RESEARCH ARTICLE

Adequacy of food consumption in elderly Alzheimer's disease in a community of Southern Brazil: a Cross-sectional study [version 1; peer review: 2 not approved]

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Abstract

Background: Alzheimer's disease (AD) is the most common cause of dementia, with a multifactorial etiology, in which the person has great difficulty identifying feelings of hunger, satiety, and feeding, which may affect their nutritional status. Pathologically, it is associated with neurodegeneration of synapses followed by neuronal loss, accompanied by glial proliferation surrounded by neurofibrillary tangles, beta-amyloid peptide (Aβ) deposition, inflammation and cerebrovascular injury hindering the ability to perform activities of daily living. This study aimed to analyze quantitatively the differences between an elderly group with AD and a control group, in terms of macro and micronutrient consumption evaluation.
Methods: the study involved 69 participants who were assessed via collection of anthropometric measurements (weight, height and body mass index) with nutritional status being assessed by 24-hour food recall and three-day food record. Cognitive assessments were performed using the Mini-Mental State Examination (MMSE) and Clinical Dementia Ranting (CDR).
Results: The intake of lipids in patients with severe dementia, was lower ($p < 0.05$). The consumption of proteins showed a decrease with demential advance. For vitamins, there was a significant difference (p

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<0.05) in the amount of thiamine, niacin, vitamin D, E and K and calcium, chromium and iodine minerals, which were significantly reduced in AD patients (p <0.05).
Conclusions: Decreases in macronutrient and micronutrient consumption may result in a consequent impairment of nutritional status, dementia progression, and decreased quality and life expectancy of elderly patients with AD.

Keywords
Alzheimer's Disease, Food Intake, Dementia, Macronutrients, Vitamins, Cognition

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Introduction

Alzheimer's disease (AD) is the most common type of dementia, characterized by cognitive and neuropsychiatric manifestations that result in progressive disability[1,2]. Several factors contribute to the neurodegenerative process of AD. Lack of availability of certain nutrients, abnormal protein processing and neuronal membrane degeneration accelerate dysfunction and synaptic loss from the onset of disease[3].

The medial temporal lobe is an area of the brain that is involved in the regulation of food intake[4], and a site where neurodegeneration is typically present[5]. The behavioral modification of the AD patient interferes directly with the reduction of food consumption and the absorption of essential nutrients for the normal functioning of the body[6].

The synthesis of synaptic membranes is dependent on various nutrients, such as folate, vitamin B12, vitamin B6, vitamin E, vitamin C and selenium. Inadequate ingestion and metabolism of these nutrients is related to increased phospholipid degradation and degeneration of neuronal membranes responsible for synapse loss[3].

The nutritional status and food intake in the elderly differ by their cognitive, physical and biological capabilities. Taste reduction, difficulty in swallowing and xerostomia may be the result of neuropathological disorders, senility, or even induced by polypharmacy due to a higher incidence of chronic diseases resulting in them being more likely to be taking a combination of pharmaceuticals[7,8].

Since the nutritional status directly affects the patient's physiological condition, causing the elderly to be more susceptible to decreased biological function, making them more susceptible to falls, fractures, protein-energy malnutrition, and associated nutritional deficiencies[6], this study had the intention of analyze the food intake in patients with AD, comparing it with the control group, identifying micro and macronutrient deficits that lead to worsening dementia and decreased quality of life.

Methods

Ethics and approval

This study was approved by the Ethics Committee of the State University of Midwest (UNICENTRO) under number 026/2011. Patients and their caregivers received information on the purpose, methods, risks and benefits of research and gave written informed consent to participate in the study. Patients with any nutritional changes were referred to the Nutrition School Clinic of UNICENTRO for follow-up.

Sample

A cross-sectional study with a control group was performed, with 37 elderly controls and 32 AD patients.

The elderly were attended and diagnosed clinically with AD in the health centers through the Unified Health System (SUS) and were passed through a confirmed diagnosis issued by a geriatric doctor or neurologist, and assisted by the Association of Studies, Research and Assistance to People with Alzheimer's (AEPAPA) in Guarapuava, Paraná.

The control group, in turn, was formed by healthy elderly participants of exercise groups and cultural activities for seniors, provided by the Social Welfare Department of the city of Guarapuava. A Mini-Mental State Examination (MMSE) was performed and evaluated by a trained healthy professional, applied to the health elderly as exclusion criteria for the control group. Also, they agreed in participate of the study and gave written informed consent in participate in the study.

Patients and elderly without AD not found in their homes on three attempted visits on different days of the week, those who died, the residents of the countryside, and those who had moved to other cities, in addition to those unwilling or who rejected the survey after the first visit, along with those who did not agree with the terms of the Free and Informed Consent Term (TCLE) were excluded from the study.

Data collection

The study occurred between August 2013 to June 2014, with an initial sample of 55 AD patients attending SUS in Guarapuava, and after applying the exclusion criteria, the sample consisted of 32 patients. The sample of healthy elderly was initially 50 participants, of which 13 declined to continue due to the amount of data that needed to collected and the time it would take to collect this data, consisting of 37 patients. All remaining individuals after applying exclusion criteria completed the study.

Assessment of nutritional status and food intake

To evaluate the nutritional status, anthropometric measurements were collected: weight (kg), height (m), body mass index - BMI (kg/m^2). Weight and height were collected according to the methods recommended by the Food and Nutrition Surveillance System - SISVAN[9]. When weighing and stature measurement was not possible due to the patient's health conditions, weight and height were estimated using theoretical formulas, using arm circumference (AC) and calf circumference (CC) measures, knee height and subscapular cutaneous fold. The formulae used to estimate weight were:

a) For men: weight = {[1.73 × Arm Circumference (cm)] + [0.98 × Calf Circumference (cm)] + [0.37 × subscapular skin fold (mm)] + [1,16 × Knee Height (cm)] = 81.69;

b) For women: weight = {[0.98 × Arm Circumference (cm)] + [1.27 × Calf Circumference (cm)] + [0.4 × subscapular skin fold (mm)] + [0.87 × Knee Height (cm)] = 62.35}.

