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ORIGINAL ARTICLE

Probiotics Supplementation on Cardiac Remodeling Following Myocardial Infarction: a Single-Center Double-Blind Clinical Study

Jalal Moludi[1,2] · **Somaieh Saiedi**[3] · **Behzad Ebrahimi**[4] · **Mohammad Alizadeh**[5] · **Yaser Khajebishak**[4] · **Sevda Saleh Ghadimi**[5]

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Abstract

Adverse cardiac remodeling after myocardial infarction (MI) can lead to the syndrome of heart failure (HF). Recently, changes in gut microbiota composition (dysbiosis) have appeared as a novel candidate that may be linked to the development of CR and HF. The aim of this trial was to evaluate the effects of probiotics administration on attenuating CR in patients with MI. A single-center double-blind placebo-controlled stratified randomized clinical study was conducted in 44 subjects with MI who underwent percutaneous coronary intervention (PCI). Patients were randomly assigned to take, with lunch, either a probiotic capsule containing 1.6×10^9 colony-forming unit (CFU) of bacteria (treatment group) or capsules contained inulin (control group) over 3 months. CR biomarkers (including serum procollagen III, transforming growth factor beta (TGF-β), trimethylamine *N*-oxide (TMAO), and matrix metallopeptidase 9 (MMP-9)) were assessed. Echocardiography results were measured at baseline and after the intervention. Significant decreases were seen in serum TGF-β concentrations (-8.0 ± 2.1 vs. -4.01 ± 1.8 pg/mL, $p = 0.001$) and TMAO levels (-17.43 ± 10.20 vs. -4.54 ± 8.7 mmol/L, $p = 0.043$), and there were no differences were seen in MMP-9 (-4.1 ± 0.12 vs. $-4.01 + 0.15$ nmol/mL, $p = 0.443$) and procollagen III levels (-1.35 ± 0.70 vs. $0.01 + 0.3$ mg/L, $p = 0.392$) subsequent to probiotics supplementation compared with the placebo group. Improvements in echocardiographic indices were also greater in the probiotics group as compared with that in the control group, but not at a significant level. Regression analysis revealed that baseline left ventricular ejection fraction (LVEF), and changes of procollagen III, predicted 62% of the final LVEF levels. Probiotics administration may have a beneficial effect on the cardiac remodeling process in patients with myocardial infarction. Iranian Registry of Clinical Trials (IRCT): IRCT20121028011288N15

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✉ Jalal Moludi
jmoludi@yahoo.com

✉ Mohammad Alizadeh
mdalizadeh@gmail.com

1 School of Nutrition Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran

2 Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

3 Nutrition Research Center, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran

4 Maragheh University of Medical Sciences, Maragheh, Iran

5 Department of Clinical Nutrition, School of Nutrition and Food Sciences, Faculty of Nutrition and Food Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

Keywords Heart failure · Cardiac remodeling · Microbiome · Probiotics · Myocardial infarction · Rehabilitation

Introduction

Heart failure (HF) is a public health concern with a poor prognosis and a high mortality rate [1]. Myocardial infarction (MI) is a leading cause of HF, which can progress rapidly after MI and may continue or develop by a process called cardiac remodeling [2, 3]. Although the routine use of antiplatelet therapy and percutaneous coronary intervention (PCI) is standard in the management of patients with MI, the incidence of HF among patients with MI is still unacceptably high [4]. Additionally, although angiotensin-converting enzyme (ACE) inhibitors, β-receptor blocking agents, and aldosterone receptor antagonists are useful in reducing the incidence of

HF, their efficacy is not optimum. Some patients continue to have HF symptoms [5]. Accordingly, there is a must for supplementary treatment methods and strategies.

In recent years, many studies have identified mechanisms linking the role of gut microbiota in low-grade inflammation in cardiovascular disease (CVD) settings [6]. Many studies also have uncovered a relationship between gut microbiota and the progression of HF [7]. Studies have revealed that altered intestinal microbiota (dysbiosis) is involved in the development of HF [7, 8]. Additionally, a large body of evidence is being collected regarding probiotics administration and beneficial effects on the reduction of cardiovascular risk factors [9].

More recently, increased levels of trimethylamine *N*-oxide (TMAO), a gut bacterial metabolite, has been suggested as a novel risk factor in HF development [10, 11]. Alteration in gut barrier function (dysbiosis) also leads to increased TMAO levels (a "colorless amine oxide produced from betaine, choline, and carnitine via gut microbiota metabolism" [12]). It has been also found that TMAO levels are substantially higher in individuals with HF compared with that in control subjects [10]. Additionally, TMAO induced cardiac hypertrophy and cardiac fibrosis [13]. Furthermore, TMAO levels are strongly associated with gut microbiota and it has been found that gut microbiota modulation using probiotics can lead to a decrease in TMAO levels [14].

Probiotic supplements have been shown to be effective in preventing HF and adverse outcomes [15]. However, there have been few randomized controlled trials regarding the effect of probiotics administration in attenuating cardiac remodeling following myocardial infarction (MI). The current research study builds on the immune-regulatory, anti-inflammatory, and antiproliferative effects of probiotics [16, 17]. Hence, the present trial was designed to assess the effects of probiotics supplementation on echocardiography and biomarkers of cardiac remodeling in subjects with MI.

