38]. The human body has an antioxidant defense system to help regulate oxidative stress [39]. Antioxidants block the process of oxidative stress by neutralizing free radicals; however, endogenous antioxidant resources are depleted with age, so there is a constant need to replenish them [37]. N-acetylcysteine increases the body’s production of glutathione, which is the brain’s most important scavenger of reactive oxidative species, also known as free radicals [40]. A report on several case studies has shown oral supplementation with NAC, in addition to folate and vitamin B12 therapy, may reduce the effects of oxidative stress and improve cognitive function in patients with memory deficits [41].

Elevated homocysteine levels may result from abnormalities in the function of enzymes involved in homocysteine metabolism or from deficiencies of the vitamin cofactors: folate, cobalamin (B12) and pyridoxine HCl (B6) [108]. Elevated homocysteine is hypothesized to be a risk factor for vascular disease. Although American Heart Association guidelines do not recommend widespread use of folic and vitamin B supplements to reduce the risk of heart disease and stroke, the guidelines do suggest eating a healthy and balanced diet, which contains 400 µg of folic acid per day [109]. Folic acid and other B vitamins are important cofactors for metabolism of homocysteine, thus homocysteine levels may be strongly influenced by a combination of dietary and genetic factors [109]. Elevated blood homocysteine may also be a useful marker for neuroinflammation. In AD, elevated homocysteine can be lowered by folic acid plus B-vitamin supplements and N-
acetylcysteine [40]. However, the recent systemic meta-analysis and meta-regression review of causative links between high homocysteine and AD did not support a causal relationship, even though individuals with AD had higher homocysteine level than controls [42].

Results from a 1-year uncontrolled open study of a nutriceutical formulation containing folic acid, B12 (N-acetylcysteine (constituents of CerefolinNAC), as well as vitamin E, S-adenosylmethionine and acetyl-L-carnitine, in 14 patients with early-stage AD indicated improvements in the Dementia Rating Scale and Clock-drawing tests, multiple domains of the Neuropsychiatric Inventory and maintenance of performance in the Alzheimer’s Disease Cooperative Study-ADL [43]. Although CerefolinNAC lacks the randomized clinical trial-based evidence provided by Axona and Souvenaid, complementary studies with constituents suggest high doses of folate sufficient to lower homocysteine offer a clinical benefit.

According to the package insert, a single dose of L-methylfolate 5 mg is three-times more effective in decreasing plasma homocysteine compared with folic acid 5 mg and $C_{\text{max}}$ is also higher (129 vs 14.1 ng) [103].

Common adverse reactions of CerefolinNAC include mild transient diarrhea, polycythemia vera, itching, transitory exanthema and the feeling of swelling of the entire body, which have been associated with methylcobalamin. Nausea, vomiting, headache, other gastrointestinal symptoms and rash (with or without mild fever) have been associated with CerefolinNAC. In addition, there are rare reports of renal stone formation with CerefolinNAC [103].

**Mediterranean diet**

The Mediterranean diet does not comprise of medical foods, however, the concept is very similar to medical food whereby a specific healthy dietary pattern is adhered to, which may help in the prevention or delay of AD progression. There are a number of dietary approaches and interventions that have been proposed for the prevention and/or treatment of AD. We included a single dietary approach (i.e., the Mediterranean diet) and its scientific evidence to give one example of possible alternative nutritional approaches that may have lower costs, lower side effects and stronger epidemiologic evidence of health outcomes.

The most common version of the Mediterranean diet was presented by Dr Walter Willett of Harvard University’s School of Public Health in the mid-1990s [44]. This diet emphasizes plant-based foods in abundance, fresh fruit as the typical daily dessert, olive oil as the principal source of fat, dairy products (principally cheese and yogurt), fish and poultry consumed in low to moderate amounts, zero to four eggs consumed weekly, red meat consumed in low amounts and wine consumed in low to moderate amounts. The total fat in this diet is 25–35% of daily calorie allowance, with saturated fat at 8% or less of daily calorie allowance [44].

A number of published studies found the benefits of adhering to the Mediterranean diet are being less likely to develop depression [45], more than 50% lowering of early death rates [46] and 83% relative reduction in the risk of developing diabetes [47]. The Seven Countries Study report also found the Cretan diet – a type of traditional Mediterranean diet consisting mostly of olive oil, bread, an abundance of fruits and vegetables, fish and moderate amounts of dairy foods and wine – can help lower death rates from heart disease [48]. The Lyon Diet Heart Study was a randomized, controlled trial with free-living subjects. Its goal was to test the effectiveness of a Mediterranean-type diet on the rate of coronary events in people who have had a first heart attack. A total of 302 experimental and 303 control subjects were randomized in the study. The results suggest that a Mediterranean-style diet may help reduce recurrent events in patients with heart disease [49]. The Mediterranean diet is low in...
saturated fat and high in monounsaturated fat and dietary fiber. The possible explanations for the results may include the healthy effect of olive oil on the heart, or the consumption of red wine containing flavonoids with powerful antioxidant properties [50]. Following the concept of ‘what is good for the heart is good for the brain’, the Mediterranean diet has been increasingly studied in patients with AD and in individuals at risk for dementia.