The formulae used for height estimation (m) in the elderly were:

a) For males: height = {64,19 - [0,04 × age (years)] + [2,02 × knee height (cm)]};

b) For women: height = {84,88 - [0,24 × age (years)] + [1,83 × knee height (cm)]}.

By three-day and 24-hour dietary recall, the caregiver noted in forms all food and drink consumed (Forms are available as part of Dataset 1[10]). The collected information was used to perform a diet analysis including values of energy, macronutrients and micronutrients provided by DietWin® software, version 2008.

All values obtained from the average of four days of each nutrient were compared with the recommendations of the Dietary Reference Intakes (DRIs), according to the gender and age of the patient. As yet there are no specific recommendations for AD patients.

Evaluated consumption in grams (g), milligrams (mg) and micrograms (mcg) of nutrients, and calculated the percentage of adequacy by the following formula: adjustment percentage = amount of ingested nutrient (g/mg/mcg)/DRI recommendation (g/mg/mcg) × 100.

Cognitive evaluation

The elderly were evaluated through the Mini Mental State Examination (MMSE) and Clinical Dementia Rating (CDR) between August 2013 to June 2014. Each category is classified based on scores - none/questionable dementia, mild dementia, moderate or severe[11]. The CDR and MMSE was applied and evaluated by a trained health professional.

Statistical analysis

Data were analyzed using the SPSS 20.0 statistical software for Windows, presented as median and interquartile range, relative and absolute frequency. Prior to the analysis, the following assumptions were tested on the numerical variables: homogeneity of the variances using the Levene test, and distribution of the data using the Shapiro-Wilk test. If the assumptions were incorrect data was analyzed using the Kruskal-Wallis test. When necessary, we used the multiple comparisons analysis with the Bonferroni level of significance correction. The Chi-square test, Fisher's Continuity Correction and Exact Correction were used to identify associations between the diagnosis of AD and clinical and sociodemographic variables. A significance level of $p < 0.05$ was adopted.

Results

In total, 69 participants took part in the study: 37 from the control group and 32 from the AD group.

Table 1 shows anthropometric characteristics of the sample, these data are characterizing this population according to CDR, age, current weight, height and BMI. Data are presented as median (P25-P75).

The age of the elderly ranged from 57 to 94 years, with a median of 74 years, with a median height of 1.58m of the total sample. Regarding body weight, the control group had the highest average, and the weight in AD patients decreased with increased severity of disease.

Regarding the sociodemographic characteristics (Table 2), 45 (65.2%) were female; 20 were married in the control group (29.0%) and in the AD group there were 7 (10.1%); 3 from the control group were unmarried (4.3%) and in the AD group there were 7 (10.0%). There were 13 widowers in the control group (18.8%), and 17 (24.5%) in the AD group.

The average family income reported by the participants in the control group was R$ (Brazilian real - BRL) 904.00 ± 8.99 (US$ 275.76) and the participants with the disease presented income around R$ 1.031.13 ± 67.29 (US$ 314.54).

Table 3 shows the comparison of carbohydrate intake. The consumption was lower in the control group but there was no difference between the groups of patients with dementia ($p > 0.05$). In contrast, with respect to the consumption of proteins, as disease progressed this concomitantly decreases. The only macronutrient that gave a significant difference ($p < 0.05$) was the lipid values, in relation to the control group (113.64% adequacy), which presented a greater consumption than CDR-1, mild dementia (86.95% adequacy) and CDR-3, severe dementia (7751% adequacy).

Table 4 presents the data in relation to the intake of vitamins. For vitamin B1, the control group consumed a median 0.77

Table 1. Anthropometric data classified as the control group and for each category of Alzheimer's disease severity. Guarapuava, PR, Brazil. 2013.

		CDR		
	Control (n=37)	**Mild (n=6)**	**Moderate (n=10)**	**Severe (n=16)**
Age (years)	67.00 (65.0-72.0)	80.0 (63.0-82.0)	80.00 (78-87)	82.00 (77.0-86.0)
Current weight (kg)	72.00 (62.9-83.2)	70.70 (60.1-75)	67.50 (59.5-77.3)	60.00 (50.0-65.9)
Height (m)	1.59 (1.56-1.63)	1.60 (1.54-1.66)	1.57 (1.55-1.62)	1.51 (1.45-1.62)
BMI (Kg/m^2)	27.90 (23.5-31.5)	26.00 (24.7-28.3)	26.30 (25.0-28.1)	24.40 (18.9-27.6)

Data are presented as median (P25-P75) CDR: clinical dementia rating (cases); BMI: Body Mass Index; n: sample number.

Table 2. Sociodemographic data classified as a control group and staging of the disease. Guarapuava, PR, Brazil. 2013.

			CDR					
	Control		Mild		Moderate		Severe	
	n	%	n	%	N	%	n	%
Gender								
Female	25	36.2	2	2.9	6	8.7	12	17.4
Male	12	17.4	4	5.8	4	5.8	4	5.8
Marital status								
Single	3	4.3	1	1.4	5	7.2	1	1.4
Married	20	29.0	2	2.9	2	2.9	3	4.3
Widowed	13	18.8	3	4.3	3	4.3	11	15.9
Co-habiting	1	1.4	0	0.0	0	0.0	1	1.4

CDR: clinical dementia rating (cases); n: sample number.

Table 3. Frequency of consumption of macronutrients classified as the control group and for each category of Alzheimer's disease severity. Guarapuava, PR, Brazil. 2013.

