

Effect of Probiotic Supplementation Along With Calorie Restriction on Metabolic Endotoxemia, and Mega Inflammation in Coronary Artery Disease Patients: A Double Blind Placebo Controlled Randomized Clinical Trial

jalal moludi (✉ jmoludi@yahoo.com)

School of Nutrition Sciences and Food Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran <https://orcid.org/0000-0002-8333-414X>

Hossein Samadi Kafil

Tabriz University of Medical Sciences

Pourya Gholizadeh

Tabriz Medical University: Tabriz University of Medical Sciences

Mohammad Alizadeh

Tabriz Medical University: Tabriz University of Medical Sciences

Hamed Jafari Vayghyan

Arak University of Medical Sciences

Research

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Abstract

Purpose: Dysbiosis has been associated with increased microbial translocation, leading to chronic inflammation in cardiovascular diseases (CVD). It has been proposed that modulation of gut microbiota by probiotic might modify metabolic endotoxemia. Therefore, the purpose of this study was to examine the effects of *Lactobacillus rhamnosus* GG (LGG) on metabolic endotoxemia, and marker of inflammation in CVD subjects.

Methods: This study was a 12-weeks randomized, double-blind, and intervention on 44 patients with CVD. Patients were randomly allocated to receive either one LGG capsule 1.6×10^9 colony-forming unit (CFU) or the placebo capsules for 12 weeks. Serum levels of interleukin-1 β (IL-1 β), Toll-like receptor 4 (TLR4), interleukin-10 (IL-10), and lipopolysaccharide (LPS), were assessed before and after the intervention.

Results: A significant decrease in IL1-Beta concentration (-1.88 ± 2.25 , vs. 0.56 ± 1.58 mmol/L, $P=0.027$), and LPS levels (-5.20 ± 2.70 vs. 2.96 ± 5.27 mg/L, $P=0.016$), was observed after the probiotic supplementation compared with the placebo. Subjects who had ≥ 2.5 kg weight loss showed significantly improved in some variables, compared to patients with < 2.5 kg weight reduction, regardless of the supplement they receive.

Conclusion: These data provide preliminary evidence that probiotic supplementation has beneficial effects on metabolic endotoxemia, and mega inflammation in subjects with CVD.

Introduction

Cardiovascular disease (CVD) is the main cause of death, morbidity, and disability in both western and developing countries, with 1 in 3 people dying from CVD [1]. Despite the enormous growth in knowledge and advances in identifying traditional risk factors such as hypercholesterolemia, homocystinemia, hypertension, and hyperglycemia, which there are many unanswered questions about other cardiovascular risk factors yet [2, 3]. Currently, the scholars have been paid attention to the role of gut microbiota alteration (dysbiosis) as one of the major etiological factors that are involved in the development of CVD [4].

Numerous data have supported the contribution of dysbiosis in the development of CVD by some mechanisms including increased gut permeability and metabolic endotoxemia [5, 6]. This can may be explained by a microbiome-derived lipopolysaccharide (LPS), a major component of the external membrane in gram-negative bacteria. LPS can pass through the intestinal mucosa to enter the bloodstream, and may represent an important mediator of chronic inflammation [7, 8]. Chronic inflammation following ME might be a possible mechanism for the association between dysbiosis and CVD, which is triggered by dysbiosis [9]. LPS triggers toll-like receptors (TLRs) activation, and induces endothelial damage through increasing the expression of surface adhesion molecules such as cluster of differentiation 14 (CD14) on inflammatory cells, as well as, stimulates the release of proinflammatory

cytokines [10]. Endotoxin may also induce plaque formation and progression of atherosclerotic lesions, and release of other molecules from endothelial cells involved in the proinflammatory process [11].

Probiotics are well-defined live microbe, which can administrated in adequate amounts and have positively effects on the health of the host by improving or restoring the gut flora [12]. In addition, probiotics exert anti-obesity and lipid-lowering effects, anti-inflammatory and anti-oxidative activity [13, 14]. In additions to probiotics help balance keeping of homeostasis in gut microbiota, they have been also considered as a possible treatment for CVD [15]. A small number of studies have explored the effects of probiotics on systemic levels of endotoxin in some clinical settings. Those that have observed the effect of probiotics administration on endotoxin levels and associated metabolic disorders have revealed conflicting results [16, 17]. Probiotics keep preserve integration of gut barrier function and decrease intestinal permeability, which consequently decline endotoxin levels [18].

