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Original Article

The Effects of Probiotic Supplementation on Gene Expression Related to Inflammation, Insulin and Lipid in Patients with Parkinson's Disease: A Randomized, Double-blind, Placebo-Controlled Trial

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Abstract

Background: This study was conducted to evaluate the effects of probiotic supplementation on gene expression related to inflammation, insulin and lipid in patients with Parkinson's disease (PD).

Methods: This randomized, double-blind, placebo-controlled clinical trial was conducted in 50 patients with PD as a pilot study. Participants were randomly allocated into two groups to take either 8×10^9 CFU/day probiotic supplements or placebo (n = 25 each group, one capsule daily) for 12 weeks. Gene expression related to inflammation, insulin, and lipid was quantified in peripheral blood mononuclear cells (PBMC) of PD patients, with RT-PCR method.

Results: After the 12-week intervention, compared with the placebo, probiotic intake downregulated gene expression of interleukin-1 (IL-1) ($P = 0.03$), IL-8 ($P < 0.001$) and tumor necrosis factor alpha (TNF-α) (P=0.04) in PBMC of subjects with PD. In addition, probiotic supplementation upregulated transforming growth factor beta (TGF-β) ($P = 0.02$) and peroxisome proliferator-activated receptor gamma (PPAR-γ) ($P = 0.03$) in PBMC of subjects with PD compared with the placebo. We did not observe any significant effect of probiotic intake on gene expression of low-density lipoprotein receptor (LDLR) and vascular endothelial growth factor (VEGF) in PBMC of patients with PD.

Conclusion: Overall, probiotics supplementation for 12 weeks in PD patients significantly improved gene expression of IL-1, IL-8, TNF-α, TGF-β and PPAR-γ, but did not affect gene expression of VEGF and LDLR, and biomarkers of inflammation and oxidative stress.

Keywords: Inflammation, insulin metabolism, Parkinson's disease, probiotics supplementation

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Introduction

Parkinson's disease (PD) is a disabling pathology that has a usually asymmetric onset, and is characterized by both motor and non-motor symptoms which affects millions of people worldwide.[1] Experimental models of PD proposed that the loss of dopaminergic neurons is extremely due to increased inflammatory cytokines especially tumor necrosis factor alpha (TNF-α).[2-4] Furthermore, the modulation of peroxisome proliferator-activated receptor gamma (PPAR-γ) activity results in neuroprotective impacts on biomarkers of oxidative stress, apoptosis, and neuroinflammation in PD.[5] On the other hand, dyslipidemia and obesity are well-established risk factors for cognitive disturbances and dementia in older adults.[6]

Epidemiological and experimental studies in human and animal models support a general schema which implicates the gut microbiota through the microbiome-gut-brain axis in the pathogenesis of common neurodegenerative diseases, including PD and Alzheimer's disease.[7,8] In addition, depletion or modulation of the gut microbiota can influence the severity of the central pathology or behavioral deficits observed in brain disorders.[9] Some studies have previously reported the beneficial effects of probiotics on metabolic and genetic diseases related to neurodegenerative disorders. Previously, we showed that 12 weeks of probiotic administration in people with multiple sclerosis (MS) significantly decreased interleukin 8 (IL-8) and TNF-α gene expressions, but did not affect expression of genes involved in insulin and

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lipid metabolism.[10] Furthermore, Steed et al[11] revealed that 6-month synbiotic supplementation in patients with active Crohn's disease led to a significant reduction in TNF-α gene expression. In another study, Hsieh et al[12] observed that reducing levels of PPAR-γ gene expression after high fructose treatment, were significantly elevated by *Lactobacillus reuteri* supplementation in animal models.

This evidence suggests the importance of probiotic supplementation on biomarkers of inflammation and oxidative stress, and gene expression related to inflammation, insulin, and lipid in patients with PD. To the best of our knowledge, data on the effects of probiotic supplementation on biomarkers of inflammation and oxidative stress, and gene expression related to inflammation, insulin and lipid in patients with PD are limited and controversial. The aim of the current survey was to evaluate the effects of probiotic supplementation on inflammation and oxidative stress biomarkers, and gene expression related to inflammation, insulin and lipid in patients with PD.