Materials and Methods

Participants

Our study protocol was conducted in accordance with the Helsinki Declaration of the World Medical Association (2000) and was accepted by the Ethics Committee of the Tabriz University of Medical Sciences (IR.TBZMED.REC.1397.184). The protocol of the study has already been published [18] and listed in the Iranian Registry of Clinical Trials (IRCT) (IRCT20121028011288N15). In brief, 44 patients with new MI who underwent percutaneous transluminal coronary angioplasty (PTCA) participated in this clinical trial. This single-center, double-blind, randomized, stratified, placebo-controlled, clinical study was carried out at the Cardiology Clinic at the Shahid Madani Heart Center, affiliated with the Tabriz University of Medical Science, Iran, from April to October 2018.

All subjects admitted for new MI were considered for involvement in the trial and screened by a cardiologist for appropriateness. Subjects were excluded if they declined to participate, had a low left ventricular ejection fraction (< 35%), or failed PCI. A third party who was unaware of the study provided a randomization sequence from allocation software.

Informed consent was obtained from each participant. In this study, patients were randomly assigned to two groups to take either *Lactobacillus rhamnosus* GG (LGG) as a probiotic ($n = 22$) or placebo ($n = 22$) for 3 months. Information related to participants' registration is shown in Fig. 1. Both contributors and researchers were blind to the allocation and to the intervention.

Patients in the intervention group received one probiotic capsule daily, including a *Lactobacillus rhamnosus* GG (LGG) 1.6×10^9 colony-forming unit (CFU) with their lunch. The placebo (control) group took capsules Inulin (by TakGen Zist Medicinal Company, Iran). Adherence to the dosage regiment was checked by asking participants to return the capsule containers.

Dietary Assessment

Dietary intake was assessed using a dietary record at months 0, 1, and 3 of intervention. We used Nutritionist IV software (The Hearst Corp., San Bruno, CA, USA) adjusted for Iranian diets to acquire nutrient intakes of participants based on these average 3-day food diaries.

Physical Activity Assessment

The physical activity assessment was carried out to monitor patients' usual physical activity levels throughout the study. The validated short-form International Physical Activity Questionnaire (IPAQ) was used to measure the participant's physical activity. Based on previous studies, physical activities were classified as low, moderate, and high [19].

Biomarker of Cardiac Remodeling

After 10 h of fasting, blood samples were taken by venipuncture of the antecubital vein, using vacuum tubes. After centrifugation, aliquots of samples were stored at – 80 °C until further analysis. Sera procollagen III, transforming growth factor beta (TGF-β), trimethylamine *N*-oxide (TMAO), and matrix metallopeptidase 9 (MMP-9) were assessed using an enzyme-linked immunosorbent assay (ELISA) (Crystal Day, Shanghai) according to the manufacturer's instructions.

