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Effects of probiotic yogurt on glycemic indexes and endothelial dysfunction markers in patients with metabolic syndrome

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ABSTRACT

Objectives: The relationship between gut microflora and metabolic syndrome components such as obesity, low-grade chronic systemic inflammation, dyslipidemia, and altered glucose metabolism is now acknowledged. The aim of this study was to assess the effects of probiotic yogurt on glycemic indexes and endothelial dysfunction markers in patients with metabolic syndrome.

Methods: This was a randomized, double-blind, placebo-controlled clinical trial of 44 patients with metabolic syndrome (22 men and 22 women), who were 20 to 65 y of age. The patients were assigned to either a treatment or control group and consumed 300 g/d of probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 or a regular yogurt for 2 mo, respectively. Each group contained 22 participants. Fasting blood glucose and serum insulin was performed to derive homeostasis model assessment of insulin resistance (HOMA-IR), insulin sensitivity (Quicki), and HOMA of β-cell function (HOMA- β). In addition, markers of vascular cell adhesion molecule cell (VCAM)-1, intercellular adhesion molecule cell (ICAM)-1, and plasminogen activator inhibitor (PAI)-1 were measured to evaluate endothelial function at the beginning and at the end of the study.

Results: Consumption of probiotic yogurt resulted in a significant reduction in the level of blood glucose and VCAM-1. Significant changes in PAI-1, VCAM-1, insulin, HOMA-IR, and Quicki were observed in the probiotic yogurt group after intervention compared with baseline.

Conclusion: Consumption of probiotic yogurt improved fasting blood glucose and partly modified serum endothelial function markers. These results suggest that regular intake of probiotic yogurt may exert positive effects on the treatment of metabolic syndrome.

Introduction

Metabolic syndrome (MetS), characterized by the coexistence of interrelated cardiovascular risk factors, greatly increases the risk for atherosclerotic cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [1–3] MetS components, including abdominal obesity, impaired glucose metabolism, dyslipidemia, and hypertension, can individually impair endothelial function. Therefore, endothelial dysfunction can be more prevalent in patients with MetS [4–7].

Endothelial dysfunction, characterized by deficiency of nitric oxide production, overexpression of adhesion molecules on the surface of endothelial cell, and inflammatory changes [8], plays a major role in the pathogenesis of atherosclerosis and T2DM and also is a predictive factor for vascular events [9–12]. Moreover, a strong relationship has been reported between MetS and increased levels of circulating adhesion molecules such as vascular cell adhesion molecule (VCAM)-1, intracellular cell adhesion molecule (ICAM)-1, and plasminogen activator inhibitor (PAI)-1 [13–18].

The relationship between gut microflora and MetS components such as obesity, low-grade chronic systemic inflammation, dyslipidemia, and altered glucose metabolism is now acknowledged [19,20]. Hence, multiple therapeutic strategies have been suggested to change the composition of the gut microbiota. One of the suggested strategies for the prevention of MetS is the manipulation of intestinal flora using probiotics [21,22]. Probiotics are defined as

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live microorganisms that exert health benefits on the host when administered in adequate amounts [23,24].

To the best of our knowledge, there is no clinical trial to date investigating the effects of probiotic yogurt consumption on the glycemic indexes and vascular endothelial dysfunction markers in patients with MetS. The aim of the present study was to investigate the effects of consuming probiotic yogurt containing probiotic strains *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 on the homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (Quicki), and β-cell function (HOMA-β) or serum levels of VCAM-1, ICAM-1, PAI-1, insulin, and fasting blood glucose (FBG) in patients with MetS.