Subjects and Methods

Participants

This randomized, double-blind, placebo-controlled clinical trial, registered in the Iranian Registry of Clinical Trials (identifier: IRCT20170513033941N34; http://www.irct.ir), was conducted among population with PD, aged 50–80 years old, diagnosed according to the clinical diagnostic criteria of the UK PD Society Brain Bank[13] and referred to the Shahid Beheshti hospital in Kashan, Iran, between October 2017 and January 2018. This study was performed according to Good Clinical Practice guidelines, and the study protocol was approved by the Research Ethics Committee of Islamic Azad University. Written informed consent was obtained from all patients before the study. Exclusion criteria were as following: taking probiotic and/or synbiotic supplements, taking antioxidant supplements and anti-inflammatory agents, suffering from depression and severe psychosis, hypothyroidism, hyperthyroidism, and being smoker.

Study Design

At baseline, to decrease potential confounding effects, all participants were stratified randomly according to age, body mass index (BMI), gender and the dosage and kind of medications. Then, subjects in each block were randomly allocated into 2 treatment groups to take either 8×10^9 CFU/day probiotic, containing *Lactobacillus acidophilus, Bifidobacterium bifidum, L. reuteri,* and *Lactobacillus fermentum* (each 2×10^9) (n = 25) or placebo (n = 25) for 12 weeks. In addition, all participants were matched according to age, BMI, gender, and the dosage and type of medications. Probiotics and placebos were produced by Lactocare Zisttakhmir Company (Tehran, Iran) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. Since the supplements and placebo capsules had similar packaging, patients and researchers were unaware of the content of the package until the end of study. Randomization assignment was done using computer-generated random numbers as blindness by a trained staff at the neurology clinic. Patients, investigators, clinical site staff and laboratory staff were all masked to treatment assignment throughout the study. All people completed 3-d dietary records (2 weeks' days and one weekend day) at weeks 1, 5, 9, and 12 of the trial. To obtain nutrient intakes of participants according to 3-d food records, we applied Nutritionist IV software (First Databank, San Bruno, CA) adapted for the Iranian food pattern.[14] Physical activity was described as metabolic equivalents (METs) in hours per day. To determine the METs for each subject, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables.

Treatment Adherence

Compliance was evaluated by counting the remaining supplements and placebos, and subtracting from the number of supplements provided to the participants. To increase compliance rate, all subjects received reminder messages on their cell phones every day to remind them to take their capsules.

Assessment of Anthropometric Parameters

Weight was measured on a balance scale (Seca, Hamburg, Germany) at baseline and after the 12-week intervention in the clinic by a trained staff member. Height was determined by a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was determined as weight in kg divided by height in meters squared.

Assessment of Outcomes

The primary outcome was gene expression related to inflammatory markers. The secondary outcome was gene expression related to insulin and lipid metabolism, and biomarkers of inflammation and oxidative stress.

Biochemical Measurements

The nitric oxide (NO) levels were assessed using Griess method[15] with inter- and intra-assay coefficient variances (CVs) of lower than 5%. Plasma glutathione (GSH) was measured using Beutler et al method[16] with inter- and intra-assay CVs less than 5%.

Isolation of Lymphocyte, RNA Extraction and cDNA Synthesis

Twenty milliliters of blood samples were collected in anti-coagulant EDTA tubes. Lymphocytes were isolated using 50% percoll solution (Sigma-Aldrich, Dorset, UK) gradient by centrifugation for 20 minutes and 3000 rpm at 4°C.[17] Total RNA was extracted based on acid guanidinium-phenol-chloroform procedure using RNX™-plus reagent (Cinnacolon, Tehran, Iran) according to the manufacturer's instructions. RNAs was treated with DNAase I (Fermentas, Lithuania) to ensure the elimination of any genomic DNA contamination. Concentration, integration, and purity of RNA samples were determined by spectrometry and gel electrophoresis. Three micrograms of total RNA was used for cDNA synthesis with random hexamer and oligo (dT) 18 primers through RevertAid™ Reverse Transcriptase (Fermantase, Canada) in total 20 µL reaction mixture.[17]