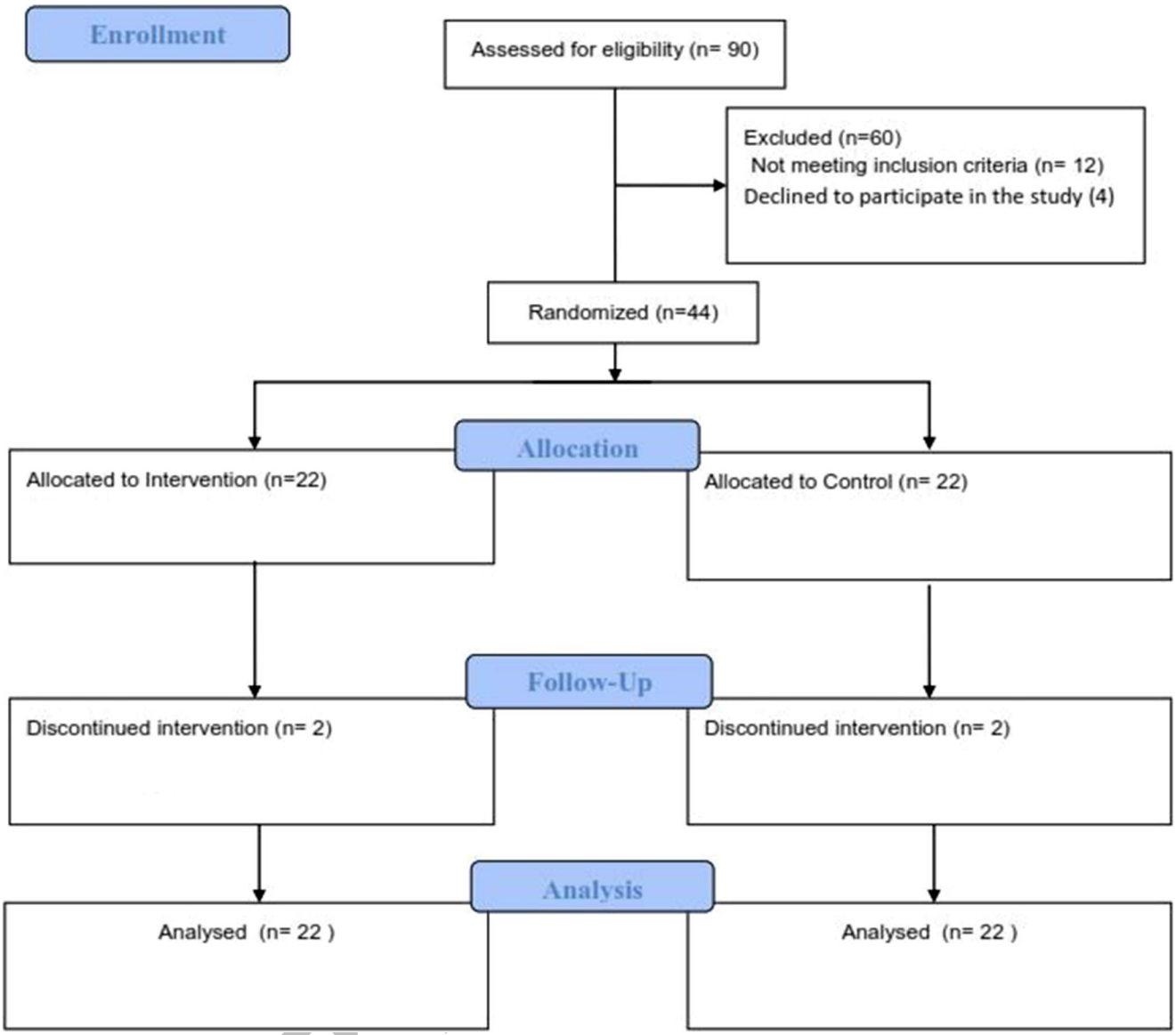

Fig. 1 Information related to participants' registration

Serum high-sensitivity C-reactive protein (hs-CRP) levels were evaluated using immunoturbidimetry.

DNA Extraction and Real-time PCR Analysis for Evaluation of Gut Microbiota

Morning fecal samples were collected from subjects before and after 3 months of intervention via a sterile container, which was then transported to the laboratory in a frozen condition and stored at – 80 °C until analysis. The quantity of Firmicutes and Bacteroidetes was assessed. Real-time PCR (SYBR green method) was performed for each sample using qRT-PCR (Rotor Gene 3000, Corbett Life Science, Mortlake, Australia). Each RT-PCR reaction contained 10 mM of each primer of Firmicutes, Bacteroidetes, and *LGG*, 5 μL SYBR Green Master mix (Fermentase, Waltham, MA, USA), and 2 μL DNA template in a 10-μL reaction volume. The Firmicutes to Bacteroidetes (F/B) ratio was then calculated for both groups.

Statistical Analysis

The data were analyzed using IBM SPSS® software (version 19; SPSS Inc., Chicago, IL) and the results were expressed as mean and standard deviation (SD). To determine the normal distribution of data, we used skewness and kurtosis. Comparison baselines were obtained using a paired sample *t* test. Analysis of covariance (ANCOVA) was employed to control for potential confounding variables. To investigate the predictors of change in EF levels, a hierarchical stepwise regression analysis was performed; the EF score was entered as

the dependent variable, and the predictors investigated included baseline EF levels, BMI, the quantity of Bacteroidetes, Firmicutes, and LGG in a stool sample, MMP9, TGF-β, procollagen III, and hs-CRP levels. A p value of less than 0.05 ($p < 0.05$) was considered statistically significant.

Results

Ninety patients with recent MI were recruited for this study, and 44 who met the inclusion criteria were included. A total of 22 patients were randomly allocated to the control group and 22 to the intervention group. Two patients in each group withdrew from the study. Based on the ITT principle, we included all participants ($n = 44$) in the analysis. Adherence to dosage in our study was over 80% of pills in both groups (Table 1).

Baseline Characteristics

Baseline demographic and medical information is shown in Table 1. There were no statistically significant differences in the parameters of weight, BMI, dietary intake, or physical activity levels between the two groups. Overall, one patient in the placebo group and two patients in the probiotic group reported symptoms including abdominal pain and stomach upset, indicating participants tolerated the probiotics well. A significant increase in *LGG* DNA expression (246.56 ± 77.40 vs. 9 ± 53.7 CFU/g, $p = 0.001$) was detected following supplementation with the LGG supplementation compared with placebo. Thus, taking probiotics resulted in an increase in the levels of the gut microbiota's bacterial genera compared with placebo.