Weight loss diets and control of traditional risk factors are the treatment choices for CVD in overweight or obesity subjects [19]. Several guiding principles are recommended following a weight loss program to achieve 5–10% weight reduction [20]. Although the few animal studies appear to support the fact that dietary intervention leads to significant improvements in endotoxin levels [21]. In addition, the effect of dietary intervention on endotoxin levels has not been studied in human studies yet.

To the best of our knowledge, there have been little or no controlled randomized trials testing the effect of probiotic supplementation along with calorie restriction on metabolic endotoxemia in coronary artery disease (CAD) subjects. Therefore, the current study evaluated the effects of probiotic supplementation along with calorie restriction on metabolic endotoxemia, and mega inflammation markers in CAD subjects.

Materials And Methods

Participants

We ran a double-blind randomized, placebo-controlled study to inspect the endotoxemia, and inflammation levels in 44 CAD subjects. This trial was done at Shahid Madani hospital, as cardiology and heart surgery hospital of Tabriz University of Medical Sciences, Tabriz, Iran. 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). All patients admitted to this hospital with a diagnosis of CAD were considered for participation in the study and screened by a cardiologist for eligibility. Patients were selected a few days after the PCI and/or conditions were stable. The inclusion criteria were as follows: having CAD; maintaining them during the course of the trial; normal diet; and Body Mass Index (BMI) =25-35 kg/m². Patients were excluded if they refused to participate or if they had a history of gastrointestinal disorders, if they had thyroid, renal, pancreatic, or liver diseases; if they were lactating or pregnant; if they are taking antibiotics, probiotics, prebiotics, and inflammatory drugs for one month before the intervention or during the intervention. A third party who

was blind to the study gave the randomization sequence extracted from allocation software. The contributors, investigators and the medical providers were blinded after assignment to interventions. Informed consent was gotten from each participant. Calculation of sample size was based on a parallel two group randomized clinical trial with measurements of LPS at two time points. Considering a type I error of $\alpha = 0.05$ and a medium to large effect size, a sample of 44 participants would suffice to demonstrate superiority of the probiotic compared to the placebo group with a power of 80% [22]. All 44 contributors completed the study and none of them dropped their participation (**Fig 1**. CONSORT Flow diagram).

Study design

A blind trial at two levels (researcher, participants), placebo-controlled, randomized, clinical design was used to examine the effect of probiotic supplementation on endotoxemia in patients with CAD. Each qualified contributor was randomly allocated into probiotic or placebo group, according to 1:1 equal proportion rule. The order of random allocation was made by random sequence software. The random numbers were kept by a free person not complicated in the assessment of the patient, or in the data collection and analysis. In the current study, patients were randomly assigned into two groups to receive whichever probiotic supplements (n=22) or placebo (n=22) for 3 months. Both participants and researchers were unaware of the treatment allocation.

Intervention

All the participants received a moderate calorie restricted dietary plan during 12 weeks' intervention period. In this study, the program was designed to enable weight loss of 7–10% of weight, at a rate of 0.5–1 kg/wk throughout the intervention. The weight-loss program was calculated to introduce a 500- to 1000-kcal energy deficit based on estimated energy requirements at the baseline of the intervention. Patients in the probiotic group received one probiotic capsule daily containing a *Lactobacillus rhamnosus* 1.6×10^9 colony-forming unit (CFU) with their lunch. In the placebo (control) group, the capsules contained maltodextrin (Tak Gen Zist Pharmaceutical Company, Tehran, Iran). The physical properties of the placebo were identical in terms of shape, color, size, packaging, and smell but contained no bacteria. Phone contacts were made to ensure adherence twice a month. Compliance to supplementation was established by requesting participants to return the medication containers.