Materials and methods

Study design and participants

This was a randomized, double-blind, placebo-controlled study conducted in Tabriz, Iran from October 2016 to March 2017. Participants were recruited through public call. Inclusion criteria included diagnosis of MetS under the supervision of a cardiologist and age range of 20 to 65 y. The following conditions resulted in exclusion from the study: present or participation in a weight loss program during the 3 mo before the beginning of the study; participation in sports activities >100 min/wk; antibiotic treatment during the previous 7 d; concurrent use of supplements (vitamins and minerals, fiber, ω-3 and pro-, pre, or synbiotics) during the previous 2 mo; chronic use of laxative (more than twice weekly); recent antihyperglycemic or lipid-lowering treatment; hormone therapy; immunomodulatory therapy (during the 30 d before the study) or consumption of contraceptive drugs or any drug that affects the neuroendocrine function, metabolism, or appetite. In addition, individuals with CVD, renal failure, diabetes, or gastrointestinal (GI) diseases; those with history of cancer, thyroid problems, GI surgery during the past year; and those who consumed alcohol, smoked, and used tobacco were excluded from participation. Prescreening of potential participants was carried out over the telephone or in person and qualified individuals were evaluated for MetS at first visit. MetS was defined according to the international criteria [25]. The cutoff for waist circumference was based on the report of the Iranian National Committee of Obesity [26]. The study was approved by Ethical Committee of Tabriz University of Medical Sciences and was registered on the Iranian Registry of Clinical Trials.

Randomization and intervention

Participants were randomly divided into two groups (22 in each group) using a block randomization procedure of size 4. Randomization was generated by Random Allocation Software (RAS), version 1.0 [27] with stratification based on age, sex, and body mass index (BMI). Both probiotic and regular yogurts contained *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Moreover, the probiotic yogurt was enriched by adding direct-vat-set cultures of *B. lactis* Bb12 and *L. acidophilus* La5 (Chr. Hansen, Hørsholm, Denmark). Participants in the treatment and control groups consumed 300 g/d of probiotic and regular yogurt for 8 wk, respectively. According to the microbiologic analysis, the average counts of *L. acidophilus* La5 and *B. lactis* Bb12 in the probiotic yogurt was 6.45×10^6 and 4.94×10^6 cfu/g, respectively, at the first day after production, which became 2.30×10^6 and 2×10^6 cfu/g, respectively, on day 7. Both probiotic bacteria showed a steady survival rate during the 7-d storage time at an average concentration of 4.41×10^6 and 3.55×10^6 cfu/g, respectively.

Laboratory analyses

Blood samples were collected after a 12-h overnight fast at the baseline and at the end of the study. Serum was separated from whole blood by centrifugation at 1000*g* for 10 min (Hettich, Germany). Serum samples were frozen immediately and maintained at −70°C until the assay. Blood samples were analyzed at the Danesh Pathobiology Laboratory (Tabriz, Iran). FBG was measured enzymatically with a commercial kit (Pars Azmun Co., Tehran, Iran) by an auto-analyzer (Hitachi 917, Boehringer Mannheim, Japan) on the sampling day. Serum insulin level was measured by chemiluminescence (Immulite 2000 insulin, Siemens, Germany) and IR was determined by the HOMA index with the formula:

$$HOMA - IR = \text{fastinginsulin}(\mu U/mL) \times \text{fastingglucose}(mg/dL)/405$$

[28].

The Quicki was calculated with the following formula:

$$1/[log(I0) + \log(G0)]$$

where G_0 and I_0 are glucose and insulin at baseline, respectively [29].

β-cell function was estimated by HOMA-β:

$$HOMA - \beta = \text{fastinginsulin}(\mu U/mL) \times 360/(\text{fastingglucose}(mg/dL) - 63)$$

[28].

Serum VCAM-1, ICAM-1, and PAI-1 levels were measured using enzyme-linked immunosorbent assay (IBL, International GmbH, Hamburg,Germany) according to the manufacturer's instructions. The inter- and intraassay coefficients of variation were 5.2% and 3.1% for VCAM-1, 7.7% and 4.1% for ICAM-1, and 5% and 4.7% for PAI-1, respectively.

Physical activity and dietary intake

Physical activity and dietary intakes were assessed at the beginning and at the end of the study. The short form of international Physical Activity Questionnaire with three levels of metabolic equivalent (MET), including walking (3.3 METs), moderate intensity (4 METs), and vigorous intensity (8 METs), was used to determine the physical activity level. The MET-min/wk was calculated by:

MET level × minutes of activity/d × d/wk.