Effect of Probiotics Versus Placebo at 3 Months

Table 2 shows pre- and post-intervention biomarkers of cardiac remodeling in the intervention and control groups. Significant decreases were seen in serum TGF-β concentrations (− 8.0 ± 2.1 vs. − 4.01 ± 1.8 pg/mL, $p = 0.001$) and TMAO levels (− 17.43 ± 10.20 vs. − 4.54 ± 8.7 pg/mL, $p = 0.043$). There were no differences seen in MMP (− 4.1 ± 0.12 vs. − 4.01 + 0.15 nmol/mL, $p = 0.443$) and procollagen III levels (− 1.35 ± 0.70 vs. 0.01 + 0.3 mg/L, $p = 0.392$) subsequent to probiotic supplementation in comparison with placebo. Accordingly, probiotics supplementation resulted in a reduction of some cardiac remodeling markers compared with that of the placebo group. A significant decrease in hs-CRP levels (− 1.44 ± 0.70 vs. 0.65 + 0.98 mg/L, $p = 0.042$) was also seen following supplementation with probiotics compared

Table 1 Patient characteristics of the study subjects

Variable	Probiotic group ($n = 22$)	Placebo group ($n = 22$)	p value
Age (years)[a]	56.70 ± 9.10	57.10 ± 7.80	0.876[d]
Weight (kg)[b] at study baseline	75.60 ± 12.30	79.20 ± 12.10	0.390[d]
Weight (kg)[b] after intervention	72.35 ± 12.40	77.54 ± 11.20	0.156
Energy (kcal/day)[b] at study baseline	2214.14 (428.6)	2060.7 (454.38)	0.257
Energy (kcal/day)[b] after intervention	1829.34 (74.6)	1776.1 (184.3)	0.225[b]
Family history of CAD, *n* (%)	13 (59)	12 (54)	0.935
Non-Smoking, *n* (%)	17 (78)	18 (82)	0.431[c]
PAL, *n* (%)			0.491[c]
Low	7 (31)	2 (10)	
Moderate	13 (59)	19 (86)	
High	2 (10)	1 (4)	
Adverse effects, *n* (%)	2(9)	1 (4.5)	0.488
Compliance rate (%)	85	80	0.721
F/B ratio before intervention	5.2 ± 2.1	5.01 ± 2.3	0.109
F/B ratio after intervention	1.01 ± 2.1	2.2 ± 2.8	*0.001*
L. rhamnosus (CFU/g) before intervention	2.93 ± 1.74	2.66 ± 1.70	0.613
L. rhamnosus (CFU/g) after intervention	248.30 ± 78.30	1.23 ± 3.83	*0.001*

CAD coronary artery diseases, *BMI* body mass index, *PAL* physical activity levels, *F/B* Firmicutes to Bacteroidetes, *CFU* colony-forming unit

[a] Values are expressed as mean (SD)

[b] Values are expressed as frequency (%)

[c] Chi-square test

[d] Independent sample *t* test

Table 2 Effect of probiotics supplementation on biomarkers of cardiac remodeling

Variable	Probiotic group ($n = 22$)	Placebo group (n = 22)	p value
TGF-β (pg/mL)			
Baseline	25.00 ± 7.10	27.01 ± 7.20	2/01 (− 7/76, 1/32), 0.613**
End	16.01 ± 5.01	23.0 ± 8.29	− 7/00 (− 10, − 1.8), 0.001***
MD (95% CI), p*	− 8.0 (− 15, − 2.1), *0.021*	− 4.0 (− 8/70, − 1/8), *0.098*	
Procollagen III (mg/L)			
Baseline	5.0 ± 2.0	4.01 ± 1.44	− 1/16 (− 0/24, 1/76), 0.535**
End	4.01 ± 1.00	4.10 ± 1.32	0/09 (− 0/25, 1/07), 0.392***
MD (95% CI), p*	− 1/35 (− 3/10, 1/14), 0.055	− 0/0 (− 1/03, 0/10), 0.620	
MMP-9 (nmol/mL)			
Baseline	17.03 ± 7.21	19.01 ± 9.1	2/00 (− 2/10, 0/12), 0.849**
End	13.02 ± 6.24	15.01 ± 9.8	2/01 (− 1/20, 1/08), 0.443***
MD (95% CI), p*	− 4.01 (− 15, − 0.12), *0.049*	− 4.1 (− 2, − 0.15), *0.001*	
TMAO (pg/ml)			
Baseline	34.56 (18.55)	39.81 (14.90)	4.53 (− 30.1, 20.9), 0.720**
End	17.30 (6.67)	34.72 (36.61)	− 17.4 (− 34.27, − 0.55), *0.043***
MD (95% CI), p*	− 17.43 (− 35.6, − 3.55), *0.019*	− 4.54 (− 15.70, 3.80), 0.165	
hs-CRP (m/dL)			
Baseline	3.1 (1.5)	2.6 (1.2)	0.44 (− 1.2, 1.9), 0.545**
End	1.65 (0.67)	1.95 (0.98)	− 0.70 (− 1.27, − 0.33), *0.042***
MD (95% CI), p*	− 1.45 (− 2.6, − 0.55), *0.001*	− 0.65 (− 1.70, 0.8.), 0.124	

CFU colony-forming unit, *TGF-β* transforming growth factor beta, *TMAO* trimethylamine *N*-oxide, *MMP-9* matrix metallopeptidase 9, *hs-CRP* high-sensitivity C-reactive protein

* p based on paired sample t test

** p based on independent sample t test

*** p based on ANCOVA adjusted for baseline values

Mean (SD) and mean difference (95% CI) are accessible for data

with placebo. Probiotics thus contribute to a decrease in biomarker levels of inflammation (Table 2).

