



Randomized Control Trials

Probiotic and selenium co-supplementation, and the effects on clinical, metabolic and genetic status in Alzheimer's disease: A randomized, double-blind, controlled trial



Omid Reza Tamtaji^a, Reza Heidari-soureshjani^b, Naghmeh Mirhosseini^c,
Ebrahim Kouchaki^{a,d}, Fereshteh Bahmani^e, Esmat Aghadavod^e,
Maryam Tajabadi-Ebrahimi^f, Zatollah Asemi^{e,*}

^a Physiology Research Center, Kashan University of Medical Sciences, Kashan, Iran

^b Student Research Committee, Shahrekord University of Medical Sciences, Shahrekord, Iran

^c School of Public Health, University of Saskatchewan, Saskatoon, SK, Canada

^d Department of Neurology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran

^e Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, Iran

^f Faculty Member of Science Department, Science Faculty, Islamic Azad University, Tehran Central Branch, Tehran, Iran

ARTICLE INFO

Article history:

Received 12 September 2018

Accepted 30 November 2018

Keywords:

Alzheimer's disease

Cognitive function

Probiotic

Selenium

Metabolic profiles

SUMMARY

Background and aims: Combined probiotic and selenium supplementation may improve Alzheimer's disease (AD) by correcting metabolic abnormalities, and attenuating inflammation and oxidative stress. This study aimed to determine the effects of probiotic and selenium co-supplementation on cognitive function and metabolic status among patients with AD.

Methods: This randomized, double-blind, controlled clinical trial was conducted among 79 patients with AD. Patients were randomly assigned to receive either selenium (200 µg/day) plus probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Bifidobacterium longum* (2×10^9 CFU/day each) (n = 27), selenium (200 µg/day) (n = 26) or placebo (n = 26) for 12 weeks.

Results: Selenium supplementation, compared with the placebo, significantly reduced serum high sensitivity C-reactive protein (hs-CRP) ($P < 0.001$), insulin ($P = 0.001$), homeostasis model of assessment-insulin resistance (HOMA-IR) ($P = 0.002$), LDL-cholesterol ($P = 0.04$) and total-/HDL-cholesterol ratio ($P = 0.004$), and significantly increased total glutathione (GSH) ($P = 0.001$) and the quantitative insulin sensitivity check index (QUICKI) ($P = 0.01$). Compared with only selenium and placebo, probiotic and selenium co-supplementation resulted in a significant increase in mini-mental state examination score ($+1.5 \pm 1.3$ vs. $+0.5 \pm 1.2$ and -0.2 ± 1.1 , respectively, $P < 0.001$). Probiotic plus selenium intake resulted in a significant reduction in hs-CRP (-1.6 ± 1.4 vs. -0.8 ± 1.0 and $+0.1 \pm 0.5$ mg/L, respectively, $P < 0.001$), and a significant increase in total antioxidant capacity ($+89.4 \pm 129.6$ vs. $+20.0 \pm 62.5$ and -0.7 ± 27.2 mmol/L, respectively, $P = 0.001$) and GSH ($+122.8 \pm 136.5$ vs. $+102.2 \pm 135.2$ and $+1.5 \pm 53.2$ µmol/L, respectively, $P = 0.001$) compared with only selenium and placebo. In addition, subjects who received probiotic plus selenium supplements had significantly lower insulin levels (-2.1 ± 2.5 vs. -1.0 ± 1.3 and $+0.7 \pm 2.0$ µU/mL, respectively, $P < 0.001$), HOMA-IR (-0.5 ± 0.6 vs. -0.2 ± 0.3 and $+0.1 \pm 0.4$, respectively, $P < 0.001$), and higher QUICKI ($+0.01 \pm 0.01$ vs. $+0.005 \pm 0.007$ and -0.002 ± 0.01 , respectively, $P < 0.006$) compared with only selenium and placebo. Additionally, probiotic and selenium co-supplementation resulted in a significant reduction in serum triglycerides (-17.9 ± 26.1 vs. -3.5 ± 33.9 and $+0.3 \pm 9.3$ mg/dL, respectively, $P = 0.02$), VLDL- (-3.6 ± 5.2 vs. -0.7 ± 6.8 and $+0.05 \pm 1.8$ mg/dL, respectively, $P = 0.02$), LDL- (-8.8 ± 17.8 vs. -8.1 ± 19.2 and $+2.7 \pm 19.0$ mg/dL, respectively, $P = 0.04$) and total-/HDL-cholesterol (-0.3 ± 0.7 vs. -0.4 ± 0.9 and $+0.3 \pm 0.6$, respectively, $P = 0.005$) compared with only selenium and placebo.

Conclusions: Overall, we found that probiotic and selenium co-supplementation for 12 weeks to patients with AD improved cognitive function and some metabolic profiles.

* Corresponding author. Fax: +98 31 55540022.

E-mail address: asemi_r@yahoo.com (Z. Asemi).

This study was registered in the Iranian website (www.irct.ir) for registration of clinical trials (<http://www.irct.ir: IRCT20170612034497N5>).

© 2018 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

Alzheimer's disease (AD) is a progressive and fatal brain disease coupled with the reduction of cognitive ability and loss of memory [1]. It is characterized by the presence of amyloid- β and hyperphosphorylated tau-containing neurofibrillary tangles in pathological brain tissue [2]. Elevated production of reactive oxygen species (ROS) and inflammatory markers, decreased antioxidant systems, and reduced efficiency in repairing mechanisms have been associated with AD [3,4]. In addition, prior studies have reported that metabolic abnormalities such as hyperinsulinemia, hyperglycemia, decreased insulin sensitivity [5] and dyslipidemia [6] are linked to the pathogenesis and progress of AD.