			CDR				
		Control	Mild	Moderate	Severe	p	Contrasts p<0.05
		(n=37)	(n=6)	(n=10)	(n=16)		
Energy consumption‡	kcal	1277.5 (1043.25-1626.25)	1338.75 (1128.5-1991.25)	1353.75 (1063.25-1795)	1324.38 (1117.5-1467)	0.976	
	%	64.95 (56.07-84.04)	90.86 (59.31-123.39)	86.07 (68.33-113.2)	85.72 (67.84-106.07)	0.070	
Carbohydrates‡	g	156.37 (118.04-216)	199.43 (161.43-282.46)	218.81 (144.37-265)	191.03 (164.21-223.05)	0.163	
	%	50.82 (43.91-55.72)	56.98 (53.22-58.35)	62.56 (57.47-78.66)	61.55 (50.78-68.54)	0.298	
Lipids*	g	42.89 (31.79-56.27)	35.13 (24.68-57.28)	38.84 (28.63-47.7)	32.24 (17.75-42.63)	0.149	
	%	113.64 (94.61-122.28)	86.95 (85.08-94.14)	104.31 (71.43-146.83)	77.51 (60.94-106.43)	0.002	Control >1;3
Proteins‡	g	65.84 (48.45-76.21)	61.4 (54.62-86.42)	62.47 (53.71-79.55)	51.07 (40.05-63.31)	0.155	
	%	82.99 (68.63-98.99)	104.36 (86.5-124.09)	100.53 (64.37-148.45)	79.89 (55.33-111.82)	0.471	
Cholesterol‡	mg	189 (142.51-232.85)	162.76 (146.91-180.63)	234.37 (176.58-277.95)	167.83 (109.76-205.76)	0.146	
	%	94.5 (71.26-116.42)	81.38 (73.45-90.31)	117.19 (88.29-138.97)	83.91 (54.88-102.88)	0.146	
Omega 3‡	g	0.34 (0.22-0.45)	0.23 (0.12-0.41)	0.3 (0.23-0.53)	0.26 (0.12-0.3)	0.205	
	%	28.18 (19.55-37.27)	14.06 (10.91-37.05)	21.25 (14.38-32.97)	21.44 (9.05-24.89)	0.142	
Omega 6‡	g	3.07 (1.94-4.71)	1.89 (1.12-5.16)	2.86 (2.65-5.62)	2.21 (1.11-2.91)	0.187	
	%	27.77 (15.86-42.82)	15.15 (7.96-46.89)	24.66 (15.91-40.63)	18.09 (9.75-24.51)	0.299	

Data are presented as median (p25-P75) CDR: clinical dementia rating (cases); Kruskall-Waliss (Bonferroni for multiple comparisons). * There was a statistical difference between elderly control subjects and elderly with mild and advanced dementia (p<0.05). ‡There was no difference between groups (p>0.05).

(0.55-0.97) mg on median, lower than that of CDR-2, which consumed a median of 1.27 (1.1-1.47) mg (p <0.05). For vitamin B3, differences were also observed: the median control group ingestion was 6.22 (4.85-8.68) mg. CDR-2 and CDR-3 patients had a significant higher (p <0.05) consumption (10.27 (8.8-14.05) and 11.22 (9.04-14.1) mg, respectively). Vitamin D, E, and K intake were higher in the control group when compared to all stages of dementia (vitamin D, p> 0.05; vitamin E, p> 0.05; vitamin K, p <0.05).

Among the minerals shown in Table 5, only calcium, chromium and iodine showed differences between the groups (p <0.05).

Table 4. Frequency of consumption of vitamins classified as control group and for each category of Alzheimer's disease severity. Guarapuava, PR, Brazil. 2013.