Table 3 presents an outline of pre- and post-intervention echocardiographic indices for both groups. Neither between-group differences nor within-group variations reached statistical significance for any variable.

Table 4 shows three models for predicting LVEF among the total study samples. Model 3 predicts approximately 62% of the changes observed in the dependent variable, making it the preferred model; it includes baseline LVEF and changes in procollagen III as independent variables. According to this model, for every 1-unit increase in procollagen III circulating levels, LVEF is expected to increase by 0.43 ($p = 0.007$) units.

Conclusion

As observed in this clinical trial, these results show, for the first time, that probiotic supplementation attenuates post-infarction remodeling, as observed by decreases in some cardiac remodeling biomarkers in MI subjects. The results also show that probiotic supplementation leads to decreased levels of TGF-β, TMAO, and hs-CRP in patients after MI. No effects were seen for probiotic supplementation in terms of echocardiographic indices.

The incidence of HF among patients with MI is common [3]. In spite of many treatment methods, HF continues to have a poor prognosis and a high mortality rate [4, 5]. Earlier studies have highlighted diet and supplementation remedies that may improve HF symptoms [20]. Therefore, identification of the effect of supplementation with probiotics to attenuate post-infarction remodeling may possibly facilitate the identification of new treatment plans for secondary prevention in patients

Table 3 Effect of probiotics supplementation on echocardiographic indices

Variable	Probiotic group ($n = 22$)	Placebo group ($n = 22$)	MD (95% CI), p value
LVEDV (cc)			
Baseline	118.4 ± 20.60	122.95 ± 31.20	− 4/50 (− 20/34, 11/25),0.577
End	110.60 ± 18.51	117.30 ± 24.62	− 6/76 (− 20/31, 6/35),0.309
MD (95% CI), p^*	− 7.77 (− 15.25, 2.1), 0.135	− 5.5 (− 15.8, 4.9), 0.263	
LVESV (cc)			
Baseline	80.16 ± 20.13	74.26 ± 19.10	− 5.2(− 16.4, 7.19), 0.426
End	77.31 ± 19.70	76.02 ± 21.1	− 1.33(− 10.2, 13.54), 0.830
MD (95% CI), p^*	− 2.81 (− 14.3, 8.1), 0.604	2.24 (− 13.1, 8.1), 0.882	
LVEF (%)			
Baseline	37.00 ± 7.1	38.31 ± 6.64	1.31 (− 5.4, 1.5), 0.527
End	39.95 ± 6.70	38.68 ± 6.91	− 2.51 (− 6.2, 1.61), 0230
MD (95% CI), p^*	− 2.95 (− 6.0, 0.68), 0.090	0.37 (− 3.1, 2.8), 0.107	

RVEDV right ventricular end-diastolic volume, *LVEDV* left ventricular end-diastolic volume, *LVEF* left ventricular ejection function

Mean (SD) and mean difference (95% CI) are presented for data

$^*p$ based on paired sample t test

$^{**}p$ based on independent sample t test

$^{***}p$ based on ANCOVA adjusted for baseline values

with MI. The core result of the current study is that taking probiotic supplements in patients with MI over a period of 3 months led to a significant decrease in some biomarkers of cardiac remodeling, but not in echocardiographic indices, as compared with the placebo group. A few studies have assessed the effects of probiotic supplementation on the cardiac remodeling process [21–23]. In one pilot study carried out among chronic HF patients who were supplemented with *Saccharomyces boulardii* as a probiotic, a significant decrease in serum inflammatory and biochemical parameters (uric acid, hs-CRP), and an improvement in the cardiovascular function (left ventricular diameter, LVEF) were seen [22]. Danilo et al. reported that the administration of *Bifidobacterium animalis* subsp. *lactis* 420 (B420), a probiotic with known anti-inflammatory properties, mitigates the pathological impact of MI in mice [24]. Additionally, in a recent study that was conducted in a mouse model subsequently inducing a MI through a blockade of a continuous coronary artery for 2 months, administration of probiotics (*LGG*) showed a significant reduction of hypertrophy in the left ventricle, as indexed by reduction of gene expression of tissue weight and atrial natriuretic peptide, and an improvement in LVEF. Another animal model study found that probiotic administration attenuated apoptosis of cardiomyocyte and myocardial and interstitial remodeling in rats with hypertension [25]. The exact mechanisms whereby probiotics may affect the cardiac remodeling process remain unclear. Probiotics may have some positive effects on metabolic endotoxemia [26, 27];

Table 4 Hierarchical multiple regression analysis for predicting final EF

Independent variables	Model 1			Model 2			Model 3		
	B	Beta	*p*	*B*	Beta	*p*	*B*	Beta	*p*
Bassline EF	0.53	0.51	*0.001*	0.49	0.48	*0.001*	0.39	0.38	*0.002*
BMI	− 0.21	− 0.36	0.176	− 0.20	− 0.34	0.210	0.03	0.05	0.734
Bacteroidetes				7.54	0.25	0.135	4.01	0.14	0.371
Firmicutes				− 3.50	− 0.16	0.340	− 3.1	− 0.15	0.322
MMP9							0.08	0.10	0.427
TGF-β							0.14	0.22	0.322
Procollagen III							2.18	0.43	*0.007*
hs-CRP							− 0.47	− 0.07	0.319
Adjusted R^2%	34			39			62		

Dependent variable was EF levels at the endpoint of the study

several peptides that are generated by probiotics may down-regulate inflammation [28]. Other mechanisms are promising as well. For example, researchers have observed a link between dysbiosis of gut microbiota and cardiac remodeling development [11, 29]. They have also found that increases in gut permeability can induce cardiac remodeling, perhaps through translocation of bacterial endotoxin inflammatory pathways or via direct activation of cardiac cells [30]. Trials would also indicate that probiotic supplementation could be a novel treatment option for the prevention of HF [22, 31].

