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RESEARCH PAPER

A randomized, double-blind, placebo-controlled, clinical trial on probiotic soy milk and soy milk: effects on epigenetics and oxidative stress in patients with type II diabetes

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Abstract This clinical trial aimed to discover the effects of probiotic soy milk and soy milk on MLH1 and MSH2 promoter methylation, and oxidative stress among type II diabetic patients. Forty patients with type II diabetes mellitus aged 35–68 years were assigned to two groups in this randomized, double-blind, controlled clinical trial. Patients in the intervention group consumed 200 ml/day of probiotic soy milk containing *Lactobacillus plantarum A7*, while those in the control group consumed 200 ml/d of conventional soy milk for 8 weeks. Fasting blood samples, anthropometric measurements, and 24-h dietary recalls were collected at the baseline and at the end of the study, respectively. Probiotic soy milk significantly decreased promoter methylation in proximal and distal MLH1 promoter region ($P < 0.01$ and $P < 0.0001$, respectively) compared with the baseline values, while plasma concentration of 8-hydroxy-2′-deoxyguanosine (8-OHdG) decreased significantly compared with soy milk ($P < 0.05$). In addition, a significant increase in superoxide dismutase (SOD) activity was observed in probiotic soy milk group compared with baseline value ($P < 0.01$). There were no significant changes from baseline in the promoter methylation of MSH2 within either group ($P > 0.05$). The consumption of probiotic soy milk improved antioxidant status in type II diabetic patients and may decrease promoter methylation among these patients, indicating that probiotic soy milk is a promising agent for diabetes management.

Keywords Type II diabetes · Probiotic · Promoter methylation · Oxidative stress

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Introduction

Type II diabetes mellitus is a metabolic disease that begins with insulin resistance in which cells cannot respond to insulin properly, and there is high blood sugar level over a prolonged period (Castro et al. 2014). Chronic hyperglycemia has been suggested as a risk factor for the production of reactive oxygen species (ROS) (Newsholme et al. 2007). Aerobic breakdown of glucose produces NADH, and the enhancement of NADH production leads to more NADH recycling by mitochondria complex I. Furthermore, this increases the chance of electron leakage and more ROS production (Banerjee and Vats 2013). ROS has an intracellular signaling effect in healthy cells, but an enhancement of ROS production or lack of antioxidant production results in oxidative stress and may cause DNA double-strand break (Waris and Ahsan 2006). ROS can

cause transition mutation in DNA because it induces deamination of cytosine to uracil or 5-hydroxyuracil, and during DNA replication, uracil and 5-hydroxyuracil pair with adenosine, resulting in G:C to A:T transition mutation (Hegde et al. 2008).

DNA repair enzymes are very important for the maintaining of DNA integrity in diabetic patients. MLH1 and MSH2 enzymes play key roles in mismatch repair, and they act as a proofreading system during DNA synthesis and repair. Therefore, the expression of these genes should increase in this condition (Haracska et al. 2000). One very important process in patients with increased oxidative stress is DNA methylation in promoter region which is an epigenetic signaling tool that cells use to keep genes in off position, and thus, removal of methyl group from MLH1 and MSH2 promoter can increase their transcription (Ingrosso et al. 2003).

Dietary factors like cruciferous vegetables and fiber have significant effects on the epigenetic programs (Young et al. 2005; Spurling et al. 2008). Dietary fiber, an effective prebiotic, can be fermented by beneficial bacteria in human's colon probiotics, and the fermentation of the fiber produces beneficial short-chain fatty acids (SCFAs) like acetate and butyrate (Cuervo et al. 2013; Reimer et al. 2004; Sun et al. 2014). SCFAs induce chromatin remodeling by global histone acetylation and certain histones deacetylation (Worthley et al. 2009; Cho et al. 2014; Kuo et al. 2014). Certain evidence indicates that the effect of butyrate on epigenetic process is expanding beyond chromatin remodeling to DNA methylation (Slawinska et al. 2014; Stein et al. 2012).

New studies have suggested that soy phytoestrogens have a protective effect against cancer by epigenetic process, in particular promoter methylation (Matsukura et al. 2011; Phillip et al. 2012; Zhang et al. 2013; Pudenz et al. 2014). Scientists believe that using soy foods like soymilk with probiotic can improve the protective effect of either one of them because: (1) Soymilk can act as a substrate for probiotics; therefore, it increases epigenetic effects of probiotics, and (2) probiotic changes isoflavones to other active metabolites and increases their absorption through a change in the intestinal environment (Teh et al. 2010; Tsangalis et al. 2005; Zielinska et al. 2014). *Lactobacillus plantarum A7*, a strain of probiotic, is also known to change the expression of many genes and has antioxidant effects, but there are no clear evidences of the mechanism of these effects (Citar et al. 2014; Li et al. 2014).

The present study combined *Lactobacillus plantarum A7* with soy milk in order to increase the effect of probiotics on oxidative stress and epigenetic processes, and for the first time, the effect of this combination on promoter methylation and DNA damage in patients with type II diabetes was found.