		Control	mild	Moderate	Severe	P	Contrasts p<0.05
		(n=37)	(n=6)	(n=10)	(n=16)		
Vitamin A‡	mcg	176.18 (75.89-274.95)	97.14 (38.86-162.06)	132.44 (24.98-250.54)	123.82 (73.92-158.32)	0.285	
	%	20.15 (9.99-34.57)	11.73 (4.32-23.15)	16.63 (3-27.84)	15.99 (9.7-22.62)	0.315	
Vitamin B1**	mg	0.77 (0.55-0.97)	1.05 (0.77-1.28)	1.27 (1.1-1.47)	0.91 (0.68-1.36)	0.025	Control <2
	%	64.77 (49.55-87.95)	87.5 (70-106.25)	106.26 (98.96-133.86)	78.92 (62.05-118.38)	0.037	Control <2
Vitamin B2‡	mg	0.89 (0.53-1.24)	0.83 (0.66-1.15)	1.13 (0.53-1.51)	1.36 (0.91-2.13)	0.042	Control <3
	%	75.45 (47.95-95)	70.01 (50.96-88.46)	97.6 (40.77-120.45)	113.71 (77.36-177.88)	0.042	Control <3
Vitamin B3***	mg	6.22 (4.85-8.68)	8.67 (2.86-11.66)	10.27 (8.8-14.05)	11.2 (9.04-14.1)	0.001	Control <2;3
	%	44.45 (34.63-62)	58.63 (17.86-72.88)	73.36 (55-97.61)	78.26 (60.61-94.88)	0.002	Control <2;3
Vitamin B5‡	mcg	0.68 (0.3-1.58)	0.12 (0.05-0.34)	0.27 (0.07-0.36)	0.33 (0.08-0.93)	0.032	Control >2
	%	13.65 (5.95-31.6)	2.48 (0.95-6.85)	3.93 (1.3-7.15)	6.58 (1.65-18.65)	0.027	Control >2
Vitamin B6‡	mg	0.6 (0.48-0.82)	0.43 (0.25-0.57)	0.55 (0.31-0.91)	0.62 (0.41-0.85)	0.362	
	%	35.94 (28.13-49)	21.63 (12.38-35.78)	39.25 (25.63-63.91)	34.59 (23.05-53.05)	0.253	
Vitamin B12‡	mg	0.9 (0.08-1.58)	0.12 (0-0.23)	0.04 (0-0.65)	0.2 (0-1.01)	0.156	
	%	37.4 (3.23-65.94)	4.84 (0-9.38)	1.82 (0-26.88)	8.33 (0-42.03)	0.156	
Vitamin C‡	mg	37.69 (21.57-62.9)	75.32 (19.21-119.27)	43.45 (30.26-65.27)	60.67 (23.65-126.85)	0.594	
	%	50.25 (28.76-83.86)	93.97 (21.34-145.45)	52.61 (33.62-87.02)	75.82 (28.86-143.99)	0.607	
Vitamin D†	mcg	0.21 (0.01-0.84)	0 (0-0)	0.04 (0-0.2)	0.04 (0-0.25)	0.010	Control >1;2;3
	%	0.04 (0-0.14)	0 (0-0)	0.01 (0-0.03)	0.01 (0-0.04)	0.010	Control >1;2;3
Vitamin E†	mg	3.44 (0.81-6.39)	0.76 (0.35-1.01)	0.84 (0.26-2.05)	1.31 (0.26-3)	0.009	Control >1;2;3
	%	22.92 (5.37-42.62)	5.08 (2.32-6.72)	5.62 (1.73-13.67)	8.7 (1.73-20)	0.009	Control >1;2;3
Vitamin H‡	mg	4.85 (1.17-8.54)	0.06 (0.03-0.09)	2.32 (0.03-6.6)	3.09 (0.07-8.81)	0.108	
	%	16.17 (3.9-28.47)	0.2 (0.08-0.28)	7.73 (0.08-22)	10.29 (0.25-29.36)	0.108	
Vitamin K†	mg	0.06 (0.02-0.13)	0.01 (0-0.02)	0 (0-0.02)	0.01 (0-0.02)	0.002	Control >1;2;3
	%	0.1 (0.02-0.19)	0.01 (0-0.02)	0.01 (0-0.05)	0.02 (0-0.04)	0.005	Control >1;2;3

Data are presented as median (P25-P75). CDR: clinical dementia rating (cases); n; sample number; Kruskall-Waliss (Bonferroni for multiple comparisons). ** There was a statistical difference between elderly control subjects and elderly with moderate dementia (p<0.05). *** There was a statistical difference between elderly control subjects and elderly with mild and advanced dementia (p<0.05). † There was a statistical difference between elderly control subjects and elderly with mild, moderate and advanced dementia (p<0.05). ‡ There was no difference between groups (p>0.05).

Discussion

Diet and nutritional factors are known modifiable factors for dementia and cognitive decline later in life, with significant associations between an inadequate nutritional status and behavioral disorders with greater impact on activities of daily living[12].

The sample was made up mostly of patients with advanced dementia (50% of AD patients), and is similar to the study of Goes *et al.*[7] where 40% of the sample were in the advanced stages of the disease. Advanced disease is characterized by severe memory loss, with no capacity to make decisions, and requires a caregiver or person who can assist in the practice of activities of daily living, and most of them can result in food imbalances and consequent malnutrition.

A high BMI in middle age is associated with an increased risk for the development of dementia[7]. The elderly studied in the control group, and in the CDR1 and CDR2 groups, were overweight and as the staging of the disease increases, BMI decreases. This overweight condition is likely related to the food preferences found for this group, carbohydrates and lipids. These preferences and dietary deficiencies could be caused by the low family income found in the study, and also due to the decrease in

Table 5. Frequency of minerals consumption classified as control group and for each category of Alzheimer's disease severity. Guarapuava, PR, Brazil. 2013.