Earlier, it was reported that the increased permeability of the gut and blood–brain barrier induced by microbiota dysbiosis may affect AD pathogenesis and other neurodegenerative disorders associated with aging [7]. In addition, selenium, which is an important trace element and plays a critical role in various redox and metabolic processes, has been shown to be used in the prevention of the onset and progression of AD [8]. The beneficial effects of single selenium administration on cognitive function in an animal model of AD [9] and probiotic containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* (2×10^9 CFU/g each for 12 weeks) on cognitive function and metabolic profiles in people with AD [10] were reported. Previous studies have demonstrated that joint probiotic and selenium supplementation is much more efficient in influencing metabolic profiles than single selenium or probiotic supplementation. The administration of selenium-enriched probiotics has shown a better effect on lipid profiles, antioxidant status, histopathological lesions, and the gene expression related to metabolic profiles in mice fed a high-fat diet, when compared to the ingestion of each supplement alone [11]. In another study, selenium-enriched probiotics supplementation in piglets, under high-temperature environments, significantly improved antioxidant status, immune function, and selenoprotein gene expression rather than administering sodium selenite or probiotics alone [12].

Considering the antioxidant and anti-inflammatory effects of probiotic and selenium, we hypothesized that probiotic and selenium might be useful on brain function and metabolic profiles in AD. However, data on studies investigating the impact of probiotic and selenium co-supplementation on biomarkers of inflammation and oxidative stress, glycemic control, lipid profiles, and gene expression related to metabolic status in AD are scarce. Therefore, this study also evaluates the effects of probiotic and selenium co-supplementation on metabolic profiles and gene expression related to metabolic status in AD.

2. Subjects and methods

2.1. Trial design and ethics statements

This was a 12-week randomized, double-blind, controlled clinical trial that was registered in the Iranian website for registration of clinical trials (<http://www.irct.ir: IRCT20170612034497N5>) and was conducted accordance with the Declaration of Helsinki between December 2017 and July 2018. Informed consent was

received from all patients. The research was approved by the ethics committee of Kashan University of Medical Sciences (KAUMS).

2.2. Participants

Participants were eligible for inclusion if they were 55–100 years of age diagnosis of AD at the Golabchi Welfare Organization (Kashan, Iran), and Madar, Shayestegan and Amin Welfare Organizations (Shahrekord, Iran). AD patients were diagnosed following the NINDS-ADRDA criteria [13] and revised criteria from the National Institute on Aging-Alzheimer's Association [14]. Patients with metabolic syndrome, diabetes, cardiovascular disease, chronic infections, consuming probiotic and/or synbiotic supplements within 8 weeks prior to the study, taking other forms of probiotics including probiotic yogurt, kefir and other fermented foods, and taking antioxidants supplements such as selenium and vitamin E were excluded.

2.3. Study design

Firstly, all patients were matched for BMI and age. Then, they were randomly assigned to receive either selenium (200 μ g/day) plus probiotic containing *L. acidophilus*, *B. bifidum*, and *Bifidobacterium longum* (2×10^9 CFU/day each) ($n = 27$), selenium (200 μ g/day) ($n = 26$) or placebo ($n = 26$) for 12 weeks. Probiotic and selenium and the placebo (starch) were produced by Tak Gen Zist Company (Tehran, Iran), Webber Naturals Pharmaceutical Company (Coquitlam, Canada) and Tak Gen Zist Company (Tehran, Iran), respectively. They were completely identical in terms of their appearance, color, shape, size, smell and taste and packaging. Random assignment was conducted using computer-generated numbers. The randomized allocation sequence, enrolling participants and allocating them into intervention groups were conducted by a trained staff at the clinic. Since the study subjects were institutionalized because of the nature of their disease, compliance was assessed through the checklist filled by a trained staff who was responsible to give the patients their supplements every day. Dietary intakes (3-day food records) of all participants were completed at baseline, weeks 4, 8 and 12 of the intervention by a trained researcher. Daily macro- and micro-nutrient intakes were analyzed by nutritionist IV software (First Databank, San Bruno, CA).

2.4. Assessment of anthropometric measures

Weight and height of participants were determined using a standard scale (Seca, Hamburg, Germany) at the beginning of the study and after 12 weeks' treatment. BMI was calculated as weight in kg divided by height in meters squared.

2.5. Assessment of outcomes

The primary outcome measurement was Mini-Mental State Examination (MMSE) and the secondary outcome measurements were biomarkers of inflammation and oxidative stress, and metabolic profiles. The MMSE was used to assess cognition in AD subjects.

2.6. Assessment of biochemical parameters

Twelve-hour fasting blood samples were taken by venipuncture at weeks 0 and 12 of the intervention at Kashan reference laboratory. Serum high sensitivity C-reactive protein (hs-CRP) levels were quantified using a commercial ELISA kit (LDN, Nordhorn, Germany) with the intra- and inter-assay coefficient variances (CVs) lower than 6.5%. Plasma nitric oxide (NO) were quantified by the Griess method [15]. Plasma total antioxidant capacity (TAC) using the method of ferric reducing antioxidant power method developed by Benzie and Strain [16], total glutathione (GSH) by the method of Beutler et al. [17] and malondialdehyde (MDA) levels using the thiobarbituric acid reactive substance method [18] with inter- and intra-assay CVs lower than 5% were evaluated. To determine fasting plasma glucose (FPG) and lipid profiles, we used available kits (Pars Azmun, Tehran, Iran) with inter- and intra-assay CVs lower than 5%. Circulating levels of insulin were determined using an ELISA kit (Monobind, California, USA) with the intra- and inter-assay CVs 3.3 and 4.8%, respectively. The homeostatic model of assessment for insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were calculated according to suggested formulas.