Materials and methods

Study design and participants

To compare the effect of consuming probiotic soy milk with soy milk alone, a randomized, placebo-controlled, double-blind, parallel design trial was used. The participants and the researcher were blind to the intervention. The participants were randomly assigned by permuted blocks randomization to one of the two 8-week intervention groups. Each day, the subjects received, in a double-blinded fashion, 200 ml/day probiotic soymilk or soy milk to supplement their usual diet. The probiotic soy milk and soy milk were identically packed, having similar test and appearance. Two weeks before commencement of the study, all participants abstained from eating yogurt or any other fermented foods. They all received a 3-day supply of their probiotic or conventional soy milk every 3 days. Compliance with soy milk consumption guidelines was checked by telephone interviews once a week.

The subjects were type II diabetic patients with fasting blood glucose (FBS) $\geq$ 126 mg/dl, blood sugar (2 h postprandial sugar) $\geq$ 200, aged from 32 to 68 years, and have been diabetic for more than 1 year. Exclusion criteria include subjects with a history of inflammatory bowel disease, infection, liver disease, rheumatoid arthritis, smoking, alcoholism, recent antibiotic therapy, and consuming multivitamin and mineral. The intervention lasted 8 weeks, with a total of 48 diabetic patients participating in this study. Written informed consent was signed by all volunteers, and a study protocol was approved by Isfahan University of Medical Sciences. The trial has been registered in the Iranian Registry of Clinical Trials, identifier: IRCT: IRCT201405265062N8 available at: http://www.irct.ir.

Intervention and anthropometric measurements

The probiotic soy milk contained 2×10^7 cfu/ml of *Lactobacillus plantarum A7*. Conventional soy milk and probiotic soy milk were produced every 3 days and distributed to the participants. The probiotic soy milks were sampled at the time of distribution and were microbiologically analyzed every 2 weeks. Samples were refrigerated at 4 °C for 3 days, and subsequent analyzing was done on the third day of storage. For counting *Lactobacillus plantarum A7*, MRS broth (MRS agar: Merck, Darmstadt, Germany, and bile: Sigma-Aldrich, Inc., Reyde, USA) was used by pour plate method. Microbiological analyses of the probiotic soymilk showed that the average colony counts of *Lactobacillus plantarum A7* on day 1 and day 3 were the same. Therefore, bacteria indicated a steady survival rate in soy milk during a 3-day storage period. All subjects entered in

a 2-week run-in period during which they had to stop taking any probiotic food or probiotic supplements. Their weight, height, waist-to-hip ratio (WHR), and medication history were recorded before and after the intervention, and information on physical activity (PA) levels and micro- and macronutrients intakes were gathered every 2 week by international physical activity questionnaires (IPAQ) and a 24-h diet recall interview, respectively. Weight was recorded by digital scale (Seca, Germany), with an accuracy of 100 g, and standing height was recorded by non-stretchable tape (Seca, Germany), with an accuracy of 0.1 cm. Body mass index (BMI) was obtained by dividing weight by the square of height. All subjects had to have a stable dietary habit, physical activity, and medication during intervention. Subjects who intended to change their dietary habits, physical activity, or body weight during the intervention period were excluded from the study.

Biochemical analysis

In order to measure 8-hydroxy-2′-deoxyguanosine (8-OHdG) and superoxide dismutase (SOD) activity at the beginning and at the end of the intervention period, after 12-h overnight fasting, 10 ml venous blood samples were obtained and put into tubes containing ethylenediaminetetraacetic acid (EDTA). Within 1 h of collection, the blood sample was centrifuged (2500 rpm for 10 min), plasma samples were collected and frozen at −80 °C until they were used for their respective analysis. Plasma 8-OHdG levels were measured using an enzyme-linked immune sorbent assay (ELISA), with commercial kit supplied by Northwest Laboratories (Northwest Life Science Specialties, LLC, Vancouver, Canada). SOD activity was measured after washing red cells 4 times by 0.9 % saline solution, according to Biorex kit method (Cat No: BXC0531A, Biorex, UK). In this procedure, the SOD activity was evaluated by measuring the dismutation of superoxide radicals produced by xanthine oxidase and xanthine (μ/g hb).