			CDR				
		Control	Mild	Moderate	Severe	p	Contrasts p<0,05
		(n=37)	(n=6)	(n=10)	(n=16)		
Calcium***	mg	299.63 (209.1-509.25)	469.27 (344.57-640.71)	559.66 (304.57-769.72)	613.32 (410.31-789.35)	0.003	Control <2;3
	%	29.96 (20.91-50.93)	46.93 (34.46-64.07)	55.97 (30.46-76.97)	61.33 (41.03-78.94)	0.003	Control <2;3
Copper‡	mg	0.77 (0.53-1.03)	0.91 (0.35-2.35)	0.7 (0.55-1.29)	0.75 (0.6-1.19)	0.574	
	%	0.09 (0.06-0.11)	0.1 (0.04-0.26)	0.08 (0.06-0.14)	0.08 (0.07-0.13)	0.574	
Chrome††	mcg	24.86 (4.56-44.43)	2.85 (1.71-5.7)	2.28 (0.01-2.85)	2.28 (0-7.88)	0.001	Control >3
	%	89.51 (20.1-222.16)	9.5 (7.2-19)	11.4 (6.65-39.81)	11.41 (0.01-30.18)	0.001	Control >3
Iron‡	mg	6.56 (4.74-8.19)	5.97 (5.06-6.67)	7.19 (5.32-9.41)	5.81 (4.28-10.14)	0.846	
	%	82 (59.22-102.38)	74.66 (63.22-83.38)	89.83 (66.44-117.63)	72.64 (53.48-126.8)	0.846	
Fluorine‡	mcg	0.01 (0-0.03)	0 (0-0)	0 (0-0.03)	0 (0-0.05)	0.716	
	%	0.33 (0.06-0.83)	0 (0-0)	0 (0-0.83)	0.1 (0-1.38)	0.753	
Folate‡	mcg	32.65 (13.9-56.79)	4.63 (1.35-24.52)	17.85 (1.35-34.31)	9.64 (1.5-38.06)	0.086	
	%	8.16 (3.48-14.2)	1.16 (0.34-6.13)	4.46 (0.34-8.58)	2.41 (0.37-9.51)	0.086	
Phosphor‡	mg	721.38 (580.19-850.38)	727.94 (568.09-826.65)	929.46 (718.58-1069.6)	883.83 (710.51-957.26)	0.088	
	%	103.05 (82.88-121.48)	103.99 (81.16-118.09)	132.78 (102.65-152.8)	126.26 (101.5-136.75)	0.088	
Iodine†††	mcg	36.48 (6.76-68.31)	1.11 (0-4.51)	3.11 (0-12.89)	16.34 (0-27.87)	0.104	
	%	24.32 (4.51-45.54)	0.74 (0-3.01)	4.14 (0-13.81)	10.9 (0-18.58)	0.104	
Magnesium‡	mg	192.53 (161.83-242.97)	217.52 (95.72-236.07)	169.84 (130.86-208.69)	199.23 (154.52-226.13)	0.485	
	%	56.09 (43.52-75.71)	55.51 (22.79-64.02)	44.2 (36.91-65.21)	51.91 (44.97-63.86)	0.394	
Manganese‡	mg	1.63 (2.14-3.45)	2 (2.33-2.49)	1.43 (1.73-3.37)	1.42 (1.97-2.67)	0.601	
	%	78.91 (110.56-169.58)	97.75 (101.2-138.33)	79.24 (91.41-146.3)	72.77 (103.89-148.47)	0.498	
Potassium‡	mg	1701.34 (1468.07-2097.32)	2227.85 (1124.25-2321.34)	1978.62 (1596.58-2395.12)	1889.39 (1437.28-2294.09)	0.405	
	%	36.2 (31.24-44.62)	47.4 (23.92-49.39)	42.1 (33.97-50.96)	40.2 (30.58-48.81)	0.405	
Selenium‡	mg	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.701	
	%	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.701	
Sodium‡	mcg	1070.16 (871.89-1465.71)	1015.84 (662.68-1130.23)	1381.1 (802.86-1678.84)	1107.22 (741.05-1469)	0.801	
	%	82.32 (67.07-112.75)	78.14 (50.98-86.94)	106.24 (61.76-129.14)	85.17 (57-113)	0.801	
Zinc‡	mcg	8.6 (6.62-11.9)	8.36 (6.35-10.65)	10.32 (8.52-11.58)	7.38 (6.65-8.84)	0.323	
	%	106.44 (71.22-119.64)	88.96 (57.7-96.82)	106.35 (92.5-136.75)	87.19 (65.05-110.5)	0.237	

Data are presented as median (P25-P75). CDR: clinical dementia rating (cases); n; sample number; Kruskall-Waliss (Bonferroni for multiple comparisons). ** There was a statistical difference between elderly control subjects and elderly with moderate dementia (p<0.05). *** There was a statistical difference between elderly control subjects and elderly with mild and advanced dementia (p<0.05). † There was a statistical difference between elderly control subjects and elderly with mild, moderate and advanced dementia (p<0.05). ‡ There was no difference between groups (p>0.05).

executive functionality and dysphagia[3], changing the consumption of proteins for carbohydrates[13].

Tieland *et al.*[12], in the Netherlands, analyzed three groups of elderly people, 707 individuals living independently in the community, of which 194 were fragile and 276 were institutionalized. The results showed that 35% of the institutionalized elderly had inadequate protein intake, below 0.7g/kg/day. As protein intake is impaired, there is an accelerated loss of muscle mass, sarcopenia[13], low hemoglobin levels, and a consequent increase in the mortality rate[14].

According to IBGE research[15] held in Brazil, the average consumption of lipids for men and women 60 years or more, is

approximately 49 grams. In the present study, the control group had a higher intake, at 42.89 grams, in comparison to CDR 3, 32.24 grams. Diets high in fat contribute significantly in β-amyloid accumulation, tau hyperphosphorylation and an inflammatory state in the peripheral organs and brain[16].

Analyzing the thiamine intake (vitamin B1), differences in consumption between groups can be seen. The control group had the lowest intake, likely related to the vitamin supplementation initiated after diagnosis of dementia. Relating IBGE data[15], the micronutrient ingestion for elderly is approximately 1.05 mg, while the amount for the AD group was far below.

In the study by Morris *et al.*[17], the total of 3,718 participants aged 65 years and over had dietary data taken, with at least two evaluations and analyzes of cognitive changes over a median of 5.5 years. In this prospective study, nutritional deficiencies of niacin (vitamin B3) were associated with AD and cognitive decline. Since the higher intake of vitamin B3 was associated with a slow rate of cognitive decline and its retardation. The nutritional recommendation for the elderly aged 60 years or more is the intake of approximately 22.7 mg/day[7].

There are studies in which the importance of vitamin D is postulated in cases of dementia[18,19] as it promotes the regulation of calcium homeostasis, β-amyloid peptide clearance, slows down and/or improves cognitive decline, and has an important antioxidant and anti-inflammatory effect[20]. In the cohort study conducted by Karakis *et al.*[18], in the USA, the final sample being composed of 1663 patients, vitamin D was associated with lower hippocampal volumes, helping to prevent brain lesions and cognitive decline. The ingestion of calciferol in the related study was impaired in all dementia phases. This may be due to their eating habits. The recommendation according to IBGE[15] for individuals over 60 years or more is on average 3.05 mcg/day.

Vitamin E eliminates free radicals, with some chemical forms have a potent anti-inflammatory effect, and the α-tocotrienol congener protects the cortical and hippocampal neurons from apoptosis. Vitamin E deficiency in human manifests primarily as peripheral sensory neuropathy, which demonstrates its essential role in the formation and maintenance of CNS function, supporting its potential use in the prevention and treatment of neurodegenerative diseases[21]. According to the recommendations of the National Institutes of Health of the United States, the recommended daily dose of vitamin E for an adult is 15mg[22]. For the IBGE[15], 3.8 mg/day is recommended for elderly individuals.

