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The Short-term Effect of a High Protein, Energy-dense Oral Liquid Supplement on Nutritional Status of Patients with Mild Alzheimer`s Disease.

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Abbreviations:

AD - Alzheimer`s Disease

AMC - Arm Muscle Circumference

BI - Barthel Index

BIA - Bioelectrical Impedance Analysis

BMI - Body Mass Index

CDT - Clock-Drawing Test

FFM - Fat Free Mass

FM - Fat Mass

MMSE - Mini Mental State Examination

MNA - Mini Nutritional Assessment

OS - Oral Supplementation

PhA - Phase Angle

TSF - Triceps SkinFold

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To my family and friends, my best wishes

All of you,

Thank you very much

“ I was hungry and you fed me,

thirst, and you gave me a drink….

sick and you took care of me…”

Matthew 25:35-36

Summary

Background

The aetiology of weight loss in Alzheimer Disease (AD), has not yet been definitively established. Although appears multifactorial, a decline in food intake may be a contributor. Nutritional supplements are often prescribed, especially to prevention for weight loss, but it has not been demonstrated that a short-term nutritional supplementation has impact in AD-patients nutritional status.

Objectives

The aim of this study was (1) to evaluate the effect of short-term administration of a high protein, energy-dense Oral liquid Supplement (OS) in patients with mild AD who were malnourished, on nutritional status and in body composition, cognitive function and functional ability; (2) to assess the long-term effect of this intervention after discontinuation of the OS.

Methods and Measurements

A 21 days prospective randomized nonblinded controlled trial was conducted in a Geriatric Unit of a Psychiatric Hospital. From an initial group of Thirty five patients, twenty were randomly assigned to the Treatment Group (TG) n=20 and Fifteen to the Control Group (CG) n=15. During the study period, the TG received during 21 consecutive days, once a day a 200 mL high protein energy-dense OS, which provided 400kcal from a mixture of protein (18% of energy), fat (17.4% of energy), and carbohydrate (42.8% of energy) plus dietetic advice. Patients in the CG received the same dietetic advice as the TG, but no OS.

The nutritional status, body composition, functional status and cognitive function were determined at baseline, 21 days (after intervention) and at 90

days of follow-up. The Mini Nutritional Assessment (MNA), anthropometric measurements, bioelectrical impedance analysis and serum biomarkers (albumin, total-protein, total-cholesterol, vitamin-B12 and folic acid) levels were obtained. Cognitive function was evaluated using the Mini Mental Status Examination (MMSE) tool and the Clock-Drawing Test (CDT). Functional status was assessed using the Barthel Index (BI).

Results

The compliance with the OS was excellent. This short-term intervention resulted in significant improvements in TG compared to CG. Mean MNA score, weight, fat and fat free mass, triceps skinfold thickness, arm muscle circumference and serum biomarkers levels of albumin and total-protein, increased moderately in TG, compared to CG. No changes were found for dependence, cognitive and functional status in both groups.

At 90 days of follow up, significant improvements in nutritional status were found (MNA score, weight, fat free mass, triceps skinfold thickness, serum albumin and total-protein levels). No significant differences in MMSE, CDT and BI studied during this period were observed.

Conclusions

High protein energy-dense OS nutrition during 21 consecutives days, can improve the nutritional status of undernourished AD-patients in the short time. The use of this OS nutrition associated with dietetic advice, can improve or maintain patient's nutritional status. No significant differences in MMSE, CDT and BI evaluated during the study period were observed.

Key words

Alzheimer’s disease; undernutrition; body weight; body composition; nutritional assessment; oral supplementation.

Resumo

Introdução

A etiologia da perda de peso na Doença de *Alzheimer* (AD), ainda não está devidamente conhecida. Vários factores parecem estar implicados e entre eles é referida a diminuição da ingestão alimentar. A suplementação nutricional é frequentemente prescrita nestes doentes, no entanto, ainda não foi demonstrado o impacto de uma suplementação nutricional de curta duração no estado nutricional em doentes de AD.

Objectivos

Os principais objectivos deste estudo são (1) avaliar o efeito da administração de uma suplementação oral líquida de curta duração, com elevada densidade energética e proteica, no estado nutricional, composição corporal, função cognitiva e estado funcional de doentes desnutridos com provável AD no estadio inicial; (2) avaliar a longo prazo o efeito da intervenção, após a interrupção da suplementação oral.

Metodologia e Procedimentos

Realizou-se durante 21 dias consecutivos, um estudo experimental randomizado não cego, numa Unidade de Geriatria dum Hospital Psiquiátrico. Dos 35 doentes seleccionados, 20 foram alocados no Grupo Intervenção (GI) n=20 e 15 no Grupo Controlo (GC) n=15. Durante o período de estudo, o GI recebeu ao longo de 21 dias consecutivos e uma vez por dia, 200mL de uma Suplementação Oral (OS) fornecendo 400Kcal, das quais 18% são de proteína, 17.4% de gordura e 42.8% de hidratos de carbono e também aconselhamento dietético padronizado.

Os doentes do GC receberam os mesmos aconselhamentos dietéticos que os doentes do GI, mas não realizaram OS. Todos os doentes completaram a toma da OS. As avaliações foram realizadas no início do estudo, aos 21 e aos 90 dias. Realizou-se a avaliação do estado nutricional e da composição corporal, utilizando-se o Mini Nutritional Assessment (MNA), antropometria, análise por bioimpedância (BIA) e marcadores biológicos plasmáticos (albumina, proteínas totais, colesterol total, vitamina-B12 e ácido fólico). A função cognitiva e estado funcional foram avaliadas através do Mini Mental Status Examination (MMSE), Prova do Relógio (PR).e pelo Índice de *Barthel* (IB). Todos os doentes foram seguidos durante 90 dias, após o periodo de intervenção.