DNA isolation

Genomic DNA was extracted from the 0.5 ml EDTA-anticoagulated whole blood by PrimePrep Genomic DNA Isolation Kit (GeNet Bio, Daejeon, Korea) and was quantified by Nano Drop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Methylation-specific digestion

The promoter methylation analysis (MA), using methylation-specific digestion enzyme and real-time polymerase chain reaction (PCR), was done according to the guidelines provided by Bettstetter et al. (2008). Two restriction digestion batches were prepared for every sample: (1) methylation quantification digestion (MQD), (2) methylation-independent calibrator digestion (CalD). Each batch contained 5 μl DNA and was digested in a 20 μl reaction volume in 10 × buffer Tango (Thermo Scientific) at 37 °C for 16 h. Digestion in MQD batch was done by 30 U endonuclease Hin6I (Thermo Scientific, ER0481) which digests only unmethylated CGCG recognition sites within the promoter, while in CalD batch, digestion was done by 15 U endonuclease XbaI and 15 U endonuclease DraI (Thermo Scientific ER0681 and ER0221, respectively), and their recognition sites must not be present within the amplicon. Following this, enzymes were deactivated by incubating at 70 °C for 20 min, and the samples were stored at 4 °C. A positive control with up to 1 μg digested DNA was built by methyltransferase enzyme (Thermo Scientific, ER0821) according to manufacturer's instruction, and nonmethylated blood DNA from healthy persons was used as a negative control for 0 % methylated. Positive and negative controls were run in parallel with our sample.

Methylation-specific quantitative real-time PCR

For the estimation of DNA methylation, 1 μl digested DNA of MQD and CalD, together with positive and negative controls, were loaded in duplicate into qPCR, using Maxima SYBR Green/qPCR Master Mix (Thermo Scientific) on a 96-well Applied Biosystems 7900 HT system. The primer sequences were (5′–3′) MLH1_prox_MS_up AAGTCGCCCTGACGCAGACGCTCCA; MLH1_prox_MS_down CTGGGTCCACTCGGGCCGGAAAACTA; MLH1_dist_MS_up CGGCATCTCTGCTCCTATTG; MLH1_dist_MS_down ATCCTTCTAGGTAGCGGGCA MSH2_MS_up CCTGGAAGCTGATTGGGTGTGGTCG CCG; MSH2_MS_down GCTTCTTTCAGGGCATGCCG GAGAAGCCG. Real-time PCR was carried out in a final volume of 10 μl containing digested DNA (1 μl), 1 μl primer (forward and reverse), and 5 μl 2 × SYBR Green PCR Master Mix. To confirm a single PCR product, melting curve analyses were done by heating PCR products from 50 to 95 °C at the end of PCR cycle. To ensure the correct size of products, the PCR products were run on 1 % gel. The cycling parameters for real-time PCR started with 10-min initial denaturation at 95 °C, then 40 cycles of 95 °C in 10 s, 60 °C in 30 s, and 72 °C in 30 s for MLH1, and 95 °C in 10 s, 58 °C in 30 s, and 72 °C in 30 s for MSH2. The proportions of promoter methylation were measured by the differences of the Ct-values from the MQD and CalD according to the formula:

$$\text{Methylation}\,\% = 2^{\Delta Ct} \times 100$$

where $\Delta Ct = Ct\ \text{Calibrator} - Ct$ methylation quantification.

All experimental data were analyzed by using the Statistical Package for Social Science version 20 (SPSS Inc., Chicago, Illinois, USA), and results were reported as mean ± standard error (SE) for quantitative data. Histogram and Kolmogrov–Smirnov tests were performed to ensure the normal distribution of variables. For variables that did not follow normal distributions, log transformation was applied. Data on dietary intakes were compared by independent samples t test between groups, and repeated measure analysis of variance (ANOVA) was used for evaluating the changes in intakes over the study period.

In this study, carbohydrate and energy intake were considered as possible confounding factors. For comparing the levels of PA and sex in two groups, Chi-square test was used, and the duration of disease and age were compared by independent t test. Analysis of covariance (ANCOVA) was used for determining any differences between two studied groups in terms of main outcomes after intervention (adjustment was made for baseline values and confounding factors). Calorie and carbohydrate intake were the possible confounding factors in this study. Paired-sample t tests were used to detect within-group differences. P value < 0.05 was defined to be statistical significance.