A study in the Netherlands[23] involved a sample of 5395 participants over 55 years of age who did not present with dementia. These individuals completed checklists about their eating habits over the past year. The indicated food would have been consumed at least twice a month. Participants were followed for 9.6 years. Of these, 465 developed dementias, of which 365 were diagnosed as having AD. Individuals with vitamin E consumption had a 25% lower risk of developing dementia. It is interesting to analyze the control group of the present study, because it was observed that when comparing the group without AD, all the dementia groups had a lower consumption of this important vitamin.

However, with regard to vitamin K, studies demonstrate the importance of activities related to the brain[24,25]. It is related to the transport of the apolipoprotein E (ApoE), a major of marker[26,27]. In the study of Carrié *et al.*[28], conducted with mice to analyze the cognitive ability of a diet low in vitamin K, a vitamin K depleted diet resulted in higher cognitive deficits. In the study by Presse *et al.*[24], a paired control case study of 31 control subjects and with AD, respectively, as in the present study, the healthy group consumed more food containing this vitamin.

The number of works that present calcium as an important underlying factor in the process functional changes of aging, especially in AD has increased[29,30]. Its dysregulation causes loss of function and mutations in the protein presenilin 1 (PS1), which is a risk factor for the onset of the disease. Other effects include alterations in the autophagic and lysosomal pathways[31,32], increased oxidative stress and consequent inflammation in neuronal cells[33,34]. Patients without neurodegeneration, however, consumed less calcium in their diet compared to those who had moderate dementia, CDR-2 and advanced dementia, CDR-3. The increased intake of dairy products in the diet of survey participants with dementia due to preference from soft textures of the food at this stage of life and stage of disease is the likely cause of this finding.

Among the micronutrients observed in Table 5, we highlight the chromium, which was less consumed by AD patients in CDR-3, as recommended by IBGE[17]. In the study by Krikorian *et al.*[34], a double-blind study was conducted in Cincinnati, USA, to evaluate whether chromium supplementation helps in memory and neuronal function in the elderly with cognitive decline. The results were that chromium picolinate supplementation may significantly improve cognitive inhibitory control and brain function in individuals who have neurodegeneration.

A cross-sectional study was conducted in Brazil in a group of 135 elderly women with mean age of 68 years, in which 42% of the women analyzed had iodine deficiency[35]. Iodine deficiency causes thyroid dysfunction, as well as hypertension, dyslipidemia, muscle wasting, frailty and neuromuscular dysfunction, reducing quality and life expectancy[36]. There is still no daily recommendation for iodine intake specifically for the elderly population.

The sample size and the fact that the values presented being an estimative from the nutritional consumption were the limitations of the study. Therefore, these results are in agreement with similar researches that consider nutritional consumption[7].

Conclusion

The nutritional status of an individual is associated with cognitive health and disease progression. According to the results, it is concluded that the progression of dementia is associated with lower levels of dietary intake of some macro and

micronutrients. Among the macros, reduced intake of lipids in mild and severe dementia can be observed. Regarding the micronutrients vitamin D intake showed a reduction in mild dementia stage. The vitamins B5, E, and K, and minerals calcium and chromium, had reduced consumption in severe dementia cases. Specific nutritional interventions can alter the development of the disease with low risk of side effects and with satisfactory results particularly in the early stages of the disease.

Data availability

All raw data was freely available on Open Science Framework site. Dataset 1: Adequacy of food consumption in elderly Alzheimer's disease in a community of Southern Brazil: a Cross-sectional study. http://doi.org/10.17605/OSF.IO/89USV[10]

Competing interests

No competing interests were disclosed.

Grant information

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References

1. Sanchez-Mut JV, Aso E, Panayotis N, *et al.*: **DNA methylation map of mouse and human brain identifies target genes in Alzheimer's disease.** *Brain.* 2013; **136**(Pt 10): 3018–27.
PubMed Abstract | Publisher Full Text | Free Full Text

2. Foidl BM, Do-Dinh P, Hutter-Schmid B, *et al.*: **Cholinergic neurodegeneration in an Alzheimer mouse model overexpressing amyloid-precursor protein with the Swedish-Dutch-Iowa mutations.** *Neurobiol Learn Mem.* 2016; **136**: 86–96.
PubMed Abstract | Publisher Full Text

3. van Wijk N, Slot RER, Duits FH, *et al.*: **Nutrients required for phospholipid synthesis are lower in blood and cerebrospinal fluid in mild cognitive impairment and Alzheimer's disease dementia.** *Alzheimers Dement (Amst).* 2017; **8**: 139–46.
PubMed Abstract | Publisher Full Text | Free Full Text

4. Josephs KA, Whitwell JL, Parisi JE, *et al.*: **Coprophagia in neurologic disorders.** *J Neurol.* 2016; **263**(5): 1008–14.
PubMed Abstract | Publisher Full Text

5. Whitwell JL: **Progression of atrophy in Alzheimer's disease and related disorders.** *Neurotox Res.* 2010; **18**(3–4): 339–46.
PubMed Abstract | Publisher Full Text

6. Volkert D, Chourdakis M, Faxen-Irving G, *et al.*: **ESPEN guidelines on nutrition in dementia.** *Clin Nutr.* 2015; **34**(6): 1052–73.
PubMed Abstract | Publisher Full Text

7. Goes VF, Mello-Carpes PB, de Oliveira LO, *et al.*: **Evaluation of dysphagia risk, nutritional status and caloric intake in elderly patients with Alzheimer's.** *Rev Lat Am Enfermagem.* 2014; **22**(2): 317–24.
PubMed Abstract | Publisher Full Text | Free Full Text