Resultados

A aderência à OS foi excelente. Esta intervenção de curta duração conduziu a melhorias significativas nos seguintes parâmetros: pontuação de MNA, peso, massa livre de gordura, massa gorda, prega cutânea tricipital, circunferência muscular do braço e os níveis plasmáticos de albumina e de proteínas totais. Não se verificaram diferenças na função cognitiva e no estado funcional. Aos 90 dias de seguimento, encontraram-se melhorias significativas no estado nutricional, na pontuação de MNA, no peso, na massa livre de gordura, na prega cutânea tricipital, e nos níveis plasmáticos de albumina e de proteínas totais mas não na função cognitiva e ao estado funcional.

Conclusões

Os resultados obtidos, sugerem que uma intervenção de curta duração, de 21 dias consecutivos, constituída por uma OS de elevada densidade energética e proteica, pode ter melhorado o estado nutricional de doentes de

Alzheimer desnutridos. A utilização desta OS associada com os aconselhamentos dietéticos, pode melhorar ou manter o estado nutricional destes doentes de Alzheimer. Durante o período de estudo avaliado, não se observaram diferenças com significado estatístico em relação ao MMSE, CDT e BI.

Palavras-chave

Doença de *Alzheimer*; desnutrição, peso corporal, composição corporal, avaliação nutricional, suplementação oral.

IntroductionAlzheimer's disease (AD) is primarily a disease of aging, affecting at least 3% of the population worldwide with an incidence that doubles every 5 years between 65 and 85 years of age in all human populations examined. It is the fourth most frequent cause of death in developed countries (1,2). On the basis of numerous epidemiologic studies in many countries, it is now a well-established phenomenon that the prevalence of AD increases exponentially with the aging population (3).

AD is an irreversible, progressive neuro-degenerative dementia. The pathologic origin and aetiology of AD have remained unknown since the dementia was originally described in 1907 by Alois Alzheimer (4). The progressive nature of AD can be described by referring to the four stages of dementia (5). The initial "mild" stage, presents with impaired memory, personality changes and spatial disorientation. During the "moderate" or second stage, confusion, agitation, insomnia, aphasia and apraxia, are common. The "severe", or third stage, is complicated by resistiveness, incontinence, eating difficulties and motor impairment. In the "terminal stage", the patient is bedfast, mute, weakened by intercurrent infectious and dysphagia (5).

During the course of the disease, a large subset of AD-patients suffers from unintentional weight loss and undernutrition. Weight loss was first described by Alois Alzheimer's who recorded a "slowly progressive decrease in body weight" in such patients. (5,6). Some investigators showed that a common clinical observation is that most AD-patients lose weight during the progression of the disease. Particularly in the final stages (7-10), approximately 40% of

patients lose 4% of body weight per year (11). Barrett-Connor *et al.* (12), after following 134 older men and 165 women for 20 years in a longitudinal study, showed that patients' weight loss started even before the onset of AD. The National Institute of Neurological and Communicative Disorders and Strokes Task Force on Alzheimer's Disease has included weight loss as a clinical feature consistent with the diagnosis of AD (13). A body weight loss greater than 5% in a year is considered a bad prognostic indicator in AD-patients (11).

Although the aetiology of weight loss in AD appears multifactorial, there are four main hypotheses to explain the cause of weight loss: failure of body weight regulation, higher resting energy expenditure, hypermetabolism and self-feeding difficulties. However, none have been proven (13-16). Poor food intake is common in older people with dementia. It may, in part account for the weight loss/low body weight that many of these patients experience. Anorexia is associated with significant muscle wasting, weakness, depression, increased susceptibility to disease complications and decreased immunocompetence. The susceptibility to pressure sore formation and to hip fracture is increased in older people with AD (17).

Involuntary loss of muscle mass is associated with weight loss, leading to reduced muscle strength and function, which gradually leads to impairment in activities of daily living (18,19). Functional status impairment has been reported to be an independent predictor for mortality in certain elderly populations such as those in an acute care setting (16, 20, 21). It is also described as an important outcome measure to monitor response to treatment and to provide prognostic information to plan future care.

In patients with mild to moderate AD, behavioural interventions have been shown to increase food intake, facilitate weight maintenance and to improve quality of life. Patients are able to maintain function, strength and energy for longer periods of time, thus reducing the burden and costs of care for staff members and family caregivers (17).

In order to assess nutrition status as part of the standard evaluation of elderly patients, a single and rapid nutrition assessment tool, the Mini Nutritional Assessment (MNA), was developed and validated (22-27). MNA is a well-validated nutritional screening and assessment tool, which can quickly and easily evaluate and identify the nutritional status of mild AD-patients at risk for malnutrition, although some of the questions need to be answered by the caregiver (28). Vellas *et al.* has shown that MNA (but not albumin) classification is correlated with weight loss in Alzheimer patients (28).

The aging process involves modifications in nutritional and physiological status, as a decrease in body weight and height, and a reduction in Fat Free Mass (FFM), associated with an increase in Fat Mass (FM) (29). Body composition of elderly with AD differs from non-AD elderly and these are more pronounced in women. AD-patients have a lower Body Mass Index (BMI), higher percentage of lean body mass, lower percentage of body fat, and higher percentage of total body water than age matched controls (17).