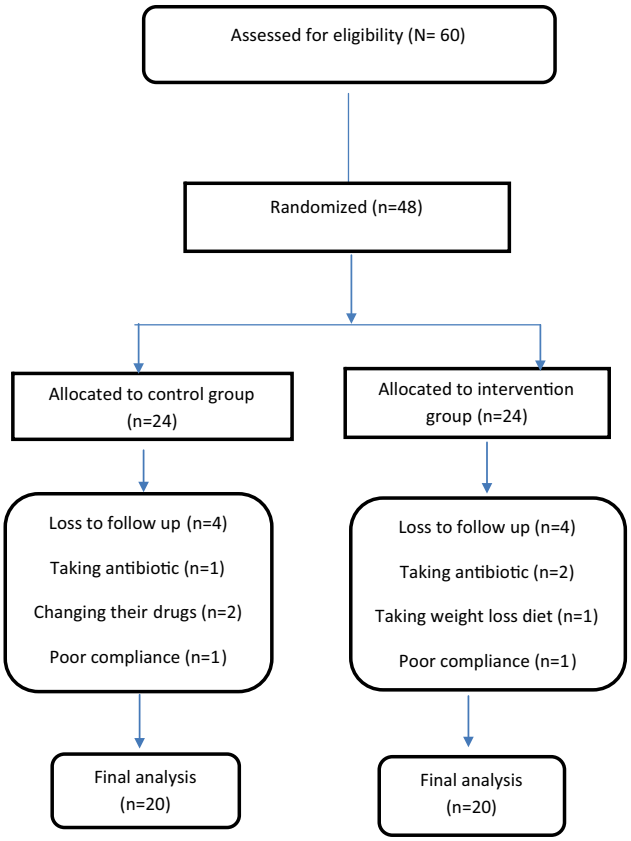

Fig. 1 Summary of patient flow

Results

Baseline data

The patients in this study received similar medications with similar dosages for a period of 8 weeks. There were no adverse reactions or symptoms in patients, and they all demonstrated good compliance. Among 48 participants in this study, 8 patients were excluded (need for antibiotic treatment, $n = 3$ change diet, $n = 1$ change drug, $n = 2$ not consuming soy milk according to plan, $n = 2$, Fig. 1). Finally, only 40 patients (male = 19 and female = 21) completed the trial ($n = 20$ for each group). There were no statistically significant differences in the baseline characteristics of the patients between the two groups (Supplementary file 1).

24-Hour diet recall

There were no significant differences between the two groups in dietary intake at the beginning of study, except for carbohydrate (Table 1). The intake of carbohydrate was significantly higher in placebo group ($P < 0.001$). Dietary intake of nutrients at the beginning and during the study did not show any significant differences within each group separately, but a statistically significant difference was found between the two groups for dietary intakes of energy and carbohydrate throughout the study.

Changes of oxidative stress markers

The baseline concentration of SOD, 8-OHdG, was not different between the two groups ($P = 0.09$ and $P = 0.71$, respectively). Within-group comparisons of SOD activity revealed that the consumption of probiotic soy milk in the intervention group increased the activity of SOD ($P < 0.01$, Fig. 2), while no significant difference was observed in the placebo group ($P = 0.13$, Fig. 2). Between-group comparison indicated significant increase in SOD activity in intervention group, in comparison with control group, after adjusting with baseline value, calorie, and carbohydrate intake ($P < 0.05$). There were no within-group changes between plasma concentration of 8-OHdG in intervention and control group, respectively ($P = 0.11$ and $P = 0.61$, respectively, Fig. 2), but the result of between-group comparison showed that there were statistically significant reductions in intervention group when compared to control group on plasma concentration of 8-OHdG at the end of the study, when it was adjusted for energy and carbohydrate intake and baseline values ($P < 0.05$, Fig. 2).

Table 1 Reported nutrient intake of participants on probiotic soy milk and soy milk groups at baseline and throughout the study

Variables	Intervention $N = 20$			Placebo $N = 20$			P value[b]
	Before	Throughout the study	P value[a]	Before	Throughout the study	P value[a]	
Carbohydrate (g/day)	275.5 ± 5.53	269 ± 5.01	0.349	309.70 ± 6.54	307.72 ± 5.75	0.663	0.00
Protein (g/day)	61.60 ± 1.80	62.42 ± 1.33	0.457	62.22 ± 1.19	63.13 ± 0.98	0.419	0.777
Fat (g/day)	90.55 ± 1.92	90.60 ± 1.95	0.980	92.89 ± 2.59	92.23 ± 2.18	0.699	0.473
Calorie (Kcal/day)	2105.85 ± 33.48	2095.19 ± 20.09	0.790	2173.45 ± 32.11	2182.65 ± 30.95	0.727	0.023
Fiber (g/day)	17.67 ± 0.61	18.63 ± 0.65	0.172	18.76 ± 0.84	18.61 ± 0.51	0.853	0.301
B12 (μg/day)	2.45 ± 0.22	2.25 ± 0.16	0.466	2.36 ± 0.19	2.26 ± 0.30	0.814	0.765
Folic acid (μg/day)	259.95 ± 14.76	291.66 ± 11.34	0.078	267.99 ± 13.66	277.29 ± 9.47	0.614	0.401

Data are mean ± SE

[a] Obtained from paired t test

[b] Obtained from independent samples t test for the comparing of dietary intakes throughout the study between two groups