In addition, a moderate calorie restriction diet plan intended for all the participants during 12 weeks' intervention period. The program was planned to facilitate weight loss of 7–10% of weight, at an amount of 0.5–1 kg/wk throughout the intervention. Calorie intake was planned based on individual features of the subjects and with the aim of daily energy restriction (500 kcal fewer than the total energy requirements [TEE] estimated by Mifflin–St Jeor equation). The diets were provided 55 ~ 60% of TEE from carbohydrate, 10 ~ 15% from protein, and 25 ~ 35% from fat.

Dietary assessment

Dietary intake was assessed using a dietary record at month 0, 1 and 3 of the intervention. We used Nutritionist IV software adjusted for Iranian diets to acquire nutrient intakes of participants based on the average of three-day food diaries.

Physical Activity Assessment

The physical activity assessment was gotten to monitor patient's 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.

Assessment of anthropometric indices

Body weight was assessed via a scale with 250 gr accuracy (Seca, Hamburg, Germany) and patients were measured while wearing a minimum dress and without shoes. Height without shoes was measured by a tape with 0.5-cm accuracy. BMI was computed by dividing weight (Kg) by height² (m). To avoid measurement bias, all measurements were taken by a trained dietitian.

Biochemical variables

After an overnight fasting (12 hours), blood was collected and supplements was provided to the participants. Blood serum was obtained from whole blood through centrifugation at 2500 rpm for 10 min. FBS and lipid profile were examined on the day of sampling, and residual serum was stored at -20 C until the analyses were done. Endotoxin, and inflammatory markers were measured using enzyme-linked immunosorbent assay [17] kits as follows: Interleukin 1 beta (IL-1 β) (intra-assay variation = 5.8%, normal range = 0.02-6 pg/mL, inter-assay variation = 9.06%, sensitivity = 0.01 pg/mL), Toll-Like Receptor 4 (TLR-4) (normal range = 0.05-15 ng/mL, intra-assay variation = 4.58%, inter-assay variation = 7.8%, sensitivity = 0.027 ng/mL), IL-10 (normal range = 0.2-100 ng/dl, intra-assay variation = 10%), and, LPS (inter-assay CV = 10.0% , detectable range = 12.00 – 1000 ng/ml).

Statistical analysis

The data were analyzed via SPSS software (version 21; SPSS Inc., Chicago, IL) and the outcomes were stated as mean \pm SD. To determine the normal distribution of variables, we used skewness and kurtosis test. Paired samples t-test was applied for within-group comparisons (end-point vs. baseline). To adjust for several confounders, we did analysis of covariance (ANCOVA) in which the confounding effect of these variables were taken into account which was used to determine the statistically significant pairwise differences. The analyses were conducted using an intention-to-treat approach [23]. Circulating endotoxin level was intended as a primary outcome, while inflammatory markers were defined as secondary outcomes. For all statistical tests, a P value less than 0.05 was interpreted as statistically significant.

Results

In the present study, 44 patients met the inclusion and exclusion eligibility criteria. 22 patients were randomly assigned to the placebo group and 22 patients to the probiotics group. **Figure 1** are shown the study flowchart.

Baseline medical and demographic data are presented in **Table.1**. There were no significant differences between the groups regarding weight, BMI, physical activity levels, and family history of CAD.

Data on dietary intakes (macronutrient distribution, as percentage of calories, changes and dietary fiber) of the patients are summarized in **Table 2**. Intake of calories and macronutrients were significantly decreased in both groups. We found significant changes in the mean of energy intake of the groups during the intervention (decreasing of -284.40 and -384.71 Kcal in probiotics and placebo groups, respectively, $P= 0.225$). Greater decrease of dietary intake was observed in the prebiotic group in comparison to placebo group ($P>0.05$).

Table 3 provides a summary of pre- and post-intervention metabolic endotoxemia and inflammation markers in both groups. Neither the between-group differences nor the within-group variations reached statistical significance for TLR4. A significant decrease in IL1-Beta concentration (-1.88 ± 2.25 , vs. 0.56 ± 1.58 mmol/L, $P=0.027$), and LPS levels (-5.20 ± 2.70 vs. $2.96+ 5.27$ mg/L, $P=0.016$) were detected following the probiotic supplementation in comparison to placebo group, respectively. Probiotics administration was resulted in a significant decrease in biomarker levels of inflammation, and metabolic endotoxemia in comparison to placebo group.