Real-time PCR Analysis

Appropriate primers for IL-1, IL-8, TNF-α, transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), PPAR-γ and LDLR, and glyceraldehyde-3 phosphate dehydrogenase were designed (Table 1). Quantitative Real-time PCR was performed by LightCycler® 96 sequence detection systems (Roche Diagnostics, Rotkreuz, Switzerland) using 4 µL of 5× EVA GREEN I master mix (Salise Biodyne, Japan), 10 ng cDNA, 200 nM of each forward and reverse primers in final volume of 20 µL. PCR was performed through the following instruction: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at 54–62.1°C for 15 seconds and extension at 72°C for 30 seconds. The specificity of PCR products was evaluated by 1.5% agarose gel electrophoresis and melting curve analysis. All experiments were performed at least in triplicate.

Sample Size

We used a randomized clinical trial sample size formula with type one (α) and type 2 errors (β) to be 0.05 and the power of 80% to calculate sample size. Based on a previous study,[10] we used a standard deviation (SD) of 0.10-fold change and a difference in mean (d) of 0.11-fold change, considering TNF-α level as the key variable. According to the calculations, 21 individuals should be enrolled in each group. Assuming a dropout of 4 people per group, the final sample size was determined to be 25 people per group.

Statistical Methods

To determine whether the study variables were normally distributed or not, we used the Kolmogorov-Smirnov test. To detect differences in anthropometric measures, macro- and micro-nutrient intakes, gene expression related to inflammation, insulin, and lipid between 2 groups, we used Student's *t* test to independent samples. Adjustment for changes in baseline values of biochemical variables, age and baseline BMI was performed by analysis of covariance (ANCOVA).[18] Pearson Chi-square test was used for comparison of categorical variables. The *P* value of <0.05 were considered statistically significant. For all statistical analyses we used the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

Fifty subjects [probiotic (n = 25) and placebo (n = 25)] completed the trial (Figure 1).

Mean age, height, weight and BMI at week 0 and week

Table 1. Specific Primers Used for Real-Time Quantitative PCR

Gene	Primer	Product Size (bp)	Annealing Temperature (C)
GAPDH	F: AAGCTCATTTCCTGGTATGACAACG R: TCTTCCTCTTGTGCTCTTGCTGG	126	61.3
IL-1	F: GCTTCTCTCTGGTCCTTGG R: AGGGCAGGGTAGAGAAGAG	174	56
IL-8	F: GCAGAGGGTTGTGGAGAAGT R: ACCCTACAACAGACCCACAC	150	56
TNF-α	F: GTCAACCTCCTCTCTGCCAT R: CCAAAGTAGACCTGCCCAGA	188	52
TGF-β	F: TTGAGACTTTTCCGTTGCCG R: CGAGGTCTGGGGAAAAGTCT	227	56
VEGF	F: CTTCTGAGTTGCCCAGGAGA R: CTCACACACACACAACCAGG	216	54
PPAR-γ	F: ATGACAGACCTCAGACAGATTG R: AATGTTGGCAGTGGCTCAG	210	54
LDLR	F: ACTTACGGACAGACAGACAG R: GGCCACACATCCCATGATTC	223	57

Abbreviations: GAPDH, glyceraldehyde-3-Phosphate dehydrogenase; IL-1, interleukin-1; IL-8, interleukin-8; LDLR, oxidized low-density lipoprotein receptor; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor.

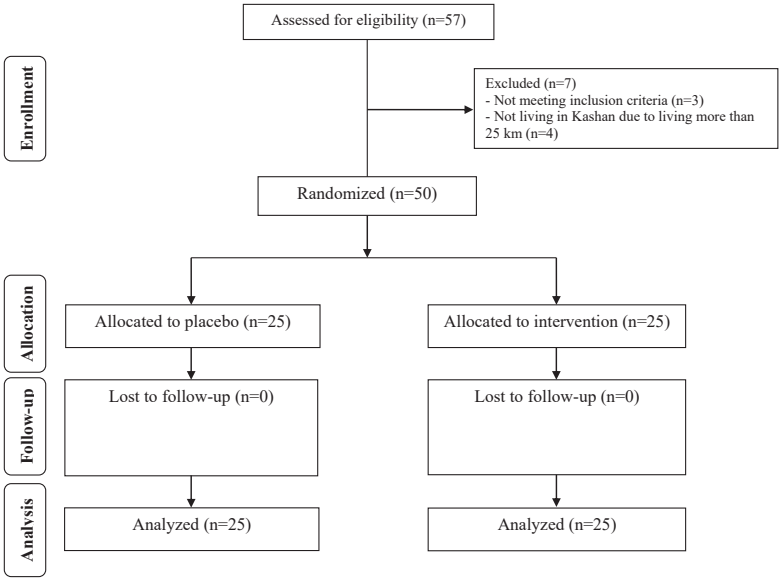

Figure 1. Summary of Patient Flow Diagram.