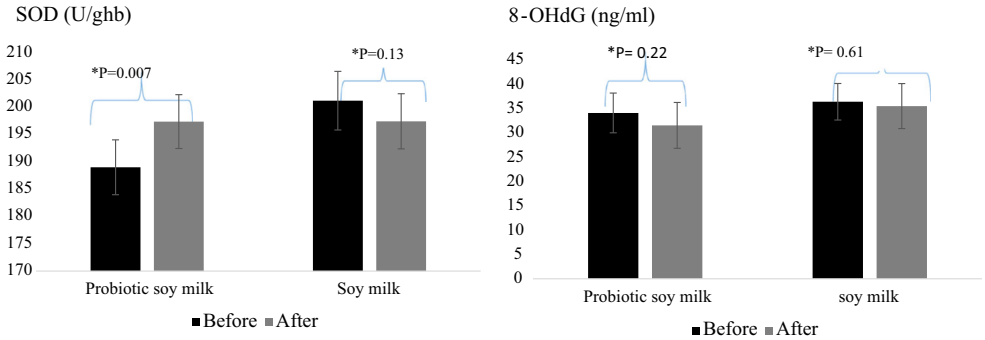

Fig. 2 Biomarkers of oxidative stress at baseline and after 8 weeks of study

Changes of promoter methylation

There was no significant difference for baseline percent of methylation in proximal and distal MLH1 promoter region ($P = 0.72$ and $P = 0.06$, respectively) between the two groups. Although the percent of methylation in two regions of MLH1 promoter decreased significantly in intervention group at the end of study ($P < 0.01$, Fig. 3), there was no significant change in control group for methylation in proximal and distal promoter region ($P = 0.12$ and $P = 0.18$, respectively, Fig. 3). Between-group comparison indicated significant reduction in methylation at two MLH1 promoter regions in probiotic soy milk group after adjusting with baseline value, calorie, and carbohydrate intake ($P < 0.05$). At baseline, percentage methylation of MSH2 promoter region was not different between two groups ($P = 0.06$, Fig. 3). Within-group comparisons revealed no significant changes in MSH2 methylation after intervention, either in the placebo group ($P = 0.874$, Fig. 3) or in the probiotic group ($P = 0.224$), even after adjusting by baseline values and covariates with ANCOVA ($P = 0.260$).

Discussion

This study revealed that the consumption of probiotic soy milk, compared to the soy milk, for 8 weeks, among patients with type II diabetes increased the activity of SOD and significantly decreased plasma 8-OHdG concentration. In addition, probiotic soy milk can reduce the value of methylation in proximal and distal MLH1 promoter region, but it was discovered that probiotic soy milk and soy milk did not have any effect on the methylation values of the MSH2 promoter region.

To the best of our knowledge, this is the first human study to compare the effect of probiotic soy milk and soy milk on oxidative stress and epigenetic consequences of diabetic patients. Majority of the patients with type II diabetes have enhancement of oxidative stress. Oxidative stress, through inducing guanine oxidation to 8-OHdG, leads to a G:C to T:A transition mutation (Giacco and Brownlee 2010). Mismatch repair (MMR) enzyme complex (in which MLH1 and MSH2 appear to play a key role) acts as a proofreading system during DNA synthesis and repairs 8-OHdG lesions (Ingrosso et al. 2003). Therefore,