Nutrition screening guidelines for AD-patients, recommend a minimum measurement of body weight and calculation of BMI (5). Anthropometric measurements have been preferred because they are easier and low cost. They provide indirect information about the amounts of subcutaneous adipose tissue and muscle mass in the body. Among all anthropometric measurements,

the BMI represents the easier and most frequently used index to identify subjects at risk for undernutrition (29,30). Many authors agree in considering this index a poor indicator of risk in the elderly (31-34) because it does not reflect regional distribution of fat or any change in fat distribution in the elderly. The BMI is generally considered to be a measurement of fatness, while it also gives information about FFM. In the elderly, FM increases whereas FFM decreases (Steen,1998) (27,35). The anthropometric measurements recommended should include height, weight, mid arm and calf circumferences, skinfolds as triceps, sub scapular and calf skin folds. Triceps SkinFold (TSF) thickness and Arm Muscle Circumference (AMC) are used as markers of endogenous fat and muscle reserves, respectively (29).

Bioelectric Impedance Analysis (BIA) is a safe, cost-effective, practical and non-invasive body composition method. It has been used in large-scale studies of body composition and assessment of fluid status. BIA measures of resistance and impedance are proportional to body water volume and the body's components with high-water concentrations, such as fat free and skeletal muscle mass. BIA assumes that the body consists of two compartments, fat and FFM. Regression analysis has been used to derive prediction models to estimate total body water and FFM. The reliability of BIA method for FFM assessment was confirmed also in healthy elderly people and equations have been developed from general healthy adult and elderly populations (36-43).

Alterations in body composition that occur with AD and ageing result in progressive loss of skeletal muscle mass, reflecting a concurrent loss of body water and a large decrease in FFM along a progressive increase in FM. Body

composition assessment allows an early detection of FFM alteration in underweight elderly and to anticipate the nutritional intervention(44-48).

Phase Angle (PhA) is an indicator based on reactance and resistance, obtained from BIA. Although its biological meaning is still not clear, PhA, has been interpreted as an indicator of membrane integrity and water distribution between the intra-and-extracellular spaces, it has also been used to predict body cell mass and appears to have an important prognostic role. PhA is a derived measure obtained from the relation between the direct measures of resistance and reactance(43-45).

Several studies have shown that cognitive deterioration can be caused by nutritional deficiencies (11, 49-51). Dementia, which is the most common cause of cognitive impairment, is defined as significant memory impairment and loss of intellectual functions, which interferes with the patients work, usual social activities or relationship and nutritional status (52). Understanding the factors that contribute to maintenance of cognitive ability is therefore of high priority (28).

Cognitive function is widely evaluated using Folstein *et al`s* test, the Mini Mental Status Examination (MMSE) validated to Portuguese population, the most extensively used cognitive screening tool (16, 53). Combining the MMSE test scores and the Clock-Drawing Test (CDT) may improve the information on a patient's functional status. The CDT join clock recognition and counting change and is less affected by education, cultural differences and measures executive function (16,20).

Functional status is an important outcome measure to monitor response to treatment and provides prognostic information to plan future care. Functional impairment has been reported to be an independent predictor for mortality in elderly populations (16,20,21). Barthel Index (BI) tool, assesses the level of independence of person`s functions in ten self-care items, bathing, eating, dressing, transfer, toileting, continence, including the assessment of bladder and bowel control(16).

In 1983 Goodwin *et al.* (54) found that healthy elderly who had low blood levels of certain vitamins (ie. folate, vitamin-B12, vitamin C and riboflavin), also scored poorly on tests of memory and nonverbal abstract thinking. It is possible that subclinical vitamin deficiencies play a role in the pathogenesis of declining neurocognitive function (55-60). The etiology of the relation between AD and Vitamin-B12 deficiency is not yet known. Vitamin-B12 deficiency, folate deficiency, or both may lead to reduced synthesis of methionine and S-adenosylmethionine, which in turn could restrict the availability of the methyl groups that are essential for the metabolism of myelin, neurotransmitters such as acetylcholine, and membrane phospholipids (4).Other micronutrients may play a protective role against AD through their antioxidant effect. These include vitamins A, E, C, and β-carotene as well as minerals such as zinc (61-64). In a prospective four years epidemiologic study of 633 elderly people, Morris *et al.* (65) found a lower incidence of AD in users of high amounts of vitamin C and vitamin E supplements than in nonusers.

Among the various markers of nutritional status, serum albumin, total-protein and total-cholesterol levels, are associated with undernutrition and,

patients with hypoalbuminaemia had higher mortality, longer hospital stay, and were more likely to be readmitted after discharge (66-69).

It is presently unclear whether the energy imbalance and the accompanying weight loss associated with AD are caused by reduced energy intake, elevated energy expenditure, or a combination of both, although it seems possible to prevent malnutrition and weight loss in many AD-patients (13). The Nutrition Intervention Guidelines for people with dementia, recommends that people with AD should maintain their weight with a diet adequate in energy (35kcal/kg/day)(17). Spindler *et al.* (70) found that subjects with AD require 34 kcals/kg/day, values similar to the above recomendations. *Pacers* (AD-patients that often move around) may need as much as 1600 kcal in excess of the standard diet (1800-2200 kcal/day) to offset weight loss (70). Extra calories can be provided by larger portions and high calorie supplements (13,71).