12 of the intervention were not different between 2 groups (Table 2).

Based on the 3-day dietary records obtained throughout the trial, we found no significant difference in mean macro- and micronutrient intakes between 2 groups (Data not shown).

Compared with the placebo, probiotic supplementation did not affect plasma NO (β 1.66; 95% CI, -1.31, 4.64; $P = 0.26$) and GSH levels (β 9.90; 95% CI, -26.26, 46.06; $P = 0.58$) (Table 3).

After the 12-week intervention, compared with the placebo, probiotic intake downregulated gene expression of IL-1 ($P = 0.03$), IL-8 ($P < 0.001$) and TNF-α ($P = 0.04$) in peripheral blood mononuclear cells) PBMC) of

Table 2. General Characteristics of Study Participants

	Placebo group (n = 25)	Probiotic group (n = 25)	*P*[1]
Gender (%)			
Male	16 (64.0)	17 (51.5)	0.76[†]
Female	9 (36.0)	8 (47.1)	
Duration of Parkinson's disease (y)	5.4 ± 2.5	5.0 ± 1.8	0.57
Levodopa therapy (%)	25 (100.0)	25 (100.0)	>0.99†
Amantadine therapy (%)	25 (100.0)	25 (100.0)	>0.99†
Age (y)	66.7 ± 10.7	66.9 ± 7.0	0.92
Height (cm)	163.9 ± 5.7	164.2 ± 4.5	0.86
Weight at study baseline (kg)	66.6 ± 6.8	67.6 ± 6.6	0.58
Weight at end-of-trial (kg)	66.5 ± 6.6	67.8 ± 6.5	0.45
BMI at study baseline (kg/m^2)	24.8 ± 2.8	25.1 ± 2.9	0.68
BMI at end-of-trial (kg/m^2)	24.8 ± 2.7	25.2 ± 2.8	0.57

Data are means ± SDs.
[1] Obtained from independent *t* test.
[†] Obtained from Pearson chi-square test.

Table 3. The Effect of Probiotic Supplementation on Biomarkers of Inflammation and Oxidative Stress in Patients With Parkinson's Disease

Variables	Placebo Group (n = 25)		Probiotic Group (n = 25)		Difference in Outcome Measures Between Probiotic and Placebo Treatment Groups[1]	
	Baseline	Week 12	Baseline	Week 12	β (95% CI)	*P*[2]
NO (μmol/L)	47.6 ± 3.2	49.1 ± 4.9	53.5±3.7	54.0±3.6	1.66 (-1.31, 4.64)	0.26
GSH (μmol/L)	603.4 ± 93.7	591.9 ± 70.3	494.9±76.9	522.2±90.0	9.90 (-26.26, 46.06)	0.58

Data are mean ±SDs.
1"Outcome measures" refers to the change in values of measures of interest between baseline and week 12. β [difference in the mean outcomes measures between treatment groups (probiotic group = 1 and placebo group = 0)].
2 Obtained from ANCOVA (adjusted for baseline values of each biochemical variables, age and baseline BMI).
GSH, total glutathione; NO, nitric oxide.