All types of walking were included and an average MET value for walking was created. The same procedure was undertaken for moderate-intensity and vigorous-intensity activities. The total physical activity MET-min/wk score was the sum of total (walking + moderate + vigorous) MET-min/wk scores. Physical activities of different intensities were ranked to three levels that were named as low (<600 MET-min/wk), moderate (≥600 MET-min/wk), and high (≥1500 MET-min/wk). Dietary intakes were assessed based on 1-d recall and 2-d records and were analyzed by the Nutritionist IV software program (First Databank Inc., Hearst Corp., San Bruno, CA, USA).

Statistical analysis

Sample size was calculated based on the change of VCAM-1 levels published in a study conducted by Tripolt et al. [30] with $\alpha = 0.05$ and a power of 90%, which computed 18 participants per group. By considering a 25% dropout, 22 individuals were assigned to each group. The experimental data were analyzed by SPSS version 21 (IBM Corp., Armonk, NY, USA) and the results were expressed as mean ± SD. Kolmogorov–Smirnov goodness of fit test was used to determine the distribution of data. The differences between the groups at the baseline were assessed using independent sample *t* tests, Mann–Whitney, and χ^2 test. Analysis of covariance (ANCOVA) was used to identify any differences between groups after intervention, adjusting the changes of BMI, calorie, and baseline values as covariates. The paired *t* test, nonparametric Wilcoxon signed-rank test, and sign test were used for assessing differences within the group. $P < 0.05$ was considered statistically significant.

Results

Figure 1 shows the flowchart for selection and analyzing of the participants in the trial. All participants completed the study and demonstrated good compliance with yogurt consumption. No side effects or complications were reported. The general characteristics of the participants in both probiotic and regular yogurt groups are listed in Table 1. There were no significant differences in terms of age, sex, BMI, and physical activity at baseline ($P > 0.05$) between either group.

Table 2 presents the daily dietary intakes at baseline and after intervention. No significant differences were observed in the daily dietary intake of energy and macronutrients at baseline and after 2 mo intervention ($P > 0.05$) within or between groups.

Table 3 presents the glycemic parameters of participants at baseline and after intervention. There were no significant differences in FBG, insulin, HOMA-IR, HOMA-β, or Quicki at baseline. Significant decreases ($P < 0.05$) in the levels of FBG, insulin, and HOMA-IR (4.78, 10.47, 14.55%, respectively) and a significant increase ($P < 0.05$) in the leveld of Quicki (3.12%) in serum was reported for the probiotic yogurt group after 8 wk of intervention compared with baseline values. Consumption of probiotic yogurt resulted in 1.40%, 0.86%, and 0.36% reductions in glucose, insulin, and HOMA-IR, respectively, compared with the control group. Results of ANCOVA showed a statistically significant difference in the level of glucose ($P = 0.001$), whereas no significant difference was observed in the level of insulin, HOMA-IR, HOMA-β, or Quicki