2.7. Isolation of lymphocyte cells

Lymphocyte cells were isolated from blood using 50% Percoll (Sigma–Aldrich, Dorset, UK). Cell count and viability test were conducted using trypan blue [19].

2.8. RNA extraction and real-time PCR (RT-PCR)

RNX-plus kit (Cinnacolon, Tehran, Iran) was used to extract RNA from blood samples. RNA suspension was frozen of -20°C until cDNA was derived. Following the extraction of total RNAs from each sample, RNA quantification was performed using UV spectrophotometer. Each sample OD 260/280 ratio was considered to be between 1.7 and 2.1, demonstrating no contamination with either protein or DNA [19]. The isolated RNA was reverse transcribed to cDNA library, using moloney murine leukemia virus reverse transcriptase (RT). Gene expressions of tumor necrosis factor alpha (TNF- α), interleukin-8 (IL-8), transforming growth factor beta (TGF- β), peroxisome proliferator-activated receptor gamma (PPAR- γ) and low-density lipoprotein receptor (LDLR) were assessed by quantitative RT-PCR in peripheral blood mononuclear cells (PBMCs), using the LightCycler technology (Roche Diagnostics, Rotkreuz, Switzerland) with SYBR green detection and Amplicon Kit (Table 1).

Table 1
Specific primers used for real-time quantitative PCR.

Gene	Primer	Product size (bp)	Annealing temperature (C)
GAPDH	F: AAGCTCAITTTCTGGTATGACAACG	126	61.3
	R: TCTTCTCTTGTGCTCTGTGCTGG		
TNF- α	F: GTCAACCTCCTCTCTGCCAT	188	52
	R: CCAAAGTAGACCTGCCAGA		
IL-8	F: GCAGAGGGTGTGGAGAAAGT	150	56
	R: ACCCTACAACAGACCCACAC		
TGF- β	F: TTGAGACTTTCCGTTGCCG	227	56
	R: CGAGGTCTCGGGAAAAGTCT		
PPAR- γ	F: ATGACAGACCTCAGACAGATTG	210	54
	R: AATGTTGGCAGTGGCTCAG		
LDLR	F: ACTTACGGACAGACAGACAG	223	57
	R: GGCCACACATCCCATGATTC		

GAPDH, glyceraldehyde-3-Phosphate dehydrogenase; IL-8, interleukin-8; LDLR, low-density lipoprotein receptor; PPAR- γ , peroxisome proliferator-activated receptor gamma; TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor beta.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were used as a housekeeping gene. Primer Express Software (Applied Biosystems, Foster City, USA) and Beacon designer software (Takapost, Tehran, Iran) were used to design primers. Relative transcription levels were calculated using the method of Pffafi.

2.9. Sample size

To calculate the sample size, we used the standard formula suggested for parallel clinical trials by considering type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%). Based on a previous study [10], we used a standard deviation (SD) of 2.80 and a difference in mean (d) of 2.25, considering MMSE as the key variable. Based on this, we needed 25 patients in each group. Assuming 20% dropouts in each group, the final sample size was determined to be 30 patients per group.

2.10. Statistical methods

Anthropometric measures, macro- and micro-nutrient dietary intakes, cognitive function, metabolic profiles and gene expression related to inflammation, insulin and lipid were compared among the three groups, using ANOVA test with Bonferoni post hoc pairwise comparisons. The normality of model residual was tested using the Kolmogorov–Smirnov test. The P-value of <0.05 were considered statistically significant. All statistical analyses used the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

3. Results

Four participants in selenium group, 3 participants in probiotic plus selenium group, and 4 participants in placebo group withdraw from the trial, due to personal reasons, and finally 79 participants [probiotic plus selenium (n = 27), selenium (n = 26) and placebo (n = 26)] completed the study (Fig. 1). On average, the rate of compliance in our study was high, such that 100% of supplements and placebos were taken throughout the study in three groups.

Mean age, height, weight and BMI at baseline and end-of-trial were not statistically different among the three groups (Table 2).

Based on the 3-day dietary records obtained at study baseline, end-of-trial and throughout the trial, we found no significant differences in mean dietary macro- and micro-nutrient intakes among the three groups (Data not shown).

3.1. Cognitive assessment

Compared with only selenium and placebo, probiotic and selenium co-supplementation resulted in a significant improvement in MMSE score ($+1.5 \pm 1.3$ vs. $+0.5 \pm 1.2$ and -0.2 ± 1.1 , respectively, $P < 0.001$) (Table 3).

3.2. Biochemical measurements

Selenium supplementation, compared with the placebo, significantly reduced serum hs-CRP ($P < 0.001$), insulin ($P = 0.001$), HOMA-IR ($P = 0.002$), LDL-cholesterol ($P = 0.04$) and total-/HDL-cholesterol ratio ($P = 0.004$), and significantly increased GSH ($P = 0.001$) and QUICKI ($P = 0.01$) (Table 3). Probiotic plus selenium intake resulted in a significant reduction in serum hs-CRP (-1.6 ± 1.4 vs. -0.8 ± 1.0 and $+0.1 \pm 0.5$ mg/L, respectively, $P < 0.001$), and a significant increase in TAC ($+89.4 \pm 129.6$ vs. $+20.0 \pm 62.5$ and -0.7 ± 27.2 mmol/L, respectively, $P = 0.001$) and GSH levels ($+122.8 \pm 136.5$ vs. $+102.2 \pm 135.2$