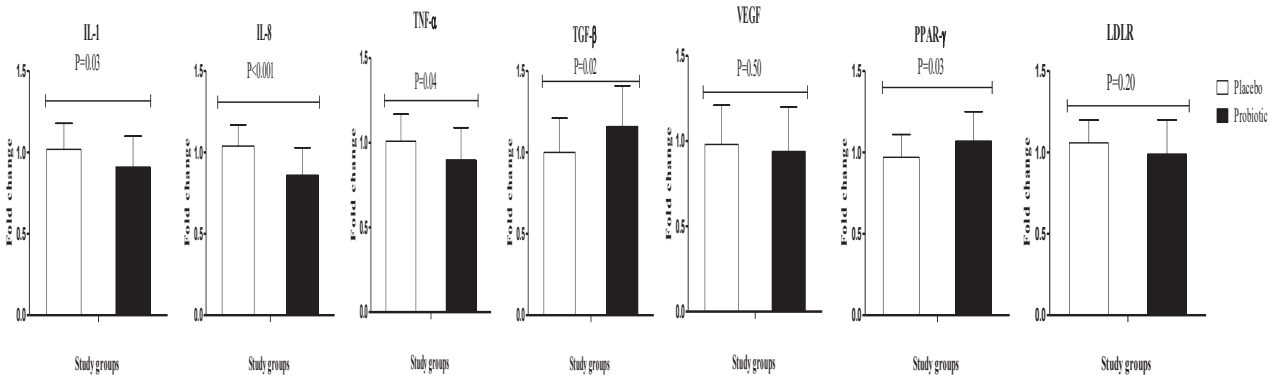

Figure 2. Effect of a 12-Week Supplementation With probiotic or placebo on Gene Expression of IL-1, IL-8, TNF-α, TGF-β, VEGF, PPAR-γ and LDLR in PBMC of Patients With Parkinson's Disease.
IL-1, interleukin-1; IL-8, interleukin-8; LDLR, oxidized low-density lipoprotein; PBMC, peripheral blood mononuclear cells; PPAR-γ, peroxisome proliferator-activated receptor gamma; TNF-α, tumor necrosis factor alpha; TGF-β, transforming growth factor beta; VEGF, vascular endothelial growth factor.

subjects with PD (Figure 2).

Probiotic supplementation upregulated TGF-β (P = 0.02) in PBMC of subjects with PD compared with the placebo (Figure 2). We did not observe any significant effect of probiotic supplementation on gene expression of VEGF in PBMC of patients with PD.

Probiotic intake upregulated PPAR-γ (P = 0.03) in PBMC of subjects with PD compared with the placebo (Figure 2). Probiotic supplementation did not affect gene expression of LDLR in PBMC of patients with PD was seen.

Discussion

In the current investigation, we evaluated the effects of probiotic supplementation on gene expression related to inflammation, insulin and lipid in individuals with PD. We found that probiotic supplementation for 12 weeks in populations with PD significantly improved gene expression of IL-1, IL-8, TNF-α, TGF-β and PPAR-γ, but did not affect VEGF and LDLR. To the best of our knowledge, this investigation is the first report of the effects of probiotic supplementation on biomarkers of inflammation and oxidative stress, and gene expression related to inflammation, insulin and lipid in populations with PD.

This study evidenced that probiotic supplementation to patients with PD for 12 weeks significantly downregulated gene expression levels of IL-1, IL-8 and TNF-α in PBMC compared with the placebo. Earlier, we have showed that probiotic supplementation for 12 weeks to patients with MS significantly decreased gene expression levels of IL-8 and TNF-α; however, it did not affect gene expression of IL-1.[10] Furthermore, supplementation with *Lactobacillus paracasei* and *L. reuteri* in an animal model significantly decreased the expression of hepatic IL-1β, IL-6 and TNF-α through suppressing the mitogen-activated protein kinase (MAPK) and nuclear factor ϰB (NF-kB) signaling pathways.[19] Gene expression of TNF-α, IL-1β and IL-6 was also up-regulated in intestinal mucositis tissues following the treatment with probiotics.[20] In another study, *Lactobacillus plantarum* significantly downregulated gene expression of IL-8 and TNF-α in HT-29 cells at 6 hours as well as 24 hours.[21] Therefore, due to their anti-inflammatory and anti-oxidative properties, probiotics may be useful to decrease the duration of neurological symptoms. Unlike, gene expression of TNF-α was not influenced with supplementation of overweight and obese people with 200 g/d yogurt enriched by *L. acidophilus*, Bifidobacterium BB12 and *Lactobacillus casei* for 8 weeks.[22] Taking probiotic capsules for 8 weeks by patients with rheumatoid arthritis decreased hs-CRP, but did not influence NO levels.[23] In addition, NO production was not changed in the groups treated with *probiotic* in herpes simplex virus type 1.[24] Different study designs, lack of considering baseline levels of biochemical variables, different dosages and types of probiotic strains as well as duration of the intervention might provide some reasons for discrepant findings. Increased inflammatory markers are associated with increased microglia activation, which in turn would result in neurodegeneration through the release of free radicals, pro-inflammatory, immunomodulatory and anti-inflammatory cytokines.[25] The dysfunctions in the immune system in terms of inflammatory cytokines production are intimately related to the nervous system alterations; therefore, controlling inflammatory markers may ameliorate the central inflammation and recovers the nervous system functions.[26] Probiotic intake may decrease gene expression levels of inflammatory markers through modulating toll-like receptors, NF-kB and MAPK pathways.[27]