Cardiac remodeling subsequent to MI is accomplished through a strong and coordinated inflammatory process via chemical signals and the recruitment of macrophages [32]. It is now understood that probiotics can suppress chronic inflammation and that this is due to the suppression of pro-inflammatory pathways and, concomitantly, induction of anti-inflammatory pathways [16, 17, 33]. According to the cardiac remodeling processes, inflammation and fibrosis are main contributors to HF and supplementation with probiotics may attenuate this process [21, 34]. The results of the current investigation show that probiotics led to decreased serum hs-CRP concentration in MI patients. hs-CRP, as an indicator of chronic low-grade inflammation, is a robust and independent predictor of HF [35]. The anti-inflammatory effects of probiotics result in the production of short-chain fatty acids (SCFA) in intestinal microbiota [26]. The results of the current study add more support to the concept that inflammation is detrimental for cardiac remodeling and that HF can be prevented by the administration of probiotics (Fig. 2). As shown in Fig. 2, dysbiosis is associated with higher gut permeability leading to metabolic endotoxemia. In addition, TMAO, as a pro-atherogenic compound, may increase the risk of developing CR. Probiotics treatment can reduce LPS absorption and TMAO levels and subsequent CR developments.

Previous studies have found that TMAO promotes cardiac hypertrophy and fibrosis by activating the TGF-β1/Smad3 signaling pathway [13]. Therefore, the TGF-β1/Smad3 signaling pathway was investigated to test whether probiotics modulated this signaling pathway to attenuate cardiac hypertrophy and fibrosis. Animal studies have shown favorable effects of probiotic administration on TMAO levels and metabolic endotoxemia [11, 14]. Our results confirmed these findings in patients with CAD who took LGG supplementation. In contrast with our results, Tripolt et al. reported that supplementation with probiotics containing *Lactobacillus casei* Shirota had no effect on TMAO levels in human studies [36]. Apart from this study, there has been no clinical trial which evaluated the effects of probiotic supplementation on TMAO levels. Probiotics inhibit TMA formation and have a cardioprotective effect on overload-induced HF via the reduction of TGF-β [11, 22]. Furthermore, TMAO promotes