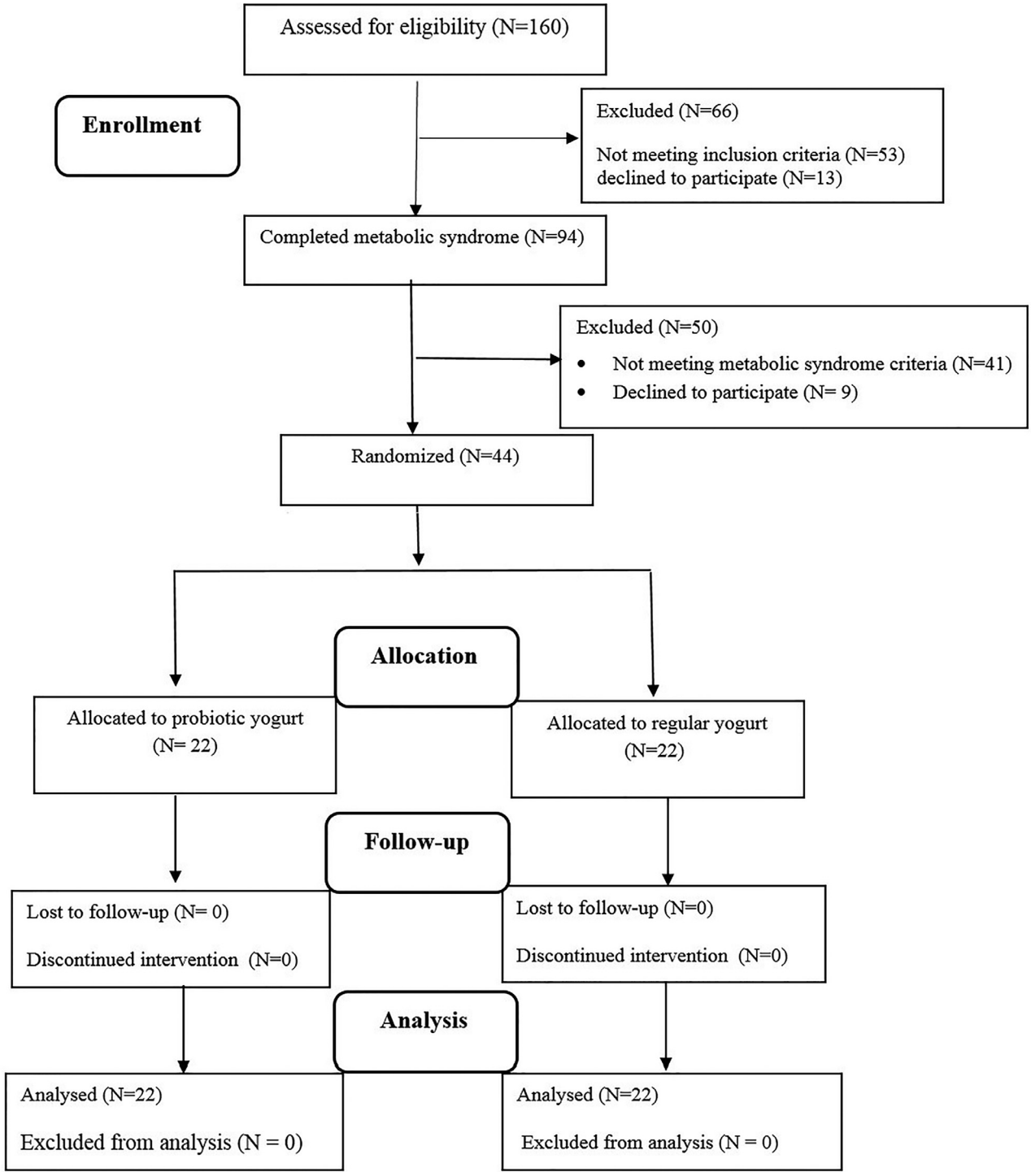

Fig. 1. Flowchart of selection of and analyzing the participants in the trial.

in serum between the two groups at the end of the study, when values were adjusted for energy, BMI, and baseline values.

Discussion

The results of the present study showed a significant decrease in the levels of glucose, important cardiovascular risk factors, in the probiotic yogurt group. This is in accordance with the findings of Barreto et al. [31], who reported that consumption of fermented milk containing a strain of *L. plantarum* for 12 wk caused a significant reduction in fasting glucose in postmenopausal women with MetS. Furthermore, Ejtahed et al. concluded that consumption of probiotic yogurt (similar to the one used in the present study) for 6 wk significantly decreased FBG levels in patients with T2DM [32]. Moreover, the results of this study are in accordance with the animal studies that have reported blood glucose improvement after consumption of probiotics [33–40]. Several animal studies have reported mechanisms by which probiotics reduce blood glucose

Table 1
General characteristics of the study participants

Variables		Probiotic yogurt (n = 22)	Regular yogurt (n = 22)	*P*-value*
Sex, n (%)	Male	11(50)	11(50)	1.00†
	Female	11(50)	11(50)	
Age (y)		6.60 ±44.05	5.70 ± 44.55	0.789
Weight (kg)		10.10 ± 87.07	12.79 ± 87.25	0.960
BMI (kg/m^2)		3.64 ± 32.09	4.02 ±32.26	0.883
Physical activity, n (%)				
Before	Low	16 (72.7)	17 (77.3)	1.00†
	Moderate	5 (22.7)	3 (13.6)	
	High	1 (4.5)	2 (9.1)	
After	Low	15 (68.2)	16 (72.7)	1.00‡
	Moderate	5 (22.7)	2 (9.1)	
	High	2 (9.1)	4 (18.2)	
P-value§		0.500	1.00	

BMI, body mass index.
Data presented as means ± SD for quantitative variable.
*P-values for independent t test.
†P-values for χ^2 test.
‡P-values based on χ^2 test on differences.
§P-values for sign test.