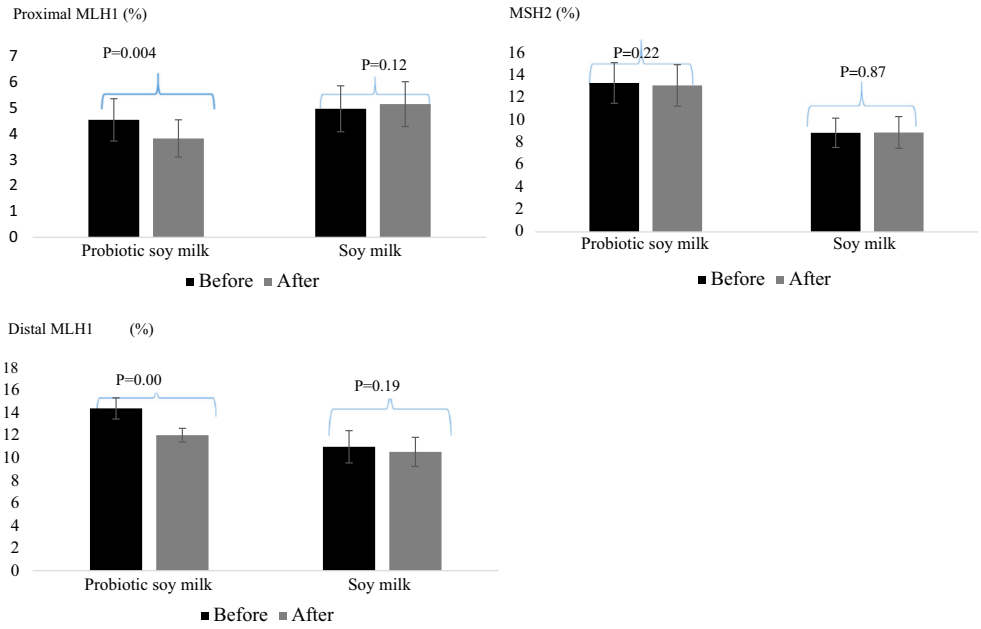

Fig. 3 Percent of promoter methylation at baseline and after 8 weeks

the expression of these genes is very important in MMR system among diabetic patients (Eccleston et al. 2011). Probiotic is believed to interfere with gene expression through different mechanisms. One of its proposed mechanisms is epigenetic processes. Epigenetic modification such as DNA methylation and histone modification can alter gene expression without making changes to the nucleotide sequence (Holliday 1993). Hypermethylation of the MLH1 promoter is the most important factor in microsatellite instability (MSI) and decreasing in MMR process. New studies have indicated that MMR deficiency caused the accumulation of potentially lethal 8-OHdG. Line studies have indicated that butyrate (SCFA produced by probiotic in colon) can reduce promoter methylation in colorectal cancer cells, and *Bifidobacterium breve* can reduce the expression of interleukins genes in intestinal culture model with epigenetic processes, including the enhancement of DNA methylation and the inhibition of histone acetylation. A study by Castaneda-Gutierrez et al. (2014) revealed that a nutritional mixture containing *Lactobacillus rhamnosus* increased the expression of genes related to lipid metabolism in rats, but did not have any effect on DNA methylation. In another study by Worthley et al. (2009), the consumption of synbiotics supplementation was associated with the reduction in MINT2 PMR (percentage of methylated reference) within normal colorectal mucosa, but their intervention did not have any effect on other genes like CDKN2A, MINT, MINT31, MGMT, and MLH1.

In the present study, dietary intervention reduced the MLH1 promoter methylation significantly; however, probiotic had no effect on MSH2 promoter region. New evidence has shown that butyrate can reach a concentration of 100 mmol/L in proximal colon after consuming probiotic supplement, but this concentration declines throughout the colon because butyrate would be rapidly used up by colonocytes (Neish 2009). A higher dosage of probiotic bacteria and a longer period of supplementation would result in a higher concentration of butyrate, leading to beneficial effects on the methylation MSH2 promoter. ROS in a living cell can attack nucleic acids, and 8-OHdG is the most frequently detected markers of nuclear and mitochondrial DNA lesion (Kryston et al. 2011). A significant increase in plasma 8-OHdG was seen in diabetic patient compared to normal subjects (Araki and Nishikawa 2010).

In the present study, probiotic, with a significant decrease in MLH1 promoter methylation, can increase its expression and enhance the activity of MMR process. Therefore, this process might cause a decrease in 8-OHdG concentration.

Collected data have shown that probiotic soy milk consumption for 8 weeks did not affect plasma 8-OHdG levels within each group; however, significant between-group differences were detected. The effect of probiotics on oxidative stress has previously been evaluated in humans (Asemi et al. 2012; Ejtahed et al. 2011, 2012) and animal models (Yadav et al. 2007; Toral et al. 2014). To the best of our knowledge, this study is the first study assessing the effect of probiotics soy milk on mitochondrial DNA lesion among diabetic patients. In a study by Asemi et al. (2012), probiotic yogurt consumption for 9 weeks decreased the plasma concentration of 8-oxo-G among pregnant women. Epp Songisepp et al. (2005) indicated that probiotic *Lactobacillus fermentum* ME-3 caused

significant improvement of blood total antioxidative activity (TAA) and total antioxidative status (TAS) in healthy volunteers. Such results have been found with consumption of fermented goat milk by *Lactobacillus fermentum ME-3* for 3 weeks among healthy subjects (Kullisaar et al. 2003). The antioxidative effect of probiotic could be as a result of scavenging of superoxide anion radicals, free radicals, and hydrogen peroxide, and the inhibition of ascorbate autoxidation and chelation with metal ion (Lin and Yen 1999).

In the present study, the result of within-group comparison in intervention group and between-group comparisons indicated significant increase in terms of erythrocyte SOD activity in intervention group. Similar effects have been indicated in other studies such as administration of *Lactobacillus brevis BJ20* for 4 weeks among healthy subjects (Kang et al. 2012) and the consumption of probiotic yogurt containing *Lactobacillus acidophilus La5* and *Bifidobacteriumlactis Bb12* in diabetic patients for 6 weeks (Ejtahed et al. 2011). However, in another study, multispecies probiotics supplementation did not have any effect on SOD activity among healthy elderly participants (Valentini et al. 2014). This difference in findings can be described by the distinction between probiotic strain and dosage, or differences between participants. New evidence has shown that probiotic *Lactobacillus* exhibits antioxidative benefits under different in vitro and in vivo conditions (Kullisaar et al. 2002). *Lactobacillus* expresses Mn-superoxide dismutase (MnSOD) activity, can effectively eliminate hydroxyl and peroxyl radicals, and has the complete glutathione system necessary for glutathione recycling, transporting, and synthesis. Therefore, an increase in SOD activity may be directly because of antioxidant effect of *Lactobacillus plantarum A7.*

A short duration of intervention, the absence of control group that did not consume soy milk, and a limited number of examined genes were limitations in this study which must be considered while interpreting the results. Therefore, to fully confirm the positive effects of probiotics soy milk on the epigenetic process and oxidative stress in the management of diabetes disease, more investigations, with more numbers of examined genes, longer duration, and control group without soy milk should also be incorporated.