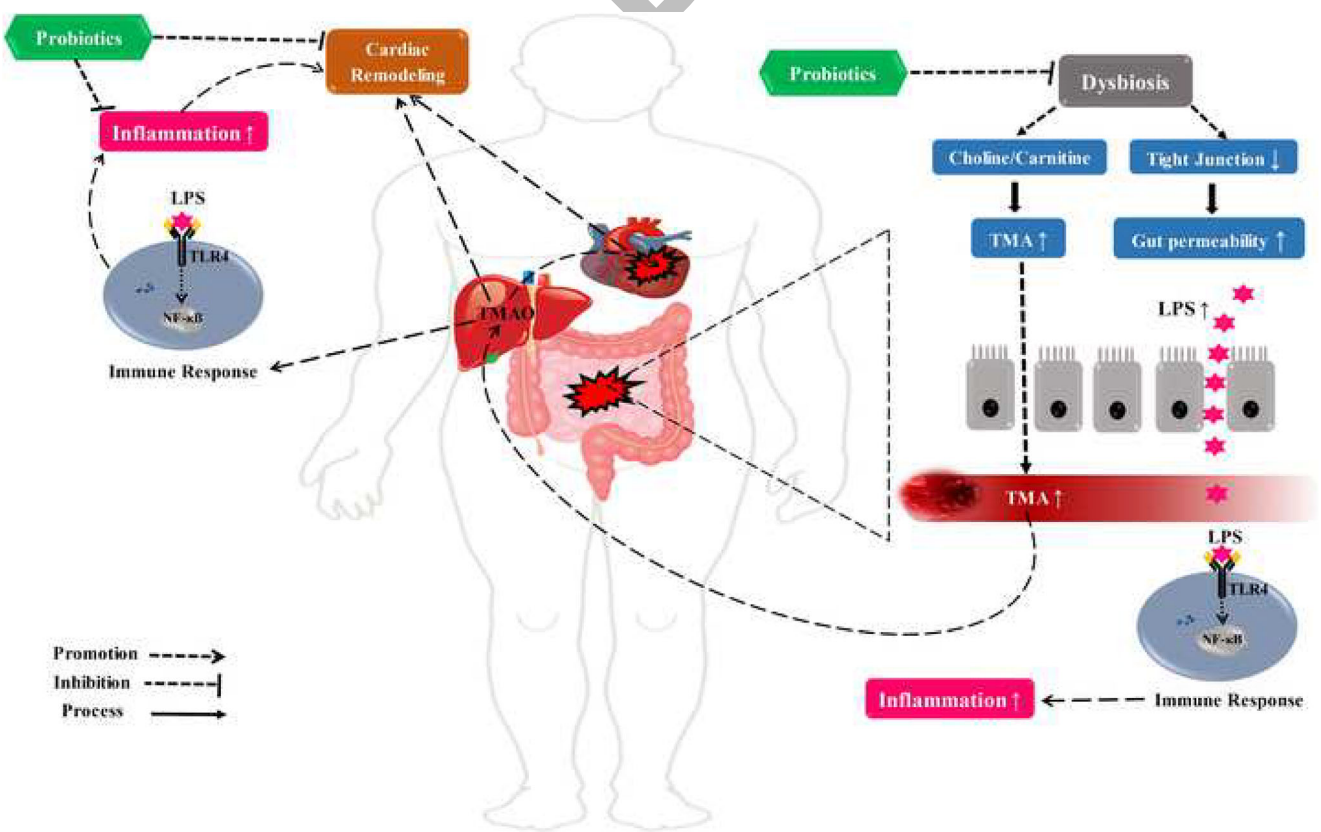

Fig. 2 Dysbiosis associated with higher gut permeability

inflammation in adipose tissue and contributes to inflammation of the arteries and leads to atherosclerosis and CR [37].

While the contribution of dysbiosis to the progression of CR is recognized [7, 11], little is known about the effect of probiotic administration on the CR process. As far as we know, no clinical trial has been conducted on the effects of probiotics in patients with MI. Former studies have proposed a direct association of dysbiosis with a greater risk of HF [9, 15, 22]. Gut microbiota plays a pivotal role in the formation of harmful metabolites such as TMAO. It has been found that TMAO levels are related to gut microbiota at phylum as well as family levels [11]. In contrast, the current study did not find a statistically significant correlation between TMAO levels with gut microbiota. This may be due to the fact that the current study did not measure whole gut microbiota and therefore, this association was not able to be observed.

Several limitations of this study should be taken into account. First, the sample size is low, which restricts the generalizability of the results. In addition, these results can only be applied to one strain of probiotics (LGG). Secondly, the period of intervention is moderately short as well. We were unable to supply probiotics for more than 3 months. Longer supplementation in future studies is indicated to examine and further affirm the positive effects of probiotics on the CR process.

In conclusion, probiotics may attenuate post-infarction remodeling as indexed by the decrease in some cardiac remodeling biomarkers in patients with MI. Probiotic supplementation also led to a decrease in TMAO levels and markers of chronic inflammation. The present trial of 3 months' supplementation with probiotics has a promising effect on CR in patients with MI following PCI. Further studies are indicated to amplify the current result.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest

Ethical Approval Our study was in agreement with the Helsinki Declaration of the World Medical Association (2000) and was accepted by our local ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1397.184) and also was listed in the Iranian Registry of Clinical Trials (IRCT) (IRCT20121028011288N15).

Informed Consent Informed consent was obtained from all individual participants included in the study using the opt-out procedure.

Abbreviations *MI,* myocardial infarction; *LVEF,* left ventricle ejection fraction; *ELISA,* enzyme-linked immunosorbent assay; *HF,* heart failure

References

1. Savarese, G., & Lund, L. H. (2017). Global public health burden of heart failure. *Cardiac Failure Review, 3*(1), 7.
2. Vigo, D., Thornicroft, G., & Atun, R. (2016). Estimating the true global burden of mental illness. *The Lancet Psychiatry, 3*(2), 171–178.
3. Garza, M. A., Wason, E. A., & Zhang, J. Q. (2015). Cardiac remodeling and physical training post myocardial infarction. *World Journal of Cardiology, 7*(2), 52.
4. Hellermann, J. P., et al. (2003). Incidence of heart failure after myocardial infarction: is it changing over time? *American Journal of Epidemiology, 157*(12), 1101–1107.
5. Flesch, M., & Erdmann, E. (2006). The problem of polypharmacy in heart failure. *Current Cardiology Reports, 8*(3), 217–225.
6. Serino, M., et al. (2014). Far from the eyes, close to the heart: dysbiosis of gut microbiota and cardiovascular consequences. *Current Cardiology Reports, 16*(11), 540.
7. Kamo, T., et al. (2017). Dysbiosis and compositional alterations with aging in the gut microbiota of patients with heart failure. *PLoS One, 12*(3), e0174099.
8. Kitai, T., Kirsop, J., & Tang, W. W. (2016). Exploring the microbiome in heart failure. *Current Heart Failure Reports, 13*(2), 103–109.
9. Ebel, B., et al. (2014). Impact of probiotics on risk factors for cardiovascular diseases. A review. *Critical Reviews in Food Science and Nutrition, 54*(2), 175–189.
10. Tang, W. W., et al. (2014). Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. *Journal of the American College of Cardiology, 64*(18), 1908–1914.
11. Tang, W. W., et al. (2015). Intestinal microbiota-dependent phosphatidylcholine metabolites, diastolic dysfunction, and adverse clinical outcomes in chronic systolic heart failure. *Journal of Cardiac Failure, 21*(2), 91–96.
12. Wang, Z., et al. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature, 472*(7341), 57.
13. Li, Z., et al. (2019). Gut microbe-derived metabolite trimethylamine N-oxide induces cardiac hypertrophy and fibrosis. *Laboratory Investigation, 99*(3), 346–357.
14. Boutagy, N. E., et al. (2015). Probiotic supplementation and trimethylamine-N-oxide production following a high-fat diet. *Obesity, 23*(12), 2357–2363.
15. Zabell, A., & Tang, W. W. (2017). Targeting the microbiome in heart failure. *Current Treatment Options in Cardiovascular Medicine, 19*(4), 27.
16. Shi, L. H., et al. (2016). Beneficial properties of probiotics. *Tropical Life Sciences Research, 27*(2), 73.
17. Tanner, G., et al. (2018). Current and future uses of probiotics. *Journal of the American Academy of PAs, 31*(5), 29–33.
18. Moludi, J., et al. (2019). The efficacy and safety of probiotics intervention in attenuating cardiac remodeling following myocardial infraction: literature review and study protocol for a randomized, double-blinded, placebo controlled trial. *Contemporary Clinical Trials Communications, 15*, 100364.