A Washington Heights–Inwood Columbia Aging Project (WHICAP) was the first to report a beneficial effect of the Mediterranean diet on incidence of AD [51]. Over 2000 individuals older than 65 years of age had a complete assessment of cognitive functions and dietary habits and were followed-up for an average of 4 years. The study found that higher adherence to the Mediterranean diet was significantly associated with a lower risk of development of AD, even after adjustment for age, sex, ethnicity, education, APOE genotype, caloric intake, smoking, comorbidity index and BMI. Compared with individuals in the lowest tertile of the Mediterranean diet score (score 0–3; indicating a low adherence to the Mediterranean diet), those in the middle score tertile (score 4–5) had 21% less risk for development of AD and those in the highest tertile (score 6–9; indicating a high adherence to the Mediterranean diet) had 40% less risk for development of AD, with a trend for a dose–response effect, in fully adjusted models [51]. Furthermore, among individuals who had MCI at baseline, adherence to the Mediterranean diet had a significantly reduced risk of developing AD over time [52].

Another prospective study was the Three-City Study from France [53]. A prospective cohort study of 1410 individuals aged 65 years or older, who were free from dementia at baseline, then followed-up at least once over a period of 5 years. The main original finding was that higher adherence to the Mediterranean diet was significantly associated with better global cognitive performance and episodic memory over time, especially in individuals who remained free from dementia. Nevertheless, there was no association between the adherence to the Mediterranean diet and risk of dementia or AD in older individuals in this study [53]. Another recent study from the Mayo Clinic (MN, USA) also found that the odds ratio of MCI was reduced for high vegetable intakes and high mono- plus poly-unsaturated fatty acid to saturated fatty acid ratio [54].

A meta-analysis of eight prospective studies analyzed the relationship between adherence to a Mediterranean diet, mortality and incidence of chronic disease in a primary prevention setting. The authors found that adherence to the Mediterranean diet is associated with a significant improvement in health status, as seen by a significant reduction in overall mortality (9%), mortality from cardiovascular diseases (9%), incidence of or mortality from cancer (6%) and incidence of Parkinson’s disease and AD (13%). These results seem to be clinically relevant for public health, in particular, for encouraging a Mediterranean-like dietary pattern for primary prevention of major chronic diseases associated with aging [55].

**Conclusion**

There is increasing interest in alternative ‘natural’ approaches to treating AD in addition to the current regimen of pharmacologic approaches. This has spurred the development of medical foods to overcome metabolic deficits or address dietary deficiencies that have been associated with AD.

CerefolinNAC and Axona are the two medical foods that are currently available by prescription. CerefolinNAC’s therapeutic approach of addressing homocysteine levels and oxidative stress might benefit a variety of patients with cognitive disorders; however, more systematic studies are needed to confirm efficacy. All references in the package inserts are limited to case reports or case series. Randomized clinical trials examining folate...
supplementation (with or without B12 supplementation) have failed to demonstrate any benefit. Axona’s approach of providing an alternative energy source (ketone bodies) to the brain other than glucose demonstrates clinical benefit only in the specific group of APOE ε4-negative patients. A larger and longer Phase III study will help clearly determine its benefit, since the preliminary study was a relatively short 90-day trial compared with most AD trials, which last at least 26 weeks with open-extensions of 1 year or longer. The lack of benefit when measuring ADAS-cog in APOE ε4-positive group leads to some question on how to best select the patient in clinical practice because APOE genotype testing is not routinely done. The last medical food that may soon be available is Souvenaid, where preliminary data have shown some improvement in verbal recall task, but not in the general cognitive scale. Published studies report a trial that is of short duration, double-blind for 12 weeks, with an extension period of 12 weeks. At baseline, approximately 40% of enrollees scored 0, the lowest score on the delayed verbal recall test (Wechsler Memory Scale-Revised) – one of the two primary outcome measures. This skew in study population required the authors to use a nonparametric analysis, instead of the planned parametric assessment. A recent presentation of a second randomized clinical trial supports the clinical benefit of Souvenaid.