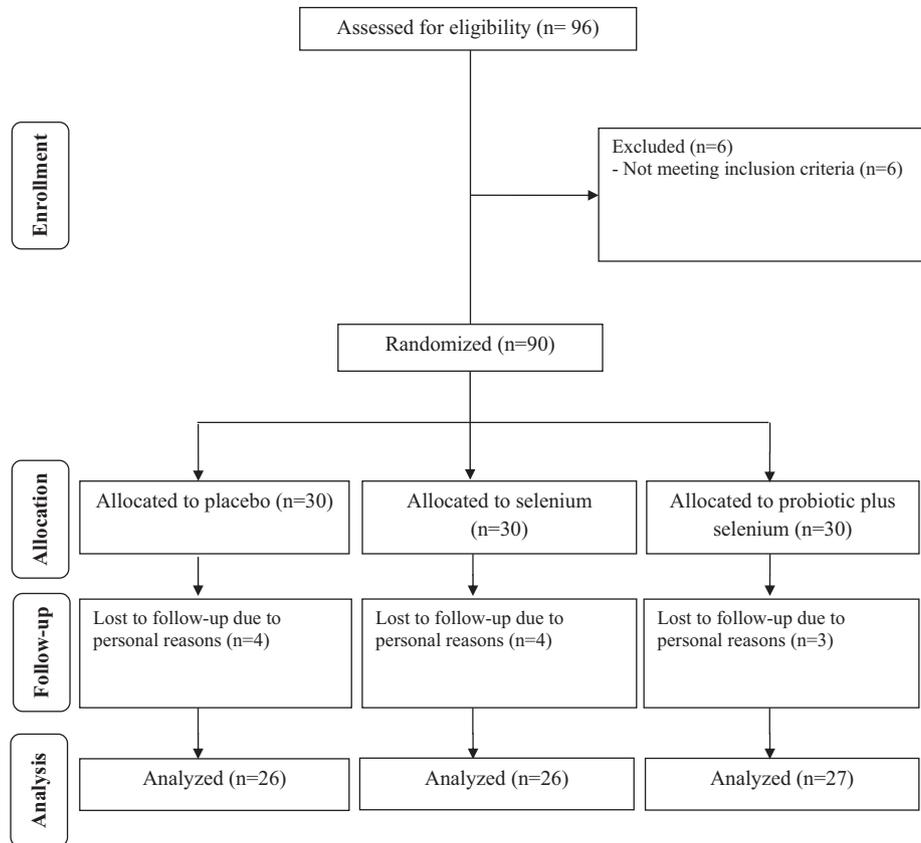


Fig. 1. Summary of patient flow.

Table 2
General characteristics of study participants.^a

	Placebo group (n = 26)	Selenium group (n = 26)	Probiotic plus selenium group (n = 27)	P ^b
Age (y)	78.5 ± 8.0	78.8 ± 10.2	76.2 ± 8.1	0.51
Height (cm)	158.9 ± 10.3	159.5 ± 5.6	159.5 ± 5.9	0.94
Weight at study baseline (kg)	54.3 ± 7.5	54.0 ± 5.4	52.9 ± 9.8	0.79
Weight at end-of-trial (kg)	54.1 ± 7.6	55.7 ± 5.5	52.8 ± 9.7	0.81
Weight change (kg)	-0.2 ± 1.2	-0.3 ± 0.5	-0.1 ± 0.5	0.68
BMI at study baseline (kg/m ²)	21.5 ± 2.4	21.2 ± 1.2	20.7 ± 3.2	0.49
BMI at end-of-trial (kg/m ²)	21.5 ± 2.5	21.1 ± 1.3	20.7 ± 3.2	0.51
BMI change (kg/m ²)	-0.1 ± 0.5	-0.1 ± 0.2	-0.1 ± 0.2	0.67

^a Data are means ± SDs.

^b Obtained from ANOVA test.

and $+1.5 \pm 53.2$ $\mu\text{mol/L}$, respectively, $P = 0.001$) compared with only selenium and placebo. In addition, subjects who received probiotic plus selenium supplements had significantly lower serum insulin levels (-2.1 ± 2.5 vs. -1.0 ± 1.3 and $+0.7 \pm 2.0$ $\mu\text{IU/mL}$, respectively, $P < 0.001$), HOMA-IR (-0.5 ± 0.6 vs. -0.2 ± 0.3 and $+0.1 \pm 0.4$, respectively, $P < 0.001$), and higher QUICKI ($+0.01 \pm 0.01$ vs. $+0.005 \pm 0.007$ and -0.002 ± 0.01 , respectively, $P < 0.006$) compared with only selenium and placebo. Additionally, probiotic and selenium co-supplementation resulted in a significant reduction in serum triglycerides (-17.9 ± 26.1 vs. -3.5 ± 33.9 and $+0.3 \pm 9.3$ mg/dL , respectively, $P = 0.02$), VLDL- (-3.6 ± 5.2 vs. -0.7 ± 6.8 and $+0.05 \pm 1.8$ mg/dL , respectively, $P = 0.02$), LDL- (-8.8 ± 17.8 vs. -8.1 ± 19.2 and $+2.7 \pm 19.0$ mg/dL , respectively, $P = 0.04$) and total-/HDL-cholesterol (-0.3 ± 0.7 vs. -0.4 ± 0.9 and $+0.3 \pm 0.6$, respectively, $P = 0.005$) compared with only selenium and placebo. Probiotic and selenium co-supplementation significantly increased MMSE score ($P = 0.007$) and QUICKI

($P = 0.03$), and significantly reduced hs-CRP ($P = 0.01$), FPG ($P = 0.04$), insulin ($P = 0.04$) and HOMA-IR ($P = 0.02$) compared with only selenium.

3.3. Gene expression related to inflammation, insulin and lipid metabolism

Probiotic and selenium co-supplementation significantly downregulated gene expression of TNF- α ($P = 0.005$) in PBMCs of patients with AD; however, it did not affect gene expression of IL-8 and TGF- β (Fig. 2).