Table 2
Daily dietary intakes at baseline and after intervention

Variables		Probiotic yogurt (n = 22)	Regular yogurt (n = 22)	*P*-value*
Energy (kcal/d)	Before	317.18 ± 2429.50	294.23 ±2404.78	0.436
	After	381.21 ± 2468.68	416. ±2391.31	
	P-value†	0.338	0.787	
Carbohydrate (g/d)	Before	79.47 ± 408.12	57.31 ±412.41	0.355
	After	85.76 ±416.38	83.11 ±401.62	
	P-value†	0.595	0.392	
Protein (g/d)	Before	12.81 ±86.95	12.59 ± 81.01	0.564
	After	17.93 ± 83.11	21.46 ± 83.85	
	P-value†	0.422	0.470	
Fat (g/d)	Before	13.31 ± 48.89	14.26 ±51.27	0.555
	After	16.58 ± 51.68	21.78± 55.71	
	P-value†	0.545	0.363	

Data presented as means ± SD.
*P-values based on analysis of covariance after adjustment for baseline values.
†P-values for independent t test.

[35,41,42]. The gut microbiota modulation, changes in intestinal environment, gut permeability, gene expression, and regulation of immune responses have been proposed as the potential mechanisms of probiotic action [41,43]. Redution in intestinal glucose absorbtion as a result of the colonization of probiotics in intestinal epithelium and their glucose usage as their major source of energy are other proposed mechanism involved in the glucose-lowering effect of probiotics [36]. Van Baarlen et al. reported that probiotics consumption directly affects the human inflammatory status and blood glucose [44]. Conversely, a number of studies relating to the effect of probiotics have failed to show any significant reduction in blood glucose levels [45–47]. The inconsistent findings regarding the effect of probiotics on blood glucose may be due to the differences in the duration of intervention, supplement dose (cfu), the type of carrier, baseline levels of glucose, sample size, and the participants' baseline characteristics [48].

Fasting insulin levels and HOMA-IR decreased significantly and the level of Quicki increased significantly from baseline to the end of intervention in the probiotic yogurt group; however, these changes were not significantly different when compared with the control group. In addition, probiotic yogurt consumption did not significantly affect β-cell function (HOMA-β) in this trial. These results are in accordance with the findings reported by Asemi et al. [43], Gøbel et al. [49], Ivey et al. [50], and Ejtehad et al. [51]. In contrast, the beneficial effects of probiotics on insulin levels and HOMA-IR have been previously reported by several authors [35,36,52,53].

The discrepancy between findings of the present study and trials that reported beneficial effects of probiotics on insulin levels and IR might be due to the difference in the study design and baseline characteristic of the patients. Studies reporting beneficial effects of probiotic supplementation on insulin levels and HOMA-IR have used models of induced diabetes or naturally occurring IR [35,36,52]. Ivey et al. hypothesized that the beneficial effects of probiotics on insulin levels and IR may be limited to pathologic states of T2DM [50]. Sun et al. [54], in a meta-analysis, examined the effect of probiotics on glucose and glycemic factors in diabetes and its associated risk factors. They concluded that probiotic consumption had a more significant effect on reducing glucose metabolism (insulin, HOMA-IR, and hemoglobin A1c) in the diabetes population compared with populations having risk factors for diabetes. A similar result has been observed in animal studies [54].

In addition, probiotic bacteria activities are highly variable and are influenced by numerous factors including probiotic–microbiome–host interaction, bacteria residing in the GI tract, and the genotype of the host. Furthermore, it has been demonstrated that the host diet can affect probiotic metabolism [50].