Results stratified by weight reduction of at minimum 2.5 kg was presented in **Table 4**. Overall, 28% of the participants, who finished the trial had at least 2.5 kg weight loss (8 patients in the probiotic group and 4 patients in the placebo group). Nevertheless, besides of what supplement they receive, subjects who reached weight loss had great decrease in some biomarkers in comparison to whom lost <2.5 kg.

Discussion

As far as we know, this is the first study to provide insight into the anti-inflammatory and anti-endotoxemia effects of probiotic in CAD patients. The results of the current study indicated, for the first time, that weight loss diet along with probiotic supplementation lead to improved endotoxemia, as described by reduction of the LPS and TLR4 levels. Probiotic supplementation also altered the gut microbiota profile. In addition, inflammatory markers were influenced favorably by probiotic supplements, among patients with CAD under a calorie-restricted diet. We also found that those who were obedient with the weight loss diet and lost a minimum of 2.5 kg by the end of the intervention had significantly improved metabolic profile in comparison to those with slight compliance with the diet.

In the current study, we ran the weight loss program plus probiotic supplementation. Although anthropometric and body composition indices reduction in the probiotic group was more advantageous than the placebo group, bur this issue did not range to statically significant levels. Similar consequences have been stated with probiotic supplementation in former studies [24]. In the current study, we were

statistically observed major changes in nutrient intakes for the period of the intervention and there was a downward trend in the total energy intake in all subjects due to the running of weight loss diet for both groups. Though weight loss was similar between the two groups, our results suggest that weight loss diet offers a favorable effect on metabolic disorders, but add-on the diet plus probiotics may promote cardiovascular risk factors (Table 4).

The other result of probiotic supplementation in CAD patients was an improvement in inflammatory markers. To the extent of our knowledge, no study has assessed the effect of probiotics supplementation on endotoxemia in patients with CAD. Former studies have stated microbial dysbiosis in CVD subjects [7]. Probiotic supplementation by modulating gut microbiota enhances the immune system function and decreases inflammation and endotoxemia [9, 16]. In our study, we established a statistically significant reduction in LPS levels in the probiotic group in comparison to placebo, but not in TLR4, which suggested probable anti-inflammatory and anti-endotoxemia properties of probiotics. Activation by LPS was resulted in an increase in plasma levels of cytokines like interleukin (IL-) 1 Beta, which was observed in CAD patients [7]. The outcomes of our study show that probiotic supplementation could led to decreased IL-1 Beta and hs-CRP levels. The anti-inflammatory effects of probiotics may involve the production of short chain fatty acids (SCFA) in the gut microbiota and the decreased expression of inflammatory cytokines [25]. This finding is consistent with the outcomes reported by Zarrati et al [26], who established that *Lactobacillus acidophilus* administration as a probiotic for 2 months resulted in a significant decrease in inflammatory markers among obese subjects. In addition, a significant decrease in hs-CRP levels was detected after the intake of probiotic yogurt for 9 weeks among pregnant women [27]. Others have failed to find a significant effect of two-month probiotic supplementation on CRP levels in polycystic ovary syndrome (PCOS) subjects [28]. The different results might be clarified by diverse dosages of probiotics supplementations and different clinical setting of individuals, who took part in those studies. Microbial translocation has been proposed to be a driver of inflammation and immune activation in CAD patients [5, 29].

Whereas the contribution of pro-inflammatory cytokines to progression of CVD is well- recognized [30], little is identified about the effect of microbial translocation (increases LPS) on the higher cytokine secretion [31]. To the best of our knowledge, no clinical trial had appraised the effects of probiotics on TLR-4 in meta-inflammation. TLR4 has been suggested to act as a molecular link among LPS, inflammation and the innate immune system [11]. It seems that probiotic supplements reduce inflammation possible via competitive mechanisms, which prevents binding LPS to theTLR4/CD14 complex [9, 32]. In addition, previous studies have indicated that calorie restriction can downregulate LPS-producing enzymes of the commensal bacteria, leading to decreased endotoxemia and inflammation [33]. There is one single study which indicates that being on a very-low calorie diet (800 Cal/day) for one month decreases LPS binding protein and zonulin in obese women [34]. Apart from that, there are few clinical trials available about the effects of calorie restriction (25-30% below TEE) on serum LPS. Therefore, we could not compare our results for LPS, to the previous studies about the effect of calorie restrictions on these factors.