This trial has shown that the consumption of 200 ml/day soy milk containing *Lactobacillus plantarum A7* can have antioxidative properties and decrease the risk of mismatch base pair in DNA among patients with type II diabetes.

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Compliance with ethical standards

Conflict of interest The authors have none to declare.

Ethical approval Written informed consent was signed by all volunteers, and the protocol for the study was approved by Isfahan University of Medical Sciences (the trial has been registered in the Iranian Registry of Clinical Trials, identifier: IRCT: IRCT2014 05265062N8 available at: http://www.irct.ir).

References

Araki E, Nishikawa T (2010) Oxidative stress: a cause and therapeutic target of diabetic complications. J Diabetes Investig 1:90–96

Asemi Z, Jazayeri S, Najafi M, Samimi M, Mofid V, Shidfar F et al (2012) Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: a randomized controlled clinical trial. Ann Nutr Metab 60:62–68

Banerjee M, Vats P (2013) Reactive metabolites and antioxidant gene polymorphisms in Type 2 diabetes mellitus. Redox Biol 11:170–177

Bettstetter M, Dechant S, Ruemmele P, Vogel C, Kurz K, Morak M et al (2008) MethyQESD, a robust and fast method for quantitative methylation analyses in HNPCC diagnostics using formalin-fixed and paraffin-embedded tissue samples. Lab Invest 88:1367–1375

Castaneda-Gutierrez E, Moser M, Garcia-Rodenas C, Raymond F, Mansourian R, Rubio-Aliaga I et al (2014) Effect of a mixture of bovine milk oligosaccharides, Lactobacillus rhamnosus NCC4007 and long-chain polyunsaturated fatty acids on catch-up growth of intra-uterine growth-restricted rats. Acta Physiol 210:161–173

Castro AV, Kolka CM, Kim SP, Bergman RN (2014) Obesity, insulin resistance and comorbidities? Mechanisms of association. Arq Bras Endocrinol Metabol 58:600–609

Cho Y, Turner ND, Davidson LA, Chapkin RS, Carroll RJ, Lupton JR (2014) Colon cancer cell apoptosis is induced by combined exposure to the n-3 fatty acid docosahexaenoic acid and butyrate through promoter methylation. Exp Biol Med 239:302–310

Citar M, Hacin B, Tompa G, Stempelj M, Rogelj I, Dolinsek J et al (2014) Human intestinal mucosa-associated Lactobacillus and Bifidobacterium strains with probiotic properties modulate IL-10, IL-6 and IL-12 gene expression in THP-1 cells. Benef Microbes 12:1–12

Cuervo A, Salazar N, Ruas-Madiedo P, Gueimonde M, Gonzalez S (2013) Fiber from a regular diet is directly associated with fecal short-chain fatty acid concentrations in the elderly. Nutr Res 33:811–816

Eccleston J, Yan C, Yuan K, Alt FW, Selsing E (2011) Mismatch repair proteins MSH2, MLH1, and EXO1 are important for class-switch recombination events occurring in B cells that lack nonhomologous end joining. J Immunol 186:2336–2343

Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V et al (2011) Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci 94:3288–3294

Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V (2012) Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 28:539–543

Giacco F, Brownlee M (2010) Oxidative stress and diabetic complications. Circ Res 107:1058–1070

Haracska L, Yu SL, Johnson RE, Prakash L, Prakash S (2000) Efficient and accurate replication in the presence of 7,8-dihydro-8-oxoguanine by DNA polymerase eta. Nat Genet 25:458–461

Hegde ML, Hazra TK, Mitra S (2008) Early steps in the DNA base excision/single-strand interruption repair pathway in mammalian cells. Cell Res 18:27–47

Holliday R (1993) Epigenetic inheritance based on DNA methylation. Exs 64:452–468

Ingrosso D, Cimmino A, Perna AF, Masella L, De Santo NG, De Bonis ML et al (2003) Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. Lancet 361:1693–1699

Kang YM, Lee BJ, Kim JI, Nam BH, Cha JY, Kim YM et al (2012) Antioxidant effects of fermented sea tangle (Laminaria japonica) by Lactobacillus brevis BJ20 in individuals with high level of gamma-GT: a randomized, double-blind, and placebo-controlled clinical study. Food Chem Toxicol 50:1166–1169

Kryston TB, Georgiev AB, Pissis P, Georgakilas AG (2011) Role of oxidative stress and DNA damage in human carcinogenesis. Mutat Res 711:193–201

Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, Kairane C et al (2002) Two antioxidative lactobacilli strains as promising probiotics. Int J Food Microbiol 5(72):215–224

Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M (2003) Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 90:449–456

Kuo SM, Chan WC, Hu Z (2014) Wild-type and IL10-null mice have differential colonic epithelial gene expression responses to dietary supplementation with synbiotic Bifidobacterium animal is subspecies lactis and inulin. J Nutr 144:245–251