Endothelial dysfunction parameters of participants at baseline and at the end of the study are listed in Table 4. There were no significant differences in VCAM-1, ICAM-1, and PAI-1 levels at the beginning of the study between either group ($P > 0.05$). Consumption of probiotic yogurt resulted in a significant reduction ($P < 0.05$) in VCAM-1 and PAI-1 (by 42.24% and 14.74%, respectively) compared with the baseline values. Change in the level of ICAM-1 was not statistically significant at the end of the study compared with the baseline in either group ($P > 0.05$). Results of ANCOVA showed a statistically significant difference in the level of VCAM-1 in serum at the end of the study ($P = 0.001$); whereas no significant changes were observed in the levels of ICAM-1 and PAI-1 in serum between either group at the end of the study, adjusted for energy, BMI, and baseline values ($P > 0.05$).

Adhesion molecules including VCAM-1, ICAM-1, and PAI-1 are vascular inflammatory markers for endothelial dysfunction and are elevated in MetS [55–57]. A positive association has been demonstrated between high levels of adhesion molecules and CVDs [58–60]. To our knowledge, this was the first randomized controlled trial to investigate the effects of probiotic yogurt on VCAM-1, ICAM-1, and PAI-1 levels in patients with MetS. The results of this study revealed that consumption of probiotic yogurt containing *L. acidophilus* La5 and *B. lactis* Bb12 for 8 wk resulted in significant redutions in VCAM-1 and PAI-1 levels in the in serum of patients with MetS. However, no significant changes were seen in PAI-1 and ICAM-1 in patients at the end of the study in either group. In a 12-wk study of patients with MetS conducted by Tripolt et al. [30], the consumption of a milk drink containing a probiotic strain significantly decreased VCAM-1 levels. However, no improvements were observed in Quicki, HOMA-IR, or β-cell function, nor in the parameters used to assess endothelial dysfunction. Hlivak et al., in a clinical study, reported that long-term (56-wk) oral administration of capsules containing probiotics resulted in a significant reduction in ICAM-1 of adults [61]. In an in vivo study conducted on the lipopolysaccharide-induced mouse, *L. plantarum* Lp91 significantly down-regulated the expression of VCAM-1 and ICAM-1 [62]. In a study by Angulo et al. [63] conducted on colitic rats, it was demonstrated that *L. casei* down-regulated the expression of ICAM-1.

It has been reported that even a mild derangement in glucose metabolism may be associated with endothelial dysfunction

Table 3
Glycemic parameters of participants at baseline and after intervention

Variables*	Probiotic yogurt (n = 22)	Regular yogurt (n = 22)	MD (95% CI)† *P*-value
FBG (mg/dL)			
Before	10.47 ± 100.45	11.93 ± 97.82	−3.80 (−6.05 to −1.55); 0.001
After	10.58 ± 95.64	7.72 ± 97.00	
MD (95% CI)† *P*-value	− 4.81(−6.63 to −3.00); 0.001	−0.82 (−3.14 to 1.51); 0.473	
Insulin (mu/L)			
Before	3.62 ± 12.98	3.18 ± 12.30	− 0.55 (−2.36 to 1.26); 0.544
After	4.42 ± 11.62	3.60 ± 11.52	
MD (95% CI)† *P*-value	−1.36 (−2.72 to−0.01); 0.048	−0.78 (−2.03 to 0.46); 0.206	
HOMA-IR			
Before	0.99 ± 3.23	0.75 ± 2.94	−0.29 (−0.71 to 0.11); 0.154
After	1.14 ± 2.76	0.93 ± 2.77	
MD (95% CI)† *P*-value	−0.47 (−0.78 to −0.16); 0.004	−0.17 (−0.45 to 0.09); 0.193	
HOMA-β			
Before	55.01 ± 133.04	88.77 ± 150.90	23.52 (−10.09 to 57.14); 0.165
After	80.54 ± 141.23	53.82 ± 128.78	
MD (95% CI)† *P*-value	8.19 (−17.83, 34.21); 0.154	−22.12 (−49.50 to 5.27); 0.108	
Quicki			
Before	0.01 ± 0.32	0.01 ± 0.32	0.008 (−0.002 to 0.017); 0.107
After	0.02 ± 0.33	0.01 ± 0.33	
MD (95% CI)† *P*-value	0.01 (0.003 to 0.019); 0.007	0.01 (−0.0005 to 0.009); 0.081	

BMI, body mass index; FBG, fasting blood glucose; HOMA, homeostasis model assessment of insulin resistance; IR, insulin resistance; MD, Mean Difference; Quicki, quantitative insulin sensitivity check index.
*Data presented as means ± SD.
†Denotes the significance of within-group changes (paired-samples *t* test).
‡Denotes the significance of between group changes (analysis of covariance test). Adjusted for baseline values and changes on body mass index and calorie.