As mentioned above, dysbiosis leads to endotoxemia and chronic inflammation. These two factors have been proposed to make subjects vulnerable to CVDs. Probiotics might reverse these effects by helping to enhance or restore health gut microbiota composition. Cui et al.[35], revealed that probiotic supplementation with *Bifidobacterium spp* in IBD patients for 2 months, meaningfully increased the stool concentration of *Bifidobacterium spp* and *Lactobacillus spp*, compared to placebo. However, in the study of Kato et al.[36], consumption of *Bifidobacterium*-fermented milk for 3 months did not alter the level of stool Bacteriodes.

Our study has some of the limitations, including not assessing the gut microbiota, as well as the duration and doses of the supplementations. Furthermore, the sample size is small, which may interfere with generalizability. So, given the gradually common use of Firmicutes/Bacteriodes ratio, the analysis of these bacterial contents may well be used in the future as indicators of a dysbiotic microbiome that contributes to low grade inflammation and CVD progression. Apart from that, this supplementation is worthy of additional survey.

Conclusion

The present trial revealed that 12 weeks' probiotic supplementation (*L. rhamnose*) has a positive effect on endotoxemia and chronic inflammation in CAD patients. In addition, our results propose that calorie restriction plus probiotic supplementations might lead to improved metabolic endotoxemia and better than probiotic supplementation alone. Further studies are needed to replicate the present findings and investigate the mechanisms of the observed effects and test the generalizability of the current findings in CAD patients and patients with other types of cardiovascular disease.

Abbreviations

MI

Myocardial infarction; LVEF:Left ventricle Ejection fraction; ELISA:enzyme-linked immunosorbent assay, HF:heart failure, BMI:Body Mass Index BP:Blood Pressure DBP:Diastolic Blood Pressure, FBS:Fasting Blood Sugar, HDL:High-Density Lipoprotein, LDL:Low-Density Lipoprotein, SBP:Systolic Blood Pressure, TC:Total Cholesterol, TG:Triglyceride

Declarations

Ethics declarations

Conflict of Interest

The authors declare that there is 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 as a proposal for PhD grade in Nutritional Sciences (IR.TBZMED.REC.1397.184) and also was listed in the Iranian Registry of Clinical Trials (IRCT) (**IRCT20121028011288N15**).

Consent for publication

All authors support the submission to this journal.

Informed Consent

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

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Author contribution

Jalal moludi designed the study. Hossein Samadi Kafil and Pourya Gholizadeh completed the entire clinical studies. Jalal moludi collected and analyzed the data. Mohammad Alizadeh prepared the manuscript. Jalal moludi conducted statistical analysis. All of authors edited the manuscript.

Availability of data and materials

All data generated and analyzed during this study are included in the manuscript.

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Tables

Table 1- General characteristics of the study subjects			
P-value	Placebo group (n=22)	Probiotic group (n=22)	Variable
0.876 ^d	57.10 ±7.80	56.70 ±9.10	Age (years)^a
0.390 ^d	79.20 ±12.10	75.60 ±12.30	Weight (kg)^b at study baseline
0.156	77.54 ± 11.20	72.35 ± 12.40	Weight (kg)^b after intervention
0.431 ^c	4 (18)	5 (22)	Smoking
0.830	760.6 (201.21)	773.7 (198.3)	Physical activity (METs)
0.527	38.31 (6.7)	37.0 (7.1)	Ejection Fraction (%)
0.431 ^c	18 (82)	17 (78)	Non-Smoking n(%)
0.491 ^c			PAL n(%)
	2 (10)	7 (31)	Low
	19 (86)	13 (59)	Moderate
	1 (4)	2 (10)	High
CAD: Coronary artery diseases, METs: Metabolic equivalents (MET-minutes/week); MD: Mean/Median of difference			
a- Values are expressed as mean (SD)			
b- Values are expressed as frequency (%)			
c- Chi-square test			
d- Independent samples t-test			