RT-PCR quantitative results showed a significant upregulation of gene expression of PPAR- γ ($P = 0.002$) and LDLR ($P = 0.003$) in PBMCs of patients with AD following probiotic and selenium co-supplementation compared with only selenium and placebo (Fig. 3). In addition, selenium supplementation significantly increased gene expression of LDLR compared with the placebo.

Table 3

Cognitive function, biomarkers of inflammation and oxidative stress, and metabolic profiles at baseline and after the 12-week intervention in patients with Alzheimer's disease that received either probiotic plus selenium, selenium supplements or placebo.

	Placebo group (n = 26)			Selenium group (n = 26)			Probiotic plus selenium group (n = 27)			P ^c
	Wk0	Wk12	Change	Wk0	Wk12	Change	Wk0	Wk12	Change	
MMSE	9.3 ± 4.1	9.1 ± 4.4	-0.2 ± 1.1	9.9 ± 4.0	10.4 ± 4.2	0.5 ± 1.2	9.4 ± 3.5	10.9 ± 3.8	1.5 ± 1.3 ^{b,a}	<0.001
hs-CRP (mg/L)	4.8 ± 1.3	4.9 ± 1.3	0.1 ± 0.5	4.9 ± 1.9	4.1 ± 1.6	-0.8 ± 1.0 ^a	5.5 ± 1.5	3.9 ± 0.9	-1.6 ± 1.4 ^{b,a}	<0.001
NO (μmol/L)	35.3 ± 3.7	35.3 ± 3.8	-0.03 ± 1.8	33.2 ± 3.8	33.8 ± 5.0	0.6 ± 5.6	33.5 ± 5.2	34.1 ± 4.8	0.6 ± 4.3	0.81
TAC (mmol/L)	834.3 ± 92.0	833.6 ± 92.8	-0.7 ± 27.2	821.2 ± 63.9	841.2 ± 62.2	20.0 ± 62.5	815.0 ± 77.4	904.4 ± 109.2	89.4 ± 129.6 ^{b,a}	0.001
GSH (μmol/L)	403.3 ± 89.5	404.8 ± 105.0	1.5 ± 53.2	444.1 ± 80.3	546.3 ± 99.3	102.2 ± 135.2 ^a	458.9 ± 96.9	581.8 ± 96.7	122.8 ± 136.5 ^{b,a}	0.001
MDA (μmol/L)	2.7 ± 0.4	2.7 ± 0.4	0.002 ± 0.2	2.8 ± 0.2	2.8 ± 0.3	0.009 ± 0.3	2.7 ± 0.3	2.6 ± 0.2	-0.1 ± 0.4	0.10
FPG (mg/dL)	88.7 ± 9.8	89.7 ± 9.5	1.1 ± 10.8	86.3 ± 13.7	87.9 ± 6.6	1.5 ± 10.0	91.7 ± 4.3	87.6 ± 8.6	-4.1 ± 9.5	0.08
Insulin (μU/mL)	11.4 ± 2.1	12.1 ± 2.1	0.7 ± 2.0	11.3 ± 2.2	10.3 ± 1.9	-1.0 ± 1.3 ^a	11.7 ± 1.3	9.7 ± 2.5	-2.1 ± 2.5 ^a	<0.001
HOMA-IR	2.5 ± 0.6	2.6 ± 0.5	0.1 ± 0.4	2.4 ± 0.5	2.2 ± 0.6	-0.2 ± 0.3 ^a	2.7 ± 0.4	2.2 ± 0.6	-0.5 ± 0.6 ^a	<0.001
QUICKI	0.33 ± 0.01	0.33 ± 0.01	-0.002 ± 0.01	0.33 ± 0.01	0.34 ± 0.01	0.005 ± 0.007	0.33 ± 0.007	0.34 ± 0.01	0.01 ± 0.01	<0.001
Triglycerides (mg/dL)	116.9 ± 38.3	117.2 ± 36.2	0.3 ± 9.3	112.4 ± 35.5	108.9 ± 26.3	-3.5 ± 33.9	126.9 ± 30.1	108.9 ± 23.3	-17.9 ± 26.1 ^a	0.02
VLDL-cholesterol (mg/dL)	23.4 ± 7.7	23.4 ± 7.2	0.05 ± 1.8	22.5 ± 7.1	21.8 ± 5.3	-0.7 ± 6.8	25.4 ± 6.0	21.8 ± 4.7	-2.6 ± 5.2 ^a	0.02
Total cholesterol (mg/dL)	185.4 ± 33.8	186.2 ± 34.1	0.8 ± 20.6	184.9 ± 37.9	177.0 ± 34.7	-7.9 ± 24.5	203.9 ± 34.2	192.0 ± 30.6	-11.9 ± 18.9	0.09
LDL-cholesterol (mg/dL)	114.1 ± 34.5	116.8 ± 34.4	2.7 ± 19.0	117.9 ± 34.9	109.7 ± 32.9	-8.1 ± 19.2 ^a	129.8 ± 31.1	121.0 ± 27.9	-8.8 ± 17.9 ^a	0.04
HDL-cholesterol (mg/dL)	47.9 ± 7.8	45.9 ± 8.9	-2.0 ± 4.3	44.5 ± 10.5	45.5 ± 8.7	1.0 ± 6.9	48.7 ± 4.5	49.2 ± 5.4	0.5 ± 5.9	0.15
Total-/HDL-cholesterol	3.9 ± 0.9	4.2 ± 1.2	0.3 ± 0.6	4.3 ± 1.2	3.9 ± 0.8	-0.4 ± 0.9 ^a	4.2 ± 0.9	3.9 ± 0.8	-0.3 ± 0.7 ^a	0.005

Data are mean ± SDs.