Some studies consistently show that providing long-term oral nutrition supplements enables an improvement in AD-patients nutritional status (18,11,72,73). The intervention period varies between 3 months (18,73) and 1-year study (11). All studies demonstrate an increase in daily energy intake and an improvement in body weight status and FFM. Vellas B. *et al.(18),* found that a 3-month oral supplementation in 46 patients with AD significantly improved energy and protein intake, resulting in a significant increase in weight and FFM, although no significant changes were found in dependence and cognitive function, or biological markers. Planas M. *et al.* (72) showed that mild AD-patients receiving 6-month nutritional supplementation with micronutrients, tend to increase their energy intake. The 1-year study by (put the authors) (11)

reported a slowing down on the deterioration of mental function. A 9-months intervention period using a comprehensive intervention strategy, including enhanced dietitian monitoring and menu changes (16), has shown to prevent weight loss in dement residents of special care units.

It has been demonstrated that a short-term 21 days supplementation study, with juice and a supplement bar, in probable AD-patients resulted in an increased nutritional intake (74). The changes resulting from the intervention were maintained after discontinuation (75). This is of major relevance, as may allow for rotating supplementation schedules in heath care services, reducing staff burden (75). Although this short-term intervention (74) favourably increased nutritional intake, to our knowledge, it has not been yet demonstrated that a short-term intervention with a high protein, energy-dense oral liquid supplement has a beneficial impact on the nutritional status of mild AD-patients.

Objectives

The aims of the present study were:

1. to evaluate the short-term effect of a high protein energy-dense oral liquid nutritional supplement, on body weight, body composition, nutritional and functional status, and cognition in patients with mild Alzheimer`s Disease;

2. to assess the long-term effect of this intervention after discontinuation of the oral supplement.

Subjects and Methods

Subjects selection

A 21 days prospective randomized nonblinded controlled trial was conducted in an accreditaded Geriatric Unit of a Psychiatric Hospital in Porto, Portugal. Thirty seven patients older than 60 years, with recently diagnosed probable mild AD on the basis of the Diagnostic Statistics Mental Disorders-IV (DMS-IV) criteria and with a weight loss higher than 5% of body weight in the previous year, were recruited.

Exclusion criteria were terminal care or severe acute illness, cancer or history of cancer in the last 5 years, enteral or parenteral nutritional support at the moment of the study, dietary advise or use of nutritional supplements in the last days prior to entering the study.

Patients were randomized in two groups to receive high protein energy--dense liquid nutritional oral supplement plus standard dietetic advice (Treatment Group (TG), n=20), or only standard dietetic advice (Control Group (CG), n=17). Two patients in the CG died during the study and their data was excluded from analysis. During this study, all the participants followed the treatment protocol in the Geriatric Unit that included folic acid and vitamin B-12 supplementation.

The study protocol was approved by the Hospital Ethical Committee. All the patients or their legal tutors gave their written informed consent to participate.

Dietary Intervention

Patients from the TG received during the study period, once a day a 200 mL high protein energy-dense liquid nutritional Oral Supplement (OS) *Resource®* 2.0 - *Novartis* (total: 400kcal/day, as 42.8% carbohydrates, 17.4% fat, and 18% protein), plus dietetic advice (Table 1).

Table 1. Oral nutritional supplementation composition *Resource®* 2.0 - *Novartis*

Nutrients	200mL (1 Pack)
Energy (Kcal)	400
Carbohydrates (g)	42.8
Protein (g)	18
Fat (g)	17.4
Vitamins	
Vitamin A (µg)	160
Vitamin D_3 (µg)	2
Vitamin E (mg)	2
Vitamin K_1 (µg)	14
Vitamin B_1 (mg)	0.28
Vitamin B_2 (mg)	0.32
Vitamin B_6 (mg)	0.4
Vitamin B_{12} (mg)	0.28
Vitamin C (mg)	12
Folic acid (µg)	40
Biotin (µg)	15
Niacin (mg)	3.6
Pantotenic acid (mg)	1.2
Minerals and Oligoelements	
Sodium (mg)	120
Potassium (mg)	320
Calcium (mg)	380
Magnesium (mg)	60
Phosphorous (mg)	180
Chlorine (mg)	140
Iron (mg)	3
Copper (mg)	3
Manganese (mg)	0.6
Iodine (µg)	30
Fluorine (µg)	300
Chromium (µg)	20
Molybdenum (µg)	16
Selenium (µg)	12
Water Content (mL)	140.4
Osmolality (mOsm/L)	543 [(1)] / 749 [(2)]
Density (g/mL)	1.11

(1) Vanilla; (**2)** Apricot.

The OS was given in the morning, between breakfast and lunch, and when not possible, in the afternoon. Two flavors were supplied, vanilla and apricot. Acceptability and tolerance of OS products was also recorded. The compliance to supplementation was controlled by the principal investigator (LS) and all patients took the study doses (a minority of patient left < 20mL of the formula). Patients in the CG received the same dietetic advice as the TG, but no OS. Participant caregivers from both groups received written information about balanced diet recommendations and advice on how to increase energy intake by using home-made food.

OS was discontinued at 21 days and patients were followed-up up to 90 days (Figure 1).