Table 2. Changes in calorie, percent from macronutrients, and dietary fiber throughout the study			
Variable	Probiotic group (n=22)	Placebo group (n=22)	P-value
Energy (Kcal/d)			
Baseline	2214.14 (428.6)	2060.7 (454.38)	153.4 (-116.1 to 422.8), 0.257**
End	1829.34 (174.6)	1776.1 (184.3)	53.27 (-32.1 to 138.68), 0.225***
MD (95% CI), <i>P</i> *	-384.71 (-567.9 to -201.6), <0.001	-284.40 (-447.40 to -121.9), <0.001	
Carbohydrates (%)			
Baseline	53.33 (13.43)	54.1 (11.51)	0.80 (-7.25, 5.78), 0.987**
End	54.72 (10.22)	53.88 (11.34)	-0.92 (-7.31, 7.36), 0.807***
MD (95% CI), <i>P</i> *	1.39 (-7.12, 7.66), 0.667	-0.25 (-6.34, 5.12), 0.672	
Protein (%)			
Baseline	13.55 (3.9)	13.99 (3.76)	0.44 (-2.21, 2.21), 0.087**
End	15.08 (3.68)	15.7 (2.76)	0.62 (-2.3, 1.14), 0.379***
MD (95% CI), <i>P</i> *	1.53 (-3.44, 2.21), 0.172	1.71 (-2.21, 0.72), 0.634	
Fat (%)			
Baseline	34.12 (9.87)	32.1 (7.72)	-2.01 (-5.03, 8.59), 0.751**
End	30.20 (10.62)	31.12 (10.85)	1.01 (-6.23, 5.20), 0.756***
MD (95% CI), <i>P</i> *	-4.08 (-7.21, 3.33), 0.662	-2.00 (-4.12, 5.31), 0.656	
Dietary fiber (g)			
Baseline	16.40 (4.25)	15.72 (3.17)	-0.68 (-1.2, 3.12), 0.559**
End	19.77 (8.05)	17.72 (6.51)	-2.01 (-2.12, 6.35), 0.342***

MD (95% CI), 3.37 (-0.06, 0.34), 0.090 2.00 (-0.06, 0.41), 0.147
*P**

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

* P based on Paired samples t-test

** P based on Independent samples t-test

*** P based on ANCOVA adjusted for baseline values

Table 3. Effect of probiotics supplementation on metabolic endotoxemia and inflammation markers			
Variable	Probiotic group (n=22)	Placebo group (n=22)	P-value
IL-10 (ng/dl)			
Baseline	5.11 ± 4.60	4.76± 3.60	0.11 (-2.4 to 2.7), 0.930**
End	5.25 ±2.91	5.78± 2.29	0.56 (-2.6 to 1.6), 0.605***
MD (95% CI), P*	0.60 (-1.4 , 2.6) 0.542	1.04 (-1.31 , 3.59) 0.399	
IL1-Beta (pg/mL)			
Baseline	5.62 ± 3.72	5.31± 2.09	-0.31 (-1.4 to 2.6), 0.723**
End	3.75 ±2.10	5.81± 3.60	2.05 (-3.8 to -0.25), 0.027***
MD (95% CI), P*	-1.88 (-3.25 , -0.48) 0.010	0.56 (-1.58 , 2.56) 0.546	
LPS (ng/ml)			
Baseline	21.92 (11.64)	26.18 (16.85)	4.25 (-13.1, 4.4), 0.335**
End	16.04 (6.8)	23.22 (12.22)	7.27 (-13.4, -1.4), 0.016***
MD (95% CI), P*	-5.92 (-10.74, -1.09), 0.019	-2.96 (-11.6, 5.7), 0.165	
TLR4 (ng/ml)			
Baseline	11.59 (7.22)	10.58 (8.30)	-0.70 (-2.4, 5.6), 0.765**
End	9.8 (7.24)	11.85 (5.6)	2.2 (-5.3, 1.8), 0.301***
MD (95% CI), P*	-1.72 (-6.21, 3.10), 0.488	1.27 (-1.7, 4.7), 0.581	
LPS: Lipopolysaccharide, Mean (SD) and Mean difference (95% CI) are presented for data.			
∞ Data analyzed for 40 patients.			
* P based on Paired samples t-test			
** P based on Independent samples t-test			
*** P based on ANCOVA adjusted for baseline values			