19. Hagströmer, M., Oja, P., & Sjöström, M. (2006). The international physical activity questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutrition, 9*(6), 755–762.
20. Zick, S. M., Blume, A., & Aaronson, K. D. (2005). The prevalence and pattern of complementary and alternative supplement use in individuals with chronic heart failure. *Journal of Cardiac Failure, 11*(8), 586–589.
21. Lai, C.-H., et al. (2016). Multi-strain probiotics inhibit cardiac myopathies and autophagy to prevent heart injury in high-fat diet-fed rats. *International Journal of Medical Sciences, 13*(4), 277.
22. Costanza, A. C., et al. (2015). Probiotic therapy with Saccharomyces boulardii for heart failure patients: a randomized, double-blind, placebo-controlled pilot trial. *International Journal of Cardiology, 179*, 348–350.
23. Singh, M., et al. (2019). Probiotic supplementation mitigates vascular remodeling in the retina. *The FASEB Journal, 33*-(1_supplement), 484.11–484.11.
24. Danilo, C., et al. (2017). Bifidobacterium animalis subsp. lactis 420 mitigates the pathological impact of myocardial infarction in the mouse. *Beneficial Microbes, 8*(2), 257.
25. Lin, P.-P., et al. (2013). Probiotic-fermented purple sweet potato yogurt activates compensatory IGF-IR/PI3K/Akt survival pathways and attenuates cardiac apoptosis in the hearts of spontaneously hypertensive rats. *International Journal of Molecular Medicine, 32*(6), 1319–1328.
26. Le Barz, M., et al. (2015). Probiotics as complementary treatment for metabolic disorders. *Diabetes & Metabolism Journal, 39*(4), 291–303.
27. Lee, S. J., et al. (2014). The effects of co-administration of probiotics with herbal medicine on obesity, metabolic endotoxemia and dysbiosis: a randomized double-blind controlled clinical trial. *Clinical Nutrition, 33*(6), 973–981.
28. Trebichavský, I., & Šplíchal, I. (2006). Probiotics manipulate host cytokine response and induce antimicrobial peptides. *Folia Microbiologica, 51*(5), 507–510.
29. Vahed, S. Z., et al. (2018). Myocardial infarction and gut microbiota: an incidental connection. *Pharmacological Research, 129*, 308–317.
30. de Punder, K., & Pruimboom, L. (2015). Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Frontiers in Immunology, 6*, 223.
31. George, A. K., et al. (2020). Dysbiotic 1-carbon metabolism in cardiac muscle remodeling. *Journal of Cellular Physiology, 235*(3), 2590–2598.
32. Gajarsa, J. J., & Kloner, R. A. (2011). Left ventricular remodeling in the post-infarction heart: a review of cellular, molecular mechanisms, and therapeutic modalities. *Heart Failure Reviews, 16*(1), 13–21.
33. Ng, S., et al. (2008). Mechanisms of action of probiotics: recent advances. *Inflammatory Bowel Diseases, 15*(2), 300–310.
34. Takano, H., et al. (2003). Implication of cardiac remodeling in heart failure: mechanisms and therapeutic strategies. *Internal Medicine, 42*(6), 465–469.
35. Araújo, J. P., et al. (2009). Prognostic value of high-sensitivity C-reactive protein in heart failure: a systematic review. *Journal of Cardiac Failure, 15*(3), 256–266.
36. Tripolt, N. J., et al. (2015). Effect of Lactobacillus casei Shirota supplementation on trimethylamine-N-oxide levels in patients with metabolic syndrome: an open-label, randomized study. *Atherosclerosis, 242*(1), 141–144.
37. Haghikia, A., et al. (2018). Gut microbiota–dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology, 38*(9), 2225–2235.