We found that probiotic supplementation for 12 weeks in people with PD upregulated gene expression of PPAR-γ in PBMC compared with the placebo, but did

not affect gene expression of LDLR. Few animal and cell line studies have reported some effects of probiotics on gene expression levels of PPAR-γ and LDLR. In a study by Liu et al,[28] it was seen that *L. reuteri* could improve the gut health of neonatal piglets by increasing colonic butyric acid levels and up-regulating the downstream molecules of butyric acid and PPAR-γ. In addition, *L. casei* supplementation in a rat model of acute liver failure significantly increased gene expression of PPAR-γ.[29] Such beneficial effects of probiotic supplementation on signaling pathway related to insulin metabolism was not reported by others. For instance, gene expression of PPAR-γ was downregulated after the intake of probiotics in rat models.[30] Dolatkhah et al[31] found that a 6-week probiotics supplementation to women with gestational diabetes mellitus did not influence insulin metabolism.

Abnormal signaling pathway related to insulin metabolism in people with PD may be correlated with extracellular events of relevance to neurodegeneration, inflammation and oxidative damage, which in turn is increasingly recognized as a main contributor to the pathogenesis of PD.[32] Therefore, probiotics due to their beneficial effects on insulin metabolism may decrease metabolic events related to diabetes and cardiovascular diseases in patients with PD. PPAR-γ plays a key function in the regulation of metabolism, including regulating insulin sensitivity, mitochondrial biogenesis, and carbohydrate and lipid homoeostasis.[33,34] In addition, it was reported that PPAR-γ has potential beneficial effects in a number of neurological disorders, such as PD, Alzheimer's disease and amyotrophic lateral sclerosis.[35] Also, simultaneous targeting of dysfunctional pathways may underlie the potent neuroprotective activity displayed by PPAR-γ agonists.[35] Probiotics intake may improve signaling pathway related to insulin metabolism by reducing cytokines and inhibiting the NF-kB pathway[36] and gut microbiota-short chain fatty acids-hormone axis.[37]

The current study had a number of strengths. Firstly, we focused on some interesting questions using a randomized, double-blind, placebo-controlled trial. The findings of improved gene expression related to inflammation and insulin in the probiotic group in our study are interesting, but need to be confirmed in a larger study. Another strength of the current study was the absence of dropout rate.

The current study had few limitations. In this study, due to funding limitations, we did not characterize the microbiota and thus could not establish whether probiotic intake over 12 weeks changed its composition. In addition, evaluating protein levels would have been more relevant. Unfortunately, we did not assess the effects of probiotic supplementation on protein levels.

Overall, probiotics supplementation for 12 weeks in PD patients significantly improved gene expression of IL-1, IL-8, TNF-α, TGF-β and PPAR-γ, but did not affect gene expression of VEGF and LDLR, and biomarkers of inflammation and oxidative stress. This suggests probiotic supplementation may confer advantageous therapeutic potential for people with PD. Further research is needed in other participants and for longer periods to determine the safety and beneficial effects of probiotic supplementation. Moreover, further studies should measure gene expression levels related to oxidative stress.

Authors' Contribution

ZA contributed to conception, design, statistical analysis and drafting of the manuscript. SB, ShO and AE contributed to conception, data collection and manuscript drafting. The final version was confirmed by all authors for submission.

Conflict of Interest Disclosures

None.