FPG, fasting plasma glucose; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-insulin resistance; HDL-cholesterol, high density lipoprotein-cholesterol; Hs-CRP, high sensitivity C-reactive protein; LDL-cholesterol, low density lipoprotein-cholesterol; MMSE, mini-mental state examination; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; VLDL-cholesterol, very low density lipoprotein-cholesterol; SGA, subjective global assessment; TAC, total antioxidant capacity.

^a Significant different from the placebo group.

^b Significant different from the selenium group.

^c Obtained from ANOVA test.

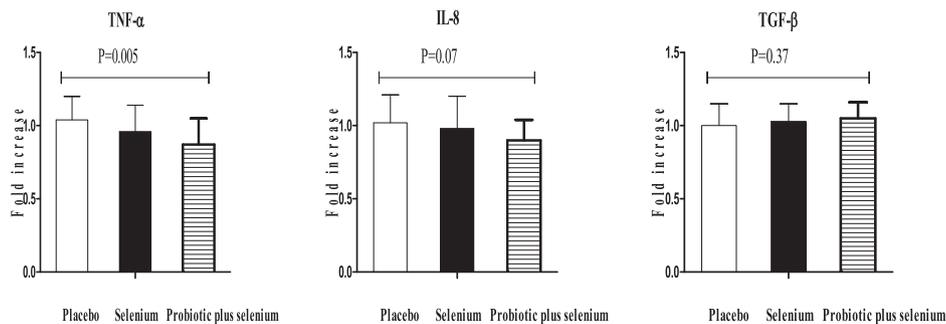


Fig. 2. Effect of the 12-week supplementation with probiotic plus selenium, selenium or placebo on expression ratio of TNF- α , IL-8 and TGF- β gene in PBMCs of patients with AD.

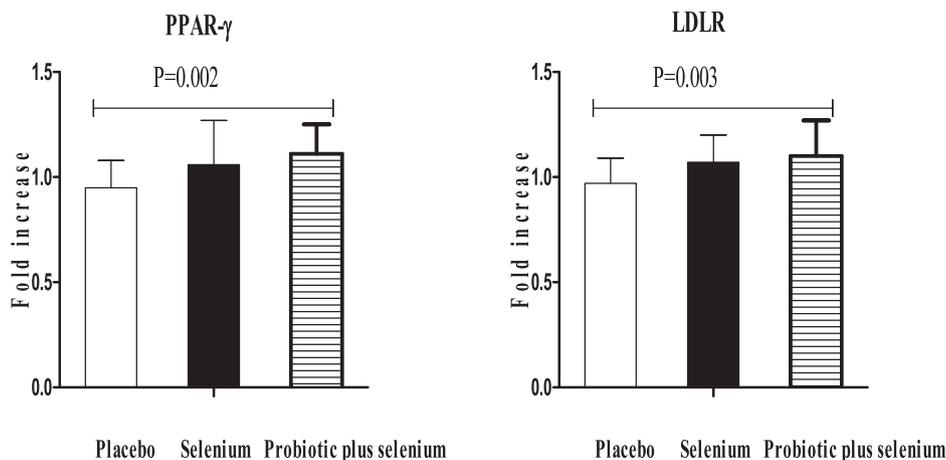


Fig. 3. Effect of the 12-week supplementation with probiotic plus selenium, selenium or placebo on expression ratio of PPAR- γ and LDLR gene in PBMCs of patients with AD. AD, Alzheimer's disease; IL-8, interleukin-8; LDLR, low-density lipoprotein receptor; PBMCs, peripheral blood mononuclear cells; PPAR- γ , peroxisome proliferator-activated receptor gamma; TNF- α , tumor necrosis factor alpha; TGF- β , transforming growth factor beta.

4. Discussion

The current study demonstrated that probiotic and selenium co-supplementation for 12 weeks to patients with AD had favorable effects on MMSE score, hs-CRP, TAC, GSH, markers of insulin metabolism, triglycerides, VLDL-, LDL-, total-/HDL-cholesterol, but it did not affect other biomarkers of inflammation and oxidative stress, FPG and other lipid profiles. In addition, co-supplementation significantly reduced gene expression of TNF- α , and significantly increased gene expression of PPAR- γ and LDLR, but did not affect gene expression of IL-8 and TGF- β . To the best of our knowledge, this study is the first evaluating the beneficial effects of probiotic and selenium co-supplementation on cognitive function, biomarkers of inflammation and oxidative stress, metabolic status and gene expression related to inflammation, insulin and lipid in patients with AD.

4.1. Effects on cognitive function

Patients with AD are susceptible to multiple complications such as increased inflammatory markers and oxidative damage, mortality [20], microvascular disease, dyslipidemia and insulin resistance [21]. Our results indicated that probiotic and selenium co-supplementation for 12 weeks to patients with AD improved MMSE score. Data on the effects of probiotic and selenium supplementation on brain behavioral phenomena are limited. We have previously shown that taking probiotic for 12 weeks by patients with AD was associated with improved cognitive function [10]. In another study, multispecies probiotic intake for 4 weeks among patients with AD significantly improved gut bacteria composition and circulating levels of tryptophan [22]. In addition, selenium-methylselenocysteine significantly ameliorated cognitive deficits by attenuating oxidative stress and metal dyshomeostasis in a murine model of AD [23]. Long-term supplementation with selenium-enriched yeast significantly improved cognitive impairment, and mitigates tau pathology in a triple transgenic mouse model of AD [9]. Probiotics may change gut microbiota and subsequently can improve cognitive function through contributing in the expression of receptors producing neurotransmitters and neuromodulators. Through inducing gut–brain axis, probiotics also may affect neurotransmitter synthesis including gamma-aminobutyric acid, norepinephrine, serotonin, dopamine and acetylcholine [24]. Selenium intake may improve normal cellular function by reduced oxidants and increased antioxidants levels in the brain [25].