8. da Silva FP, Dallepiane LB, Kirsten VR, *et al.*: **Compliance in food consumption of young and long-lived elderly of a city in southern Brazil.** *Nutr Hosp.* 2016; **33**(1): 37–42.
Reference Source

9. Brasil: **Política Nacional de Alimentação e Nutrição.** Brasília: Ministério da Saúde. 2013; 86.
Reference Source

10. Hilgemberg GR: **Adequacy of Food Consumption in Elderly Alzheimer's Disease in a Community of Southern Brazil: A Cross-Sectional Study.** *Open Science Framework.* 2018.
Data Source

11. Vos SJ, Xiong C, Visser PJ, *et al.*: **Preclinical Alzheimer's disease and its outcome: a longitudinal cohort study.** *Lancet Neurol.* 2013; **12**(10): 957–65.
PubMed Abstract | Publisher Full Text | Free Full Text

12. Tieland M, Borgonjen-Van Den Berg KJ, Van Loon LJ, *et al.*: **Dietary protein intake in community-dwelling, frail, and institutionalized elderly people: Scope for improvement.** *Eur J Nutr.* 2012; **51**(2): 173–9.
PubMed Abstract | Publisher Full Text

13. Takagi D, Hirano H, Watanabe Y, *et al.*: **Relationship between skeletal muscle mass and swallowing function in patients with Alzheimer's disease.** *Geriatr Gerontol Int.* 2017; **17**(3): 402–409.
PubMed Abstract | Publisher Full Text

14. Grant WB: **Using Multicountry Ecological and Observational Studies to Determine Dietary Risk Factors for Alzheimer's Disease.** *J Am Coll Nutr.* 2016; **35**(5): 476–89.
PubMed Abstract | Publisher Full Text

15. IBGE. Instituto Brasileiro de Geografia e Estatística, Coordenação de Trabalho e Rendimento: **Pesquisa de Orçamentos Familiares: 2008-2009.** *Análise do Consumo Alimentar Pessoal no Brasil.* Biblioteca do Ministerio do Planejamento, Orçamento e Gestão. 2011; 150.
Reference Source

16. Walker JM, Dixit S, Saulsberry AC, *et al.*: **Reversal of high fat diet-induced obesity improves glucose tolerance, inflammatory response, β-amyloid accumulation and cognitive decline in the APP/PSEN1 mouse model of Alzheimer's disease.** *Neurobiol Dis.* 2017; **100**: 87–98.
PubMed Abstract | Publisher Full Text | Free Full Text

17. Morris MC, Evans DA, Bienias JL, *et al.*: **Dietary niacin and the risk of incident Alzheimer's disease and of cognitive decline.** *J Neurol Neurosurg Psychiatry.* 2004; **75**(8): 1093–9.
PubMed Abstract | Publisher Full Text | Free Full Text

18. Karakis I, Pase MP, Beiser A, *et al.*: **Association of Serum Vitamin D with the Risk of Incident Dementia and Subclinical Indices of Brain Aging: The Framingham Heart Study.** *J Alzheimers Dis.* 2016; **51**(2): 451–61.
PubMed Abstract | Publisher Full Text | Free Full Text

19. Russ TC, Murianni L, Icaza G, *et al.*: **Geographical Variation in Dementia Mortality in Italy, New Zealand, and Chile: The Impact of Latitude, Vitamin D, and Air Pollution.** *Dement Geriatr Cogn Disord.* 2016; **42**(1–2): 31–41.
PubMed Abstract | Publisher Full Text

20. Annweiler C: **Vitamin D in dementia prevention.** *Ann N Y Acad Sci.* 2016; **1367**(1): 57–63.
PubMed Abstract | Publisher Full Text

21. Joshi YB, Praticò D: **Vitamin E in aging, dementia, and Alzheimer's disease.** *BioFactors.* 2012; **38**(2): 90–7.
PubMed Abstract | Publisher Full Text

22. Traber MG, Stevens JF: **Vitamins C and E: beneficial effects from a mechanistic perspective.** *Free Radic Biol Med.* 2011; **51**(5): 1000–13.
PubMed Abstract | Publisher Full Text | Free Full Text

23. Devore EE, Feskens E, Ikram MA, *et al.*: **Total antioxidant capacity of the diet and major neurologic outcomes in older adults.** *Neurology.* 2013; **80**(10): 904–10.
PubMed Abstract | Publisher Full Text | Free Full Text

24. Presse N, Shatenstein B, Kergoat MJ, *et al.*: **Low vitamin K intakes in community-dwelling elders at an early stage of Alzheimer's disease.** *J Am Diet Assoc.* 2008; **108**(12): 2095–9.
PubMed Abstract | Publisher Full Text

25. Ferland G: **Vitamin K, an emerging nutrient in brain function.** *BioFactors.* 2012; **38**(2): 151–7.
PubMed Abstract | Publisher Full Text

26. Lancaster C, Tabet N, Rusted J: **The *APOE* paradox: do attentional control differences in mid-adulthood reflect risk of late-life cognitive decline.** *Neurobiol*

Aging. 2016; **48**: 114–21.
PubMed Abstract | Publisher Full Text

27. Segev Y, Livne A, Mints M, *et al.*: **Concurrence of High Fat Diet and APOE Gene Induces Allele Specific Metabolic and Mental Stress Changes in a Mouse Model of Alzheimer's Disease.** *Front Behav Neurosci.* 2016; **10**: 170.
PubMed Abstract | Publisher Full Text | Free Full Text

28. Carrié I, Bélanger E, Portoukalian J, *et al.*: **Lifelong low-phylloquinone intake is associated with cognitive impairments in old rats.** *J Nutr.* 2011; **141**(8): 1495–501.
PubMed Abstract | Publisher Full Text