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References

1. Bhattacharyya KB. Hallmarks of clinical aspects of Parkinson's disease through centuries. Int Rev Neurobiol. 2017;132:1-23. doi: 10.1016/bs.irn.2017.01.003.
2. Harms AS, Lee JK, Nguyen TA, Chang J, Ruhn KM, Trevino I, et al. Regulation of microglia effector functions by tumor necrosis factor signaling. Glia. 2012;60(2):189-202. doi: 10.1002/glia.21254.
3. Kaur K, Gill JS, Bansal PK, Deshmukh R. Neuroinflammation - A major cause for striatal dopaminergic degeneration in Parkinson's disease. J Neurol Sci. 2017;381:308-14. doi: 10.1016/j.jns.2017.08.3251.
4. Tjalkens RB, Popichak KA, Kirkley KA. Inflammatory activation of microglia and astrocytes in manganese neurotoxicity. Adv Neurobiol. 2017;18:159-81. doi: 10.1007/978-3-319-60189-2_8.
5. Chaturvedi RK, Beal MF. PPAR: a therapeutic target in Parkinson's disease. J Neurochem. 2008;106(2):506-18. doi: 10.1111/j.1471-4159.2008.05388.x.
6. Doiron M, Langlois M, Dupre N, Simard M. The influence of vascular risk factors on cognitive function in early Parkinson's disease. Int J Geriatr Psychiatry. 2018;33(2):288-97. doi: 10.1002/gps.4735.
7. Mancuso C, Santangelo R. Alzheimer's disease and gut microbiota modifications: The long way between preclinical studies and clinical evidence. Pharmacol Res. 2018;129:329-36. doi: 10.1016/j.phrs.2017.12.009.
8. 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. doi: 10.3389/fnagi.2016.00256.
9. Sherwin E, Dinan TG, Cryan JF. Recent developments in understanding the role of the gut microbiota in brain health and disease. Ann N Y Acad Sci. 2018;1420(1):5-25. doi: 10.1111/nyas.13416.
10. Tamtaji OR, Kouchaki E, Salami M, Aghadavod E, Akbari E, Tajabadi-Ebrahimi M, et al. The effects of probiotic supplementation on gene expression related to inflammation, insulin, and lipids in patients with multiple sclerosis: a randomized, double-blind, placebo-controlled trial. J Am Coll Nutr. 2017;36(8):660-5. doi: 10.1080/07315724.2017.1347074.