4.2. Effects on biomarkers of inflammation and oxidative stress

Findings of the current study shown that probiotic and selenium co-supplementation for 12 weeks to patients with AD was associated with a significant decrease in hs-CRP and a significant increase in TAC and GSH, but did not affect NO and MDA levels. In addition, co-supplementation reduced gene expression of TNF- α , but did not affect gene expression of IL-8 and TGF- β . In a study conducted by Liu et al. [26], selenium-glutathione-enriched probiotics was effective in attenuating liver fibrosis by activating silent information regulator 1 signaling and attenuating hepatic oxidative stress, inflammation and mitogen-activated protein kinases)MAPK(signaling. In addition, selenium-enriched probiotics could protect the liver from fibrosis by attenuating hepatic oxidative stress, and inhibiting hepatic inflammation as well as inducing apoptosis of hepatic stellate cells [27]. Earlier, the beneficial effects of probiotic and selenium supplementation on some biomarkers of inflammation and oxidative stress in patients with AD was reported [10]. Probiotic intake may reduce inflammation and oxidative damage through their metal ion chelating ability, antioxidant systems such

as enhancing activity of superoxide dismutase and catalase, regulating signaling pathways including MAPKs and nuclear factor-kappaB, and decreased levels ROS-scavenging proteins [28]. Furthermore, selenium may attenuate inflammation and oxidative stress by the enhancing of glutathione peroxidase activity, reducing nuclear factor-kappaB activation, altering the metabolism of arachidonic acid and inhibiting MAP kinase pathways [29].

4.3. Effects on glycemic control and lipid profiles

The current study documented that taking probiotic plus selenium supplements for 12 weeks by patients with AD resulted in a significant improvement in markers of insulin metabolism, triglycerides, VLDL-, LDL- and total-/HDL-cholesterol, but it did not influence FPG, total- and HDL-cholesterol levels. In addition, co-supplementation increased gene expression of PPAR- γ and LDLR. In a meta-analysis conducted by Kasinska et al. [30], a significant effect of probiotics on decreasing HbA1c levels and HOMA-IR in patients with type 2 diabetes mellitus was seen, but did not have a significant effect on FPG, insulin, and lipid profiles. In addition, we have previously reported that selenium supplementation to patients with metabolic diseases did not influence lipid profiles [31]. The inconclusive results of different studies might be correlated to their methodology like doses, administering combined versus individual nutrients, duration of intervention and other possible confounding factors. Insulin-mimetic and antilipidemic effects of probiotic supplement may be mediated by reducing oxidative stress and pro-inflammatory markers [32], increasing β -oxidation of long-chain fatty acids in liver and muscle tissues [33]. Selenium intake may improve markers of insulin and lipid metabolism through inhibiting the expression of cyclooxygenase-2 and P-selectin, and increased gene expression of few enzymes including very long chain dehydrogenase and medium chain Acyl-CoA dehydrogenase [34].

The current study had some limitations. We did not assess the compliance to probiotic and selenium intake through quantifying fecal bacteria loads and plasma selenium levels. Due to limited funding, we did not examine the effects of probiotic and selenium co-supplementation on gene expression related to oxidative stress. In the current study, we have evaluated the effects of selenium alone, and selenium plus probiotic on clinical, metabolic and genetic status in patients with AD. A fourth group who was receiving probiotic alone would be ideal to give a full 2 \times 2 factorial design, however due to the limitations of this study, we focused on evaluating the synergistic effects of selenium and probiotic on clinical, metabolic and genetic status in patients with AD.

4.4. Conclusions

Overall, the current study demonstrated that probiotic and selenium co-supplementation for 12 weeks to patients with AD had favorable effects on MMSE score, hs-CRP, TAC, GSH, markers of insulin metabolism, triglycerides, VLDL-, LDL-, total-/HDL-cholesterol, but it did not affect other biomarkers of inflammation and oxidative stress, FPG and other lipid profiles. In addition, co-supplementation improved gene expression of TNF- α , PPAR- γ and LDLR, but did not affect gene expression of IL-8 and TGF- β .

Conflicts of interest

No conflicts are declared.

Author contributions

Z Asemi designed the research project. OR Tamtaji and R Heidari-soureshjani had principal role in performing the protocols.

E Kouchaki visited all patients for the MMSE tests. Z Asemi, E Aghadavod and F Bahmani performed measurement of metabolic biomarkers. Z Asemi and N Mirhosseini analyzed the data.

Acknowledgements

The present study was supported by a grant to Z Asemi from the Deputy of Research of Kashan University of Medical Sciences (KAUMS). Special thanks to Tak Gen Zist Company of for providing probiotics.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clnu.2018.11.034>.