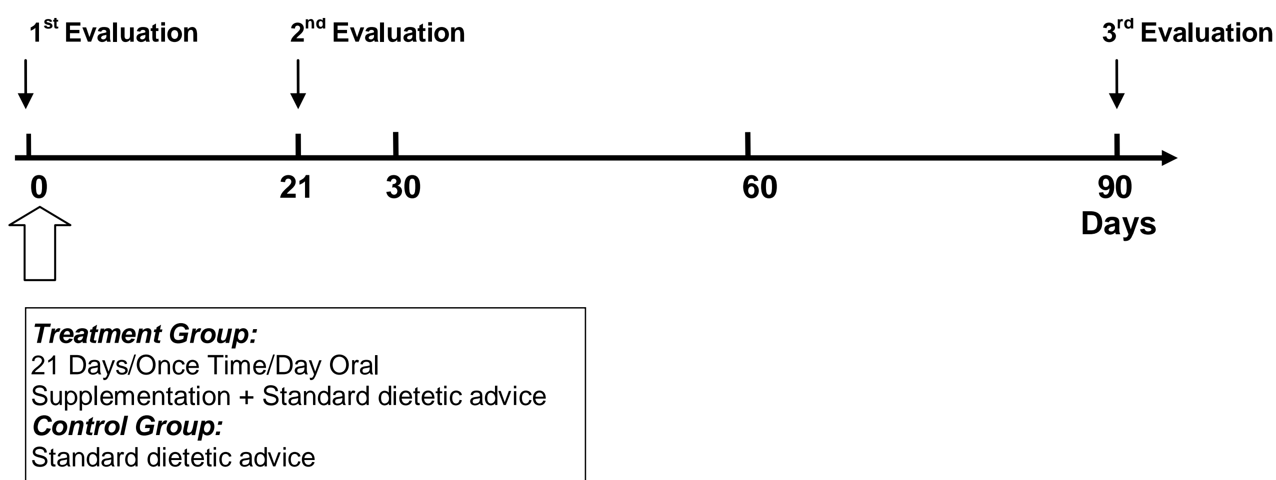

Figure 1. Study design.

Measurements

To compare the groups at baseline and to measure outcomes, the following parameters were considered: age, gender, educational level (defined as completed years of schooling), degree of dementia (using the Mini Mental State Examination, MMSE (53) the Clock-Drawing Test, CDT) (20) functional status (using the Barthel Index, BI) (21), screening and nutritional assessment (using the Mini Nutritional Assessment, MNA) (22-28) body composition assessment (using the Bioelectrical Impedance Analysis, BIA) (36-43,48). Anthropometric measurements (29,44) included biceps, Triceps (TSF), subscapular and iliac crest skinfolds (Durnin&Womersley,1974), Mid Arm Circumference (MAC), and nutritional serum biomarkers (albumin, total-protein, total-cholesterol, vitamin B-12 and folic acid) levels (57-60, 66-69, 76,77).

Data Collection

At baseline, after 21 and 90 days, body composition, anthropometry, nutritional assessment, serum biomarkers levels, functional and neuropsychological tests were performed and measured. Anthropometry measurements were made by the same researcher (LS) and recorded using a standardized protocol. Patient`s body weight was measured in light clothing without shoes, using a calibrated digital scale with 100-g precision (TEFAL, Sensitive Computer). Due to the difficulty in obtaining reliable height measurements, body height was recorded according to the mentioned in the identification card. The Body Mass Index (BMI) was calculated as body weight/height^2 (kg/m^2).

Skinfold measurements were taken using a Lange skinfold caliper (Beta Technology Incorporated Cambridge, Maryland USA), according to standard techniques. Skinfolds were measured in triplicate to the nearest 0.2mm, and the average was calculated as the absolute value (Lee and Nieman 1996). Body density was then calculated using the appropriate gender and age specific equations developed by Visser *et* al (78) and Fat Mass (FM) from Siri`s formula (79). Arm Muscle Circumference (AMC) measurements were performed on the patient`s non-dominant arm flexed to an angle of 90º at the midpoint between the acromium process and the olecranon, with a plastic tape to the nearest 0.5cm (Lee and Nieman 1996). AMC was then calculated from the formula: upper-arm circumference - (0.314×TSF thickness) = AMC (Burr *et al.*, 1984).

Fat free mass (FFM) was evaluated by BIA, using a tetra polar device (Model Akern) and a current of 800 μA at 50kHz. BIA resistance and reactance measurements were used to calculate FFM according to regression equations previously validated in the elderly population. An age-specific equation was used to estimate FFM (Roubenoff *et al.*, 1997) (36). Fat Mass (FM) was then derived by subtraction from body weight values.

An equation was used to estimate the Phase Angle (PhA) (Chumlea *et al.*, 1988) (80). All BIA measurements were taken with the patients in the supine position, with legs and leads apart. Prior to attaching pregelled electrodes for BIA measurements, the skin was cleaned with alcohol swabs.

Nutritional status was assessed by the MNA which was specifically developed to be used both as a screening and as an assessment tool in frail elderly. The European Society for Clinical Nutrition and Metabolism

recommends this tool to screen undernutrition and to assess the risk of developing undernutrition among elderly (Kondrup et al.2003) (23). It consists of 18 questions targeting mainly three areas: anthropometry (BMI, weight loss, mid--arm and calf circumferences), global assessment (lifestyle, medication, and physical and mental status), and dietary intake. An elderly patient scoring less than 17.0 out of 30 points is classified as undernourished, between 17.5 and 23.5 at-risk of undernutrition, and 24 points or more as well-nourished (Guigoz et al. 1994)(23).