Table 4
Endothelial dysfunction parameters of participants at baseline and after intervention

Variables*	Probiotic yogurt (n = 22)	Regular yogurt (n = 22)	MD (95% CI)† *P*-value
VCAM-1 (ng/m)			−463.39 (−672.56 to −254.21); 0.001
Before	813.38 ± 1646.36	644.26 ± 1427.95	
After	256.25 ± 950.91	493.34 ± 1373.41	
MD (95% CI)† *P*-value	− 695.45 (−1029.43 to −361.47); 0.001	−54.54 (−259.36 to 150.27); 0.586	
ICAM-1 (ng/m)			−16.65 (−60.12, 26.82); 0.395
Before	69.82 ± 317.27	86.34 ± 302.27	
After	97.35 ± 310.18	90.14 ± 321.23	
MD (95% CI)† *P*-value	− 7.09 (−45.96 to 31.78); 0.708	18.96 (−5.19 to 43.10); 0.118	
PAI-1 (ng/mL)			−5.48 (−18.40 to 7.43); 0.386
Before	27.65 ± 83.12	16.74 ± 81.29	
After	16.25 ± 70.86	25.38 ±76.40	
MD (95% CI)† *P*-value	−12.25 (−24.16 to −0.33); 0.044	−4.89 (−17.41 to 7.63); 0.426	

ICAM, intercellular adhesion molecule cell; MD, Mean Difference; PAI, plasminogen activator inhibitor; VCAM, vascular cell adhesion molecule cell.
*Data presented as means ± SD.
†Denotes the significance of within-group changes (paired-samples *t* test).
‡Denotes the significance of between group changes (analysis of covariance test). Adjusted for baseline values and changes on body mass index and calories.

[64–66]. Based on the results of the present study, consumption of probiotic yogurt significantly decreased fasting glucose ($P < 0.05$); therefore, the improvement of endothelial function (decreased VCAM-1 in the probiotic yogurt group) may be due to decreased blood glucose levels.

The present study did not demonstrate significant differences in the serum levels of ICAM-1 and PAI-1 between the two groups at the end of the study. To the best of our knowledge, this is the first randomized controlled trial to investigate the effects of probiotic yogurt on the levels of PAI-1 in humans and there are no clinical trials in this regard. In addition, previous evidence regarding the beneficial effects of probiotics on ICAM-1 in humans was limited to the study conducted by Hlivak et al., in which the intervention duration (56 wk) was longer and the probiotics dose (2×10^9 CFU *Enterococcus faecium* M-74 strain) was higher than the present study [61]. Similar results have been observed in animal studies. In animal studies that have reported positive results, the consumed dose of probiotics was higher than in this study [61,62]. Furthermore, in all mentioned studies, only one strain of probiotics has been used, whereas in our work, the combination of two strains has been incorporated [61,62,66]. Therefore, it seems that the difference in the intervention period, probiotic strain, dosage, and combination as well as the difference in the studied patients, influence the results of the present study. Further studies using combination of *L. acidophilus* La5 and *B. lactis* Bb12 in other dosages, another form (capsule, etc), and for a longer treatment period are needed to confirm our findings.

Conclusion

Daily consumption of a probiotic yogurt containing *L. acidophilus* La5 and *B. lactis* Bb12 for 8 wk was associated with decreased levels of blood glucose and VCAM-1 in patients with MetS. Although there was no significant change in the levels of insulin, HOMA-IR,

HOMA-β, Quicki, ICAM-1, or PAI-1 at the end of the trial, reductions of blood glucose and VCAM-1 were expected to improve the endothelial function and decrease cardiovascular risk induced by MetS.

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