References

- [1] Lewczuk P, Mroczko B, Fagan A, Kornhuber J. Biomarkers of Alzheimer's disease and mild cognitive impairment: a current perspective. *Adv Med Sci* 2015;60:76–82.
- [2] Spiess-Jones TL, Hyman BT. The intersection of amyloid beta and tau at synapses in Alzheimer's disease. *Neuron* 2014;82:756–71.
- [3] Barbagallo M, Marotta F, Dominguez LJ. Oxidative stress in patients with Alzheimer's disease: effect of extracts of fermented papaya powder. *Mediat Inflamm* 2015;2015:624801.
- [4] Gubandru M, Margina D, Tsitsimpikou C, Goutzourelas N, Tsarouhas K, Ilie M, et al. Alzheimer's disease treated patients showed different patterns for oxidative stress and inflammation markers. *Food Chem Toxicol* 2013;61:209–14.
- [5] Arrieta-Cruz I, Gutierrez-Juarez R. The role of insulin resistance and glucose metabolism dysregulation in the development of Alzheimer's disease. *Rev Invest Clin* 2016;68:53–8.
- [6] Presecki P, Muck-Seler D, Mimica N, Pivac N, Mustapic M, Stipcevic T, et al. Serum lipid levels in patients with Alzheimer's disease. *Coll Antropol* 2011;35(Suppl 1):115–20.
- [7] Jiang C, Li G, Huang P, Liu Z, Zhao B. The gut microbiota and Alzheimer's disease. *J Alzheimers Dis* 2017;58:1–15.
- [8] Hatfield DL, Tsuji PA, Carlson BA, Gladyshev VN. Selenium and selenocysteine: roles in cancer, health, and development. *Trends Biochem Sci* 2014;39:112–20.
- [9] Zhang ZH, Wen L, Wu QY, Chen C, Zheng R, Liu Q, et al. Long-Term dietary supplementation with selenium-enriched yeast improves cognitive impairment, reverses synaptic deficits, and mitigates tau pathology in a triple transgenic mouse model of Alzheimer's disease. *J Agric Food Chem* 2017;65:4970–9.
- [10] Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, Tamtaji OR, et al. Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial. *Front Aging Neurosci* 2016;8:256.
- [11] Nido SA, Shituleni SA, Mengistu BM, Liu Y, Khan AZ, Gan F, et al. Effects of selenium-enriched probiotics on lipid metabolism, antioxidative status, histopathological lesions, and related gene expression in mice fed a high-fat diet. *Biol Trace Elem Res* 2016;171:399–409.
- [12] Gan F, Chen X, Liao SF, Lv C, Ren F, Ye G, et al. Selenium-enriched probiotics improve antioxidant status, immune function, and selenoprotein gene expression of piglets raised under high ambient temperature. *J Agric Food Chem* 2014;62:4502–8.
- [13] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on alzheimer's disease. *Neurology* 1984;34:939–44.
- [14] Jack Jr CR, Albert MS, Knopman DS, McKhann GM, Sperling RA, Carrillo MC, et al. Introduction to the recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 2011;7:257–62.
- [15] Tatsch E, Bochi GV, Pereira Rda S, Kober H, Agertt VA, de Campos MM, et al. A simple and inexpensive automated technique for measurement of serum nitrite/nitrate. *Clin Biochem* 2011;44:348–50.
- [16] Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 1996;239:70–6.
- [17] Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. *J Lab Clin Med* 1985;105:581–4.
- [18] Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med* 1990;9:515–40.
- [19] Dunkley PR, Jarvie PE, Robinson PJ. A rapid Percoll gradient procedure for preparation of synaptosomes. *Nat Protoc* 2008;3:1718–28.
- [20] Schelke MW, Hackett K, Chen JL, Shih C, Shum J, Montgomery ME, et al. Nutritional interventions for Alzheimer's prevention: a clinical precision medicine approach. *Ann N Y Acad Sci* 2016;1367:50–6.
- [21] Sridhar GR, Lakshmi G, Nagamani G. Emerging links between type 2 diabetes and Alzheimer's disease. *World J Diabetes* 2015;6:744–51.
- [22] Leblhuber F, Steiner K, Schuetz B, Fuchs D, Gostner JM. Probiotic supplementation in patients with Alzheimer's dementia -an explorative intervention study. *Curr Alzheimer Res* 2018;15:1106–13.
- [23] Xie Y, Liu Q, Zheng L, Wang B, Qu X, Ni J, et al. Se-methylselenocysteine ameliorates neuropathology and cognitive deficits by attenuating oxidative stress and metal dyshomeostasis in Alzheimer model mice. *Mol Nutr Food Res* 2018;62:e1800107. <https://doi.org/10.1002/mnfr.201800107>.
- [24] Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* 2012;13:701–12.
- [25] Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* 2009;7:65–74.
- [26] Liu Y, Liu Q, Hesketh J, Huang D, Gan F, Hao S, et al. Protective effects of selenium-glutathione-enriched probiotics on CCl4-induced liver fibrosis. *J Nutr Biochem* 2018;58:138–49.
- [27] Liu Y, Liu Q, Ye G, Khan A, Liu J, Gan F, et al. Protective effects of Selenium-enriched probiotics on carbon tetrachloride-induced liver fibrosis in rats. *J Agric Food Chem* 2015;63:242–9.
- [28] Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant properties of probiotic bacteria. *Nutrients* 2017;9.
- [29] Duntas LH. Selenium and inflammation: underlying anti-inflammatory mechanisms. *Horm Metab Res* 2009;41:443–7.
- [30] Kasinska MA, Drzewoski J. Effectiveness of probiotics in type 2 diabetes: a meta-analysis. *Pol Arch Med Wewn* 2015;125:803–13.
- [31] Tabrizi R, Akbari M, Moosazadeh M, Lankarani KB, Heydari ST, Kolahdooz F, et al. The effects of selenium supplementation on glucose metabolism and lipid profiles among patients with metabolic diseases: a systematic review and meta-analysis of randomized controlled trials. *Horm Metab Res* 2017;49:826–30.
- [32] Hsieh FC, Lee CL, Chai CY, Chen WT, Lu YC, Wu CS. Oral administration of *Lactobacillus reuteri* GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. *Nutr Metab (Lond)*. 2013;10:35.
- [33] Huang Z, Mu C, Chen Y, Zhu Z, Chen C, Lan L, et al. Effects of dietary probiotic supplementation on LXRalpha and CYP7alpha1 gene expression, liver enzyme activities and fat metabolism in ducks. *Br Poult Sci* 2015;56:218–24.
- [34] Kim JE, Choi SI, Lee HR, Hwang IS, Lee YJ, An BS, et al. Selenium significantly inhibits adipocyte hypertrophy and abdominal fat accumulation in OLETF rats via induction of fatty acid beta-oxidation. *Biol Trace Elem Res* 2012;150:360–70.