Cognitive function was evaluated with a previously validated tool for the Portuguese population - the Mini Mental State Examination (MMSE) (53), and also with the Clock-Drawing Test (CDT) (20, 21). MMSE assess orientation, memory and other cognitive skills. The score allows a classification of the severity of cognitive deficit and an evaluation of the progression of the disease. Possible scores of MMSE range from 0 to 30, with lower scores indicating worse cognitive status. A score < 23 points is usually considered to be indicative of clinically significant cognitive impairment (53). CDT measures executive function. MMSE test scores combining with CDT provide information on a patient`s functional status and cognitive function. A normal CDT almost always predicts that a person`s cognitive abilities is within normal limits. For AD-patients, this scale system is based on a 9 points score. A normal score ranges between 7 and 9 points (20,21).

Functional status was evaluated with the Barthel Index (BI). This score, allows the classification of the level of independence in ten self-care items: bathing, eating, dressing, transfer, toileting, continence, including the

assessment of bladder and bowel control. Each item was scored using a rating scale of 0,5,10. The total score of the BI is 100, level of independence (21).

Serum biomarkers (albumin, total-protein, total-cholesterol, vitamin B-12 and folic acid) levels were measured at baseline, 21 and 90 days. Albumin and total-protein were measured enzimatically. Total-cholesterol was determined by routine technique in use at Hospital. Vitamin B-12 and folic acid were determined by chemiluminescence.

Statistical Analysis

Differences in categorical data were evaluated by Pearson chi-square test. Kolmogorov-Smirnov test was used to assess the normality of distribution. Comparison of baseline characteristics between the two groups and the differences between groups after intervention were carried out using the independent samples t-test or the Mann-Whitney *U* test.

All p-values were based on two-sided tests and $p < 0.05$ was considered to be significant. All statistical analyses were performed using SPSS 14.0 for Windows.

Results

Sample Characteristics

The TG and CG were comparable for age, sex, education and for the baseline nutritional status parameters as weight, body composition, cognitive and functional status and also for the serum biomarkers levels evaluated ($p>0.05$) (Table 2). All the participants were classified as undernourished by MNA (score < 17.0). The low BMI for TG (19.2±2.7kg/m^2, range 13.5-26.3) and for CG (19.5±1.3kg/m^2, range 17.1-21.4) confirmed the undernourished status by MNA score (11.6±3.8, range 4.0-17.0) for TG and (13.0±1.8, range 10.0-16.5) for CG. The mean MMSE, CDT and BI scale scores showed moderate dementia and moderate dependence.

Table 2. Baseline characteristics of patients*.

Variables	**Treatment Group (n= 20)** Mean ± SD	**Control Group (n= 15)** Mean ± SD
Age (years)	79.4 ± 6.9	78.4 ± 5.2
Sex (M/F)	5/15	4/11
Height (cm)	161.6 ± 5.6	160.2 ± 10.1
Weight (kg)	49.7 ± 11.2	51.2 ± 5.6
Education (years of schooling)	3 ± 1.7	3 ± 1.8
MNA (score)	11.6 ± 3.8	13 ± 1.8
BMI (kg/m^2)	19.2 ± 2.7	19.5 ± 1.3
FFM (kg)	37.6 ± 5.7	38.8 ± 4.2
FFM (%)	77.1 ± 9.8	76.0 ± 6.3
FM (%)	35.9 ± 5.9	37.2 ± 5.4
PhA (º)	2.9 ± 0.5	3.1 ± 0.4
TSF (mm)	10.2 ± 6.0	11.9 ± 3.6
AMC (cm)	20.1 ± 3.1	19.2 ± 3.8
Albumin (%)	52.9 ± 3.1	53.7 ± 3.2
Total-Protein (g/dL)	6.3 ± 0.5	6.3 ± 0.5
Total-Cholesterol (mg/dL)	199.1 ± 36.9	195.2 ± 39.7
Vitamin B-12 (pg/mL)	193.9 ± 83.5	201.8 ± 119.8
Folic Acid (ng/mL)	7.5 ± 5.3	6.4 ± 3.0
MMSE (score)	17 ± 7	18 ± 5
CDT (score)	0.7 ± 1.0	0.7 ± 0.8
BI (score)	59.2 ± 18.1	56.6 ± 10.6

SD: standard deviation; MNA: mini nutritional assessment; MMSE: mini mental state examination; CDT: Clock-drawing test; BI: Barthel index; BMI: body mass index; FFM: fat free mass; FM: fat mass; PhA: phase angle; TSF: triceps skinfold thickness; AMC: arm muscle circumference;

* There were no significant differences between the two groups.

Treatment changes from baseline to 21days

The compliance with the OS was excellent. This short-term intervention resulted in significant improvements in TG compared to CG (Table 3). Mean MNA (score), weight (kg), FM (%), TSF thickness (mm), AMC (cm) and serum biomarkers levels albumin (%) and total-protein (g/dL), significantly increased in TG compared to CG ($p<0.05$). No changes were found for dependence, cognitive and functional status in both groups.

Table 3. Changes of nutritional, functional and cognitive parameters, between baseline and 21st day.