Li C, Nie SP, Zhu KX, Ding Q, Li C, Xiong T et al (2014) Lactobacillus plantarum NCU116 improves liver function, oxidative stress and lipid metabolism in rats with high fat diet induced non-alcoholic fatty liver disease. Food Funct 5:3216–3223

Lin MY, Yen CL (1999) Antioxidative ability of lactic acid bacteria. J Agric Food Chem 47:1460–1466

Matsukura H, Aisaki K, Igarashi K, Matsushima Y, Kanno J, Muramatsu M et al (2011) Genistein promotes DNA demethylation of the steroidogenic factor 1 (SF-1) promoter in endometrial stromal cells. Biochem Biophys Res 412:366–372

Neish AS (2009) Microbes in gastrointestinal health and disease. Gastroenterology 136:65–80

Newsholme P, Haber EP, Hirabara SM, Rebelato EL, Procopio J, Morgan D et al (2007) Diabetes associated cell stress and dysfunction: role of mitochondrial and non-mitochondrial ROS production and activity. J Physiol 583:9–24

Phillip CJ, Giardina CK, Bilir B, Cutler DJ, Lai YH, Kucuk O et al (2012) Genistein cooperates with the histone deacetylase inhibitor vorinostat to induce cell death in prostate cancer cells. BMC Cancer 12:145

Pudenz M, Roth K, Gerhauser C (2014) Impact of soy isoflavones on the epigenome in cancer prevention. Nutrients. 6:4218–4272

Reimer RA, Pelletier X, Carabin IG, Lyon MR, Gahler RJ, Wood S (2004) Faecal short chain fatty acids in healthy subjects participating in a randomised controlled trial examining a soluble highly viscous polysaccharide versus control. J Hum Nutr Diet 25:373–377

Slawinska A, Siwek MZ, Bednarczyk MF (2014) Effects of synbiotics injected in ovo on regulation of immune-related gene expression in adult chickens. Am J Vet Res 75:997–1003

Songisepp E, Kals J, Kullisaar T, Mandar R, Hutt P, Zilmer M et al (2005) Evaluation of the functional efficacy of an antioxidative probiotic in healthy volunteers. Nutr J 4:22

Spurling CC, Suhl JA, Boucher N, Nelson CE, Rosenberg DW, Giardina C (2008) The short chain fatty acid butyrate induces promoter demethylation and reactivation of RARbeta2 in colon cancer cells. Nutr Cancer 60:692–702

Stein K, Borowicki A, Scharlau D, Schettler A, Scheu K, Obst U et al (2012) Effects of synbiotic fermentation products on primary chemoprevention in human colon cells. J Nutr Biochem 23:777–784

Sun J, Wu Q, Sun H, Qiao Y (2014) Inhibition of histone deacetylase by butyrate protects rat liver from ischemic reperfusion injury. Int J Mol Sci 15:21069–21079

Teh SS, Ahmad R, Wan-Abdullah WN, Liong MT (2010) Enhanced growth of lactobacilli in soymilk upon immobilization on agrowastes. J Food Sci 75:M155–M164

Toral M, Gomez-Guzman M, Jimenez R, Romero M, Sanchez M, Utrilla MP et al (2014) The probiotic Lactobacillus coryniformis CECT5711 reduces the vascular pro-oxidant and pro-inflammatory status in obese mice. Clin Sci 127:33–45

Tsangalis D, Wilcox G, Shah NP, Stojanovska L (2005) Bioavailability of isoflavone phytoestrogens in postmenopausal women consuming soya milk fermented with probiotic bifidobacteria. Br J Nutr 93:867–877

Valentini L, Pinto A, Bourdel-Marchasson I, Ostan R, Brigidi P, Turroni S et al. (2014) Impact of personalized diet and probiotic supplementation on inflammation, nutritional parameters and intestinal microbiota—The "RISTOMED project": randomized controlled trial in healthy older people. Clin Nutr 34:593–602

Waris G, Ahsan H (2006) Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 5:14

Worthley DL, Le Leu RK, Whitehall VL, Conlon M, Christophersen C, Belobrajdic D et al (2009) A human, double-blind, placebo-controlled, crossover trial of prebiotic, probiotic, and synbiotic supplementation: effects on luminal, inflammatory, epigenetic, and epithelial biomarkers of colorectal cancer. Am J Clin Nutr 90:578–586

Yadav H, Jain S, Sinha PR (2007) Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition 23:62–68

Young GP, Hu Y, Le Leu RK, Nyskohus L (2005) Dietary fibre and colorectal cancer: a model for environment–gene interactions. Mol Nutr Food Res 49:571–584

Zhang Y, Li Q, Chen H (2013) DNA methylation and histone modifications of Wnt genes by genistein during colon cancer development. Carcinogenesis 34:1756–1763

Zielinska D, Kolozyn-Krajewska D, Goryl A, Motyl I (2014) Predictive modelling of Lactobacillus casei KN291 survival in fermented soy beverage. J Microbiol. 52:169–178