Future perspective

Preliminary studies of medical foods have largely been conducted in mild AD, so results cannot be generalized to all stages of AD. The potential benefit of medical foods in MCI is also unclear. An important issue to reiterate is that the FDA does not require the same high level of testing for approval of medical foods as it does for prescription medications. The efficacy demonstrated so far for medical foods is at best comparable with current symptomatic medications and then so only in select populations. Medical foods are generally considered safe and have a minimal side-effect profile compared with drugs; however, careful use after a discussion about risks and benefits with the physician is still recommended. At the present, the prescription of medical foods should be considered as an adjunct to and not a replacement for current medication use.

Finally, there is increasing evidence that specific dietary patterns, especially the Mediterranean diet, show promise for reducing the risk of developing dementia as well as reducing symptom burden after diagnosis. Although challenging, the Mediterranean diet can be adopted into many diets. Many medical foods are founded on sound nutritional principles and current hypotheses of disease, but larger clinical studies are needed to fully establish efficacy. What is clear is that the concept of ‘what is good for the heart is good for the brain’ may be particularly true when considering nonpharmacological approaches to AD and other diseases of the aging brain.

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Papers of special note have been highlighted as:

- of interest


Clin Pract (Lond). Author manuscript; available in PMC 2013 January 27.


Websites


Practice Points

- General definition and availability: medical foods are a special category of products intended for special dietary management of diseases or conditions that have distinctive nutritional requirements or metabolic deficiencies, based on medical evaluation and scientific principles. Their ingredients are generally regarded as safe. Currently, there are three medical foods that claim to have benefit for Alzheimer’s disease (AD) and memory impairment: Axona®, Souvenaid® and CerefolinNAC®.

- Axona and its scientific evidence: Axona provides neurons with an alternative energy source to glucose, the ketone body β-hydroxybutyrate. Evidence from a Phase II multicenter randomized clinical trial (RCT) showed significant improvement in cognitive testing (measured by the Alzheimer Disease Assessment Scale-cognitive subscale) at 90 days in APOE ε4 allele-negative patients.

- Souvenaid and its scientific evidence: Souvenaid contains precursors and supporting nutrients thought to enhance membrane and synaptic formation and function in AD. Evidence from a multicenter RCT showed improvement in a verbal recall task, but not Alzheimer Disease Assessment Scale-cognitive subscale in mild dementia at 12 weeks.

- CerefolinNAC and its scientific evidence: CerefolinNAC addresses hypotheses regarding the role of homocysteine and oxidative stress related to memory loss that may lead to AD. Evidence is largely limited to case reports reporting benefits.

- A Mediterranean diet and its scientific evidence: the Mediterranean diet is not strictly medical food, but has a similar concept of using dietary patterns to help prevent or delay the onset and symptoms of AD. Evidence from two RCTs showed adherence to the Mediterranean diet had a reduced risk of AD and better global cognitive performance. A meta-analysis also found that adherence to the Mediterranean diet reduced incidence of AD by 13% as well as overall mortality owing to AD.

- Medical foods and their practical points and limitation: all three medical foods are relatively new to the market. More extensive RCT studies will provide clearer information on the benefit of each product. Medical foods are not meant to replace commonly prescribed medications, but rather to be used in conjunction with medication as adjuvant therapies.
Figure 1.
Proposed mechanism of action of Axona®.
BHB: β-hydroxybutyrate.
Figure 2.
Proposed mechanism of action for Souvenaid®.
Figure 3.
Proposed mechanism of CerefolinNAC®.
Table 1

Supporting evidence for medical foods and the Mediterranean diet to treat Alzheimer’s disease.

<table>
<thead>
<tr>
<th>Medical foods</th>
<th>Evidence to support the benefits in dementia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axona®</td>
<td>Phase II multicenter RCT showed significant improvement of ADAS-cog at 90 days in APOE ε4-negative patients</td>
</tr>
<tr>
<td>Souvenaid®</td>
<td>Multicenter RCT showed improvement in verbal recall task on WMS-R, but not ADAS-cog in mild dementia at 12 weeks</td>
</tr>
<tr>
<td>CerefolinNAC®</td>
<td>Case studies report supplement of CerefolinNAC to folate and B12 may improve cognitive function. However, two meta-analysis studies of effect of folic acid, with or without vitamin B, on the prevention of cognitive decline in memory impairment patients both did not find any benefit of folic acid with or without vitamin B over placebo</td>
</tr>
<tr>
<td>Mediterranean diet</td>
<td>Two RCTs showed those who adhered to the Mediterranean diet had significantly less risk of Alzheimer’s disease, and better global cognitive performances. Meta-analysis found adherence to Mediterranean diet reduced overall mortality and incidence of Alzheimer’s disease (13%)</td>
</tr>
</tbody>
</table>

ADAS-cog: Alzheimer’s Disease Assessment Scale-Cognitive; RCT: Randomized clinical trial; WMS-R: Wechsler Memory Scale-Revised.