Variables	Treatment Group (n= 20) Mean ± SD			Control Group (n= 15) Mean ± SD			P Between Differences
	1st Day	21st Day	*Difference	1st Day	21st Day	*Difference	
MNA (score)	11.6 ± 3.8	13.1 ± 3.3	1.4 ± 0.8	13.8 ± 1.8	13.8 ± 1.8	- 0.0 ±0.1	< 0.001
Weight (kg)	49.7 ± 11.2	51.8±10.8	2.1 ± 1.6	51.2 ± 5.6	51.2 ± 5.5	0.0 ± 0.9	< 0.001
BMI (kg/m^2)	19.2 ± 2.7	20.1 ± 2.7	0.9 ± 0.7	19.5 ± 1.3	19.5 ± 1.4	0.0 ± 0.4	< 0.001
FFM (kg)	37.6 ± 5.7	37.6 ± 5.7	0.0 ± 0.0 [a]	38.8 ± 4.2	38.8 ± 4.2	0.0 ± 0.0	n.s.
FFM (%)	77.1 ± 9.8	73.8 ± 9.2	- 3.3 ± 2.4	76.0 ± 6.3	76.0 ± 6.5	- 0.0 ± 1.4	< 0.001
FM (%)	35.9 ± 5.9	36.2 ± 5.9	0.3 ± 0.4	37.2 ± 5.4	37.3 ± 5.5	0.1 ± 0.4	0.008
PhA (º)	2.9 ± 0.5	2.8 ± 0.5	- 0.3 ± 0.1	3.1 ± 0.4	3.0 ± 0.4	- 0.1 ± 0.1	n.s.
TSF (mm)	10.2 ± 6.0	10.6 ± 5.8	0.4 ± 0.5	11.9 ± 3.6	11.9 ± 3.6	- 0.0 ± 0.1	< 0.001
AMC (cm)	20.1 ± 3.1	20.3 ± 2.9	0.2 ± 0.4	19.2 ± 3.8	19.0 ± 3.8	- 0.2 ± 0.8	0.024
Albumin (%)	52.9 ± 3.1	53.9 ± 2.5	1.1 ± 1.5	53.7 ± 3.2	53.1 ± 3.1	- 0.7 ± 1.7	< 0.001
Total-Protein (g/dL)	6.3 ± 0.5	6.6 ± 0.5	0.2 ± 0.3	6.3 ± 0.5	6.4 ± 0.4	0.0 ± 0.3	0.027
Total-Cholesterol (mg/dL)	199.1 ± 36.9	198.0 ± 32.5	-1.2 ± 6.2	195.2 ± 39.7	192.9 ± 38.3	- 2.2 ± 9.0	n.s.
Vitamin B-12 (pg/mL)	193.9 ± 83.5	426.4 ± 200.8	232.5 ± 220.5	201.8 ± 119.8	467.5 ± 190.6	220.9 ± 310.9	n.s.
Folic Acid (ng/mL)	7.5 ± 5.3	10.2 ± 5.1	2.7 ± 3.8	6.4 ± 3.0	10.9 ± 4.0	4.0 ± 5.8	n.s.
MMSE (score)	17.7 ± 7.0	17.7 ± 7.0	0.0 ± 0.0 [a]	18.1 ± 5.3	18.1 ± 5.3	0.0 ±0.0 [a]	n.s.
CDT (score)	0.75 ± 1.02	0.75 ± 1.02	0.00 ± 0.00 [a]	0.73 ± 0.79	0.73 ±0.79	0.00 ±0.00 [a]	n.s.
BI (score)	59.2 ± 18.1	59.2 ± 18.1	0.0 ± 0.0 [a]	56.6 ± 10.6	56.0 ± 11.0	- 0.7 ± 2.6	n.s.

SD: standard deviation; MNA: mini nutritional assessment; MMSE: mini mental state examination; CDT: Clock drawing test; BI: Barthel index; BMI: body mass index; FFM: fat free mass; FM: fat mass; PhA: phase angle; TSF: triceps skinfold thickness; AMC: arm muscle circumference.
* Difference calculated by values at 21 days minus 1st day of study.
[a] t Cannot be computed because the standard deviations of both groups are 0.
n.s. no significant.

Overall Study Changes from 21 to 90 Days

After discontinuing the short-term intervention, significant improvements in nutritional status were found for MNA score (1.4±2.3), weight (0.4±2.4kg), FM (0.3±1.1%), TSF (0.1±1.5mm), serum albumin (1.5±1.9%) and total-protein (0.1±0.4g/dL) levels (Table 4). After the intervention, the total MNA score lowered in one patient (1 point) of the CG and was maintained in all the others. In the TG, the total MNA score was maintained in one patient and improved in all the others (1 point – 45%, 2 points – 30% and 3 points - 20%). No significant differences in MMSE, CDT and BI were observed during this period.

Table 4. Changes of nutritional, functional and cognitive parameters, between 21days and 90 days of study.