11. Steed H, Macfarlane GT, Blackett KL, Bahrami B, Reynolds N, Walsh SV, et al. Clinical trial: the microbiological and immunological effects of synbiotic consumption - a randomized double-blind placebo-controlled study in active Crohn's disease. Aliment Pharmacol Ther. 2010;32(7):872-83. doi: 10.1111/j.1365-2036.2010.04417.x.
12. 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(1):35. doi: 10.1186/1743-7075-10-35.
13. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992;55(3):181-4.
14. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Sabihi SS, Esmaillzadeh A. Effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized clinical trial. Nutrition. 2014;30(11-12):1287-93. doi: 10.1016/j.nut.2014.03.008.
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(4):348-50. doi: 10.1016/j.clinbiochem.2010.12.011.
16. Beutler E, Gelbart T. Plasma glutathione in health and in patients with malignant disease. J Lab Clin Med. 1985;105(5):581-4.
17. Gmelig-Meyling F, Waldmann TA. Separation of human blood monocytes and lymphocytes on a continuous Percoll gradient. J Immunol Methods. 1980;33(1):1-9.
18. Mansournia MA, Altman DG. Invited commentary: methodological issues in the design and analysis of randomised trials. Br J Sports Med. 2018;52(9):553-5. doi: 10.1136/bjsports-2017-098245.
19. Hsu TC, Huang CY, Liu CH, Hsu KC, Chen YH, Tzang BS. *Lactobacillus paracasei* GMNL-32, *Lactobacillus reuteri* GMNL-89 and L. reuteri GMNL-263 ameliorate hepatic injuries in lupus-prone mice. Br J Nutr. 2017;117(8):1066-74. doi: 10.1017/s0007114517001039.
20. Yeung CY, Chan WT, Jiang CB, Cheng ML, Liu CY, Chang SW, et al. Amelioration of Chemotherapy-Induced Intestinal Mucositis by Orally Administered Probiotics in a Mouse Model. PLoS One. 2015;10(9):e0138746. doi: 10.1371/journal.pone.0138746.
21. Dhanani AS, Bagchi T. Lactobacillus plantarum CS24.2 prevents *Escherichia coli* adhesion to HT-29 cells and also down-regulates enteropathogen-induced tumor necrosis factor-alpha and interleukin-8 expression. Microbiol Immunol. 2013;57(4):309-15. doi: 10.1111/1348-0421.12038.
22. Zarrati M, Salehi E, Nourijelyani K, Mofid V, Zadeh MJ, Najafi F, et al. Effects of probiotic yogurt on fat distribution and gene expression of proinflammatory factors in peripheral blood mononuclear cells in overweight and obese people with or without weight-loss diet. J Am Coll Nutr. 2014;33(6):417-25. doi: 10.1080/07315724.2013.874937.
23. Zamani B, Golkar HR, Farshbaf S, Emadi-Baygi M, Tajabadi-Ebrahimi M, Jafari P, et al. Clinical and metabolic response to probiotic supplementation in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled trial. Int J Rheum Dis. 2016;19(9):869-79. doi: 10.1111/1756-185x.12888.
24. Khani S, Motamedifar M, Golmoghaddam H, Hosseini HM, Hashemizadeh Z. In vitro study of the effect of a probiotic bacterium *Lactobacillus rhamnosus* against herpes simplex virus type 1. Braz J Infect Dis. 2012;16(2):129-35.
25. Gupta V, Garg RK, Khattri S. Levels of IL-8 and TNF-alpha decrease in Parkinson's disease. Neurol Res. 2016;38(2):98-102. doi: 10.1080/01616412.2015.1133026.
26. Frischer JM, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. Brain. 2009;132(Pt 5):1175-89. doi: 10.1093/brain/awp070.
27. Wu Y, Zhu C, Chen Z, Chen Z, Zhang W, Ma X, et al. Protective effects of *Lactobacillus plantarum* on epithelial barrier disruption caused by enterotoxigenic Escherichia coli in intestinal porcine epithelial cells. Vet Immunol Immunopathol. 2016;172:55-63. doi: 10.1016/j.vetimm.2016.03.005.
28. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation. 1983;67(5):968-77.
29. Wang Y, Xie J, Li Y, Dong S, Liu H, Chen J, et al. Probiotic *Lactobacillus casei* Zhang reduces pro-inflammatory cytokine production and hepatic inflammation in a rat model of acute liver failure. Eur J Nutr. 2016;55(2):821-31. doi: 10.1007/s00394-015-0904-3.
30. Mei L, Tang Y, Li M, Yang P, Liu Z, Yuan J, et al. Co-Administration of Cholesterol-Lowering Probiotics and Anthraquinone from *Cassia obtusifolia* L. Ameliorate Non-Alcoholic Fatty Liver. PLoS One. 2015;10(9):e0138078. doi: 10.1371/journal.pone.0138078.
31. Binienda ZK. Neuroprotective effects of L-carnitine in induced mitochondrial dysfunction. Ann N Y Acad Sci. 2003;993:289-95; discussion 345-9.
32. Athauda D, Foltynie T. Insulin resistance and Parkinson's disease: A new target for disease modification? Prog Neurobiol. 2016;145-146:98-120. doi: 10.1016/j.pneurobio.2016.10.001.
33. Moran-Salvador E, Lopez-Parra M, Garcia-Alonso V, Titos E, Martinez-Clemente M, Gonzalez-Periz A, et al. Role for PPARgamma in obesity-induced hepatic steatosis as determined by hepatocyte- and macrophage-specific conditional knockouts. FASEB J. 2011;25(8):2538-50. doi: 10.1096/fj.10-173716.
34. Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W. Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell. 1992;68(5):879-87.
35. Carta AR. PPAR-gamma: therapeutic prospects in Parkinson's disease. Curr Drug Targets. 2013;14(7):743-51.
36. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006;116(11):3015-25. doi: 10.1172/jci28898.
37. Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem. 2013;288(35):25088-97. doi: 10.1074/jbc.M113.452516.