Table 4- Changes in biomarkers and variables among the patients who completed the study, stratified by a decrease of 2.5 kg in body weight

MD (95% CI), P-value ^a	weight loss \leq 2.5 (n=32)	weight loss \geq 2.5 (n=12)	Variable
-14.33 (-1.1 to -27.2), 0.032	111.52 \pm 15.16	97.28 \pm 20.10	FBS (mg/dl)
-18.23 (-13.14 to 50.21), 0.241	159.66 \pm 47.11	140.83 \pm 43.16	Total cholesterol (mg/dl)
-25.52 (-11.1 to 62.33), 0.174	160.83 \pm 59.25	134.34 \pm 37.16	TG (mg/dl)
-15.11 (-15.1 to 45.2), 0.326	87.44 \pm 44.39	72.36 \pm 44.16	LDL (mg/dl)
-2.31 (-2.22 to 7.10), 0.353	44.31 \pm 7.59	42.00 \pm 6.27	HDL (mg/dl)
-9.13 (-0.26 to 18.31), 0.056	122.47 \pm 12.16	113.33 \pm 13.18	SBP (mmHg)
-0.99 (-8.66 to 6.51), 0.794	77.10 \pm 8.10	78.83 \pm 7.16	DBP(mmHg)
-3.21 (1.47to 6.2), 0.035	16.71 \pm 4.33	13.50 \pm 3.80	BDI
-2.46 (-7.7 to 2.31), 0.301	39.28 \pm 7.23	41.75 \pm 6.13	EF (l)
-0.65 (0.16 to 0.24), 0.011	1.79 \pm 1.25	1.14 \pm 0.45	hs-CRP (mg/dl)
-11.83 (-35.1 to 59.71), 0.616	137.00 \pm 72.39	125.16 \pm 44.16	MDA (mg/dl)
-25.83 (-83.1 to 31.32), 0.367	193.81 \pm 89.50	219.66 \pm 64.83	TAC (mg/dl)
-2.91 (-16.1 to 22.2), 0.769	26.81 \pm 33.29	23.90 \pm 10.18	TMAO (mmHg)
-0.84 (-1.21 to 2.94), 0.429	5.10 \pm 3.43	4.17 \pm 1.93	IL1-Beta (mmHg)
-2.04 (-0.44 to 4.50), 0.101	6.09 \pm 3.99	4.05 \pm 2.11	IL-10 (mmHg)
-5.74 (-0.22 to 11.54), 0.059	16.25 \pm 9.75	10.47 \pm 4.30	MMP (mmHg)
-0.77 (-6.1 to 8.81), 0.833	20.18 \pm 10.42	19.50 \pm 11.50	TGF-Beta
-0.32 (-1.09 to 0.44), 0.404	4.50 \pm 1.23	4.17 \pm 1.07	Procollagen
-0.27 (-6.7 to 7.2), 0.938	19.68 \pm 9.75	19.41 \pm 11.42	LPS (mg/dl)
-1.00 (-3.1 to 5.40), 0.650	11.11 \pm 6.39	10.11 \pm 6.81	TLR4 (mg/dl)
-71.46 (-161.1 to 18.2), 0.116	110.17 \pm 123.50	181.66 \pm 151.83	L. Rhamnosus (CFU)
-0.08 (-0.24 to 0.07), 0.309	0.28 \pm 0.22	0.37 \pm 0.27	Bacteroid (CFU)
-0.08 (-0.13 to 0.30), 0.777	0.32 \pm 0.31	0.23 \pm 0.30	Firmicutes(CFU)

FBS: Fasting blood glucose, BDI: Beck Depression Inventory, HDL: high density lipoprotein; LDL: low density lipoprotein, TG: Triglyceride, TC: Total cholesterol, SBP: Systolic blood pressure, DBP: diastolic blood pressure, MMP-9: Matrix metalloproteinase 9, hs-CRP: high sensitivity C-reactive protein, MDA: malondialdehyde, TAC: total antioxidant capacity. For other abbreviations see table 4