Variables	**Treatment Group (n= 20)** Mean ± SD		**Control Group (n= 15)** Mean ± SD		P Between Differences
	90th Day	*Difference	90th Day	*Difference	
MNA (score)	14.5 ± 2.9	1.4 ± 2.3	13.3 ± 1.6	-0.5 ± 0.6	0.003
Weight (kg)	52.1 ± 11.1	0.4 ± 2.4	49.9 ± 5.6	-1.3 ± 2.1	0.002
BMI (kg/m^2)	20.2 ± 3.3	0.2 ± 1.0	19.0 ± 1.4	-0.5 ± 0.8	0.002
FFM (kg)	38.1 ± 5.4	0.4 ± 1.8	39.7 ± 3.9	0.8 ± 1.1	n.s.
FFM (%)	74.6 ± 11.1	0.8 ± 5.4	79.9 ± 8.5	3.9 ± 4.0	n.s.
FM (%)	36.5 ± 6.0	0.3 ± 1.1	37.4 ± 5.9	0.1 ± 0.7	0.012
PhA (º)	2.8 ± 0.5	-0.1 ± 0.2	2.8 ± 2.8	-0.2 ± 0.2	0.047
TSF (mm)	10.7 ± 5.9	0.1 ± 1.5	11.5 ± 2.9	-0.4 ± 1.0	0.047
AMC (cm)	20.1 ± 3.2	-0.2 ± 1.7	18.8 ± 3.9	-0.2 ± 2.9	n.s.
Albumin (%)	55.4 ± 1.4	1.5 ± 1.9	53.1 ± 2.7	0.3 ± 1.4	0.014
Total-Protein (g/dL)	6.6 ± 0.5	0.1 ± 0.4	6.2 ± 0.2	-0.2 ± 0.3	0.019
Total-Cholesterol(mg/dL)	187.2 ± 26.3	-10.8 ± 23.3	186.9 ± 34.5	17.7 ± 17.7	n.s.
VitaminB-12(pg/mL)	257.8 ± 57.6	140.3 ± 127.1	607.4 ± 300.9	192.1 ± 266.1	n.s.
Folic Acid (ng/mL)	14.1 ± 9.1	3.9 ± 6.6	12.3 ± 4.8	3.1 ± 3.6	n.s.
MMSE (score)	17.7 ± 7.0	0.0 ± 0.0[a]	18.1 ± 5.3	0.0 ± 0.0[a]	n.s.
CDT (score)	0.75 ± 1.02	0.00 ± 0.00[a]	0.73 ± 0.79	0.00 ± 0.00[a]	n.s.
BI (score)	59.2 ± 18.1	0.0 ± 0.0[a]	56±11.0	0.0 ± 0.0[a]	n.s.

SD: standard deviation; MNA: mini nutritional assessment; MMSE: mini mental state examination; CDT: Clock drawing test; BI: Barthel index; BMI: body mass index; FFM: fat free mass; FM: fat mass; PhA: phase angle; TSF: triceps skinfold thickness; AMC: arm muscle circumference.
* Difference calculated by values at 90th days - 21 days of study.
[a] t Cannot be computed because the standard deviations of both groups are 0. n.s. no significant.

Discussion

This short-term prospective study in undernourished patients with mild AD, demonstrates that an high protein, energy-dense oral liquid supplement significantly increases MNA scores, weight, FM, TSF, serum albumin and total-protein levels and has no effect on MMSE, CDT, BI scores. An improvement in AD-patients nutritional status had already been shown after long-term oral supplementation with commercial formulas (11,18,72,73) and also after a comprehensive intervention strategy (16).

A previous study conducted in AD-patients, showed that a short-term supplementation of 21 days, resulted in an increased nutritional intake (74). According to our knowledge, our results are the first to show that a short-term 21 consecutive days supplementation (74) has a favourable impact on the nutritional status of AD elderly patients.

At 90 days of follow up, further improvements emerged in the AD-patients nutritional status. If further confirmed, this effect could have important implication in clinical practice, as it may allow for rotating nutritional supplementation schedules in heath care services, reducing treatment burden, as suggested before (75).

There is a potential for bias in this study due to the small sample sizes, particularly for CG. Thus, the absence of statistical significance for some variables may reflect a lack of power rather than a lack of physiological effect (type-II errors). However, this low sample size was enough to detect significant differences between the two groups.

Another major goal of the study was to test whether OS could affect the cognitive function or functional status. No such positive effects on cognition or on functional status were found using MMSE, CDT and BI at 21 days or at 90 days follow-up. The low duration of this experiment could be responsible for the lack of effect, since Corey-Bloom J. *et al.* showed a decrease of about 3 points on the MMSE in patients with AD after one year intervention (80).

PhA is an indicator of function and general health (not only an indicator of body composition or nutritional status) (you need a reference here). The mean PhA values of this sample (2.97±0.56º for women and 3.04±0.09º for men), were below the recommended values for healthy elders (5.64±1.02º for women and 6.19±0.97 for men) (43), which is in line with the undernourished status of the participants. According to my knowledge, there are no published PhA values for AD-patients. It is generally accepted that the accuracy of BIA depends on the variation included in the prediction equation and on using a specific prediction equation validated for a specific population. Thought the equations used in the present analysis, were choosen as the best approach they could not be accurate enough to calculate the FFM and PhA in this particular group of patients. This study did not show differences in FFM and PhA during the overall intervention.

Another positive finding was the maintenance of the MNA score in the CG during the study period, in 95% of the patients. As all the participants initiated a hospital stay at the beginning of the study, the absence of the already described deterioration of nutritional status during the hospitalization (81) can be explained by the standard dietetic advice which was provided to all participants.

In conclusion, this study showed that the nutritional status of mild probable AD--patients can be improved by providing dietary advice and, specially, a high protein energy-dense oral liquid supplement. However, larger scale studies are required to confirm these effects.

Conclusions

This study suggests that providing a high protein energy-dense liquid supplement, for a short period of time (21 days),,to undernourished elderly patients with probable mild AD, has a favorable impact on their nutritional status. Oral supplementation was associated with a significant improvement in MNA score and serum biomarkers levels (albumin and total-protein), but had no effect in cognition and functional status. After discontinuing the supplementation, significant further improvements in nutritional status were found for MNA score, weight, FM, TSF thickness, serum albumin and total-protein levels.

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