29. Frazier HN, Maimaiti S, Anderson KL, *et al.*: **Calcium's role as nuanced modulator of cellular physiology in the brain.** *Biochem Biophys Res Commun.* 2017; **483**(4): 981–7.
PubMed Abstract | Publisher Full Text | Free Full Text

30. Overk CR, Rockenstein E, Florio J, *et al.*: **Differential calcium alterations in animal models of neurodegenerative disease: Reversal by FK506.** *Neuroscience.* 2015; **310**: 549–60.
PubMed Abstract | Publisher Full Text | Free Full Text

31. McBrayer M, Nixon RA: **Lysosome and calcium dysregulation in Alzheimer's disease: partners in crime.** *Biochem Soc Trans.* 2013; **41**(6): 1495–502.
PubMed Abstract | Publisher Full Text | Free Full Text

32. Lee JH, McBrayer MK, Wolfe DM, *et al.*: **Presenilin 1 Maintains Lysosomal Ca^{2+} Homeostasis via TRPML1 by Regulating vATPase-Mediated Lysosome Acidification.** *Cell Rep.* 2015; **12**(9): 1430–44.
PubMed Abstract | Publisher Full Text | Free Full Text

33. Supnet C, Bezprozvanny I: **Neuronal calcium signaling, mitochondrial dysfunction, and Alzheimer's disease.** *J Alzheimers Dis.* 2010; **20 Suppl 2**: S487–98.
PubMed Abstract | Publisher Full Text | Free Full Text

34. Krikorian R, Eliassen JC, Boespflug EL, *et al.*: **Improved cognitive-cerebral function in older adults with chromium supplementation.** *Nutr Neurosci.* 2010; **13**(3): 116–22.
PubMed Abstract | Publisher Full Text

35. Destefani SA, Corrente JE, Paiva SA, *et al.*: **Prevalence of iodine intake inadequacy in elderly Brazilian women. A cross-sectional study.** *J Nutr Health Aging.* 2015; **19**(2): 137–40.
PubMed Abstract | Publisher Full Text

36. Boelaert K: **Thyroid dysfunction in the elderly.** *Nat Rev Endocrinol.* 2013; **9**(4): 194–204.
PubMed Abstract | Publisher Full Text

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Version 1

Reviewer Report 06 February 2019

https://doi.org/10.5256/f1000research.15038.r44054

✗ **Charlotte E. Teunissen**
Department of Clinical Chemistry, Amsterdam University Medical Centers, Amsterdam, The Netherlands

The paper should provide a clear problem statement and literature review to provide a rationale for the study. There is no overview of the literature of previous similar studies, and the added value of the current study is thus not clear.

The method to perform a 24 food recall and just 3 days food record is likely way too short to get an impression of food intake of patients, which can vary over weeks. The use of this methodology is not sustained. And since this methodology does not seem appropriate, I judge that the conclusions cannot be drawn.

The discussion mixes concepts: data obtained in longitudinal risk and epidemiological studies in pre-dementia patients address a different subject than the current study trying to study the cross sectional food intake in established dementia patients.

Alzheimer disease should be diagnosed using state of the art criteria (e.g. including biomarkers, see Jack *et al* Neurlology 2018)

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
No

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Alzheimers disease, biomarkers

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 12 November 2018

https://doi.org/10.5256/f1000research.15038.r38377

Deborah R. Gustafson
Department of Neurology, State University of New York - Downstate Medical Center, New York City, NY, USA

This manuscript describes the dietary intake and anthropometric measures of 32 AD cases and 37 controls in southern Brazil. The hypothesis is sound regarding changes and differences in both diet and anthropometric measures in AD and related disorders. In addition, there is limited knowledge of this in Brazilian samples of elderly, an underrepresented group. Despite the strengths of the research question, the reviewer has several questions.

1. The cited prevalence of AD is confounded by understanding whether the authors refer to a clinical vs neuropathological diagnosis of AD. Since there are no brain imaging data referred to nor presented, it is assumed that the AD cases referred to and included in this study are based on clinical diagnostic criteria only.

2. DietWin (a 10 year old version) is the dietary analysis software cited in the Methods section. It is unclear as to how the nutrient intake data is estimated, and whether it is appropriate for southern Brazil. While the reviewer looked to the DietWin software website, noting its apparent Brazilian application, additional details regarding DietWin would be appreciated since the nutritional comparisons are based entirely on its output. Other details of dietary/food intake data entry and analysis are missing.

3. A 3 day dietary intake was merged with one 24h dietary recall and an average across 4 days was calculated. These are 2 very different methods of acquiring dietary intake data, such that adding a 24h recall to a 3 day dietary recall is not 'summing' the same thing.

4. The case group, AD patients, is being compared to a very healthy (& younger) control group. The control group does not appear to be a worthy comparison, yet they are comprised of 'patients'. Use of the latter term is incorrect.

5. It is stated that, 'By three-day and 24-hour dietary recall, the caregiver noted in forms all food and drink consumed'. Does this mean that caregivers of BOTH AD cases and controls provided information on dietary intake, or only for those who needed help. This is differential depending on the need of the participant and could introduce a bias. It is unclear what proportion of the sample self-reported vs had a caregiver report dietary intake. This very healthy control group would not seem to need a caregiver. Secondary reporting of dietary intake data is notoriously inaccurate. In addition, it is anticipated that the cases were more likely to have a caregiver report on their behalf.

6. It is unclear what proportion of participants required estimation of anthropometry using the equations provided based on measures not requiring standing. Differences in measurement also may introduce a bias.

7. Given the small sample size, the results and discussion are speculative at best.

8. An English language edit is required.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
No

Are the conclusions drawn adequately supported by the results?
No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Nutritional, dementia and aging epidemiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for

reasons outlined above.