Values are expressed as mean (SD)

a- Independent samples t-test

Figures

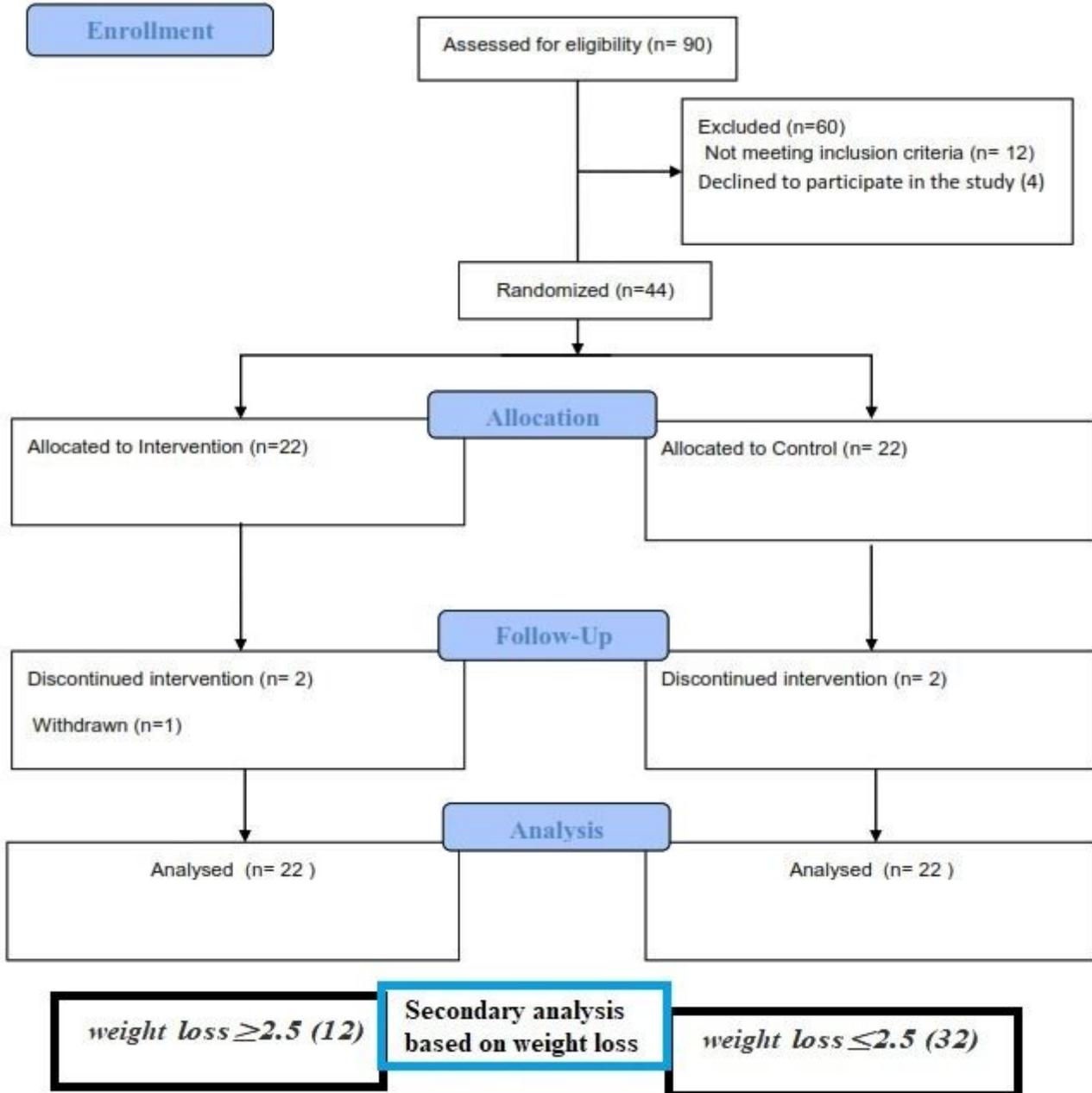


Figure 1

CONSORT Flow diagram