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# The Effects of Synbiotic Supplementation on Carotid Intima-Media Thickness, Biomarkers of Inflammation, and Oxidative Stress in People with Overweight, Diabetes, and Coronary Heart Disease: a Randomized, Double-Blind, Placebo-Controlled Trial

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**Abstract** Synbiotics are known to exert multiple beneficial effects, including anti-inflammatory and antioxidant actions. The aim of this study was to evaluate the effects of synbiotic supplementation on carotid intima-media thickness (CIMT), biomarkers of inflammation, and oxidative stress in people with overweight, diabetes, and coronary heart disease (CHD). This randomized, double-blind, placebo-controlled trial was conducted and involved 60 people with overweight, diabetes, and CHD, aged 50–85 years old. Participants were randomly allocated into two groups to take either synbiotic supplements containing three probiotic bacteria spices *Lactobacillus acidophilus* strain T16 (IBRC-M10785), *Lactobacillus casei* strain T2 (IBRC-M10783), and *Bifidobacterium bifidum* strain T1 (IBRC-M10771) ($2 \times 10^9$ CFU/g each) plus 800 mg inulin or placebo ($n = 30$ each group) for 12 weeks. Fasting blood samples were taken at baseline and after the 12-week intervention period to determine metabolic variables. After the 12-week intervention, compared with the placebo, synbiotic supplementation significantly reduced serum high-sensitivity C-reactive protein (hs-CRP) ($- 3101.7 \pm 5109.1$ vs. $- 6.2 \pm 3163.6$ ng/mL, $P = 0.02$), plasma malondialdehyde (MDA) ($- 0.6 \pm 1.0$ vs. $- 0.1 \pm 0.3$ μmol/L, $P = 0.01$), and significantly increased nitric oxide (NO) levels ($+ 7.8 \pm 10.3$ vs. $- 3.6 \pm 6.9$ μmol/L, $P < 0.001$). We did not observe any significant changes of synbiotic supplementation on other biomarkers of oxidative stress and CIMT levels. Overall, synbiotic supplementation for 12 weeks among people with overweight, diabetes, and CHD had beneficial effects on serum hs-CRP, plasma NO, and MDA levels; however, it did not have any effect on other biomarkers of oxidative stress and CIMT levels.

**Keywords** Synbiotic supplementation · Carotid intima-media thickness · Inflammation · Oxidative stress · Type 2 diabetes mellitus · Coronary heart disease

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## Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder with common co-morbidities, such as obesity and cardiovascular disease (CVD) [1]. CVD has been reported to account for 75% of deaths in diabetic subjects [2]. Multiple pre-existing risk factors, including CVD, diabetes, and overweight/obesity, increase vulnerability to coronary heart disease (CHD), the leading cause of death in developed countries [3]. Earlier, the relationship between carotid intima-media thickness (CIMT) and metabolic syndrome (MetS) has been assessed in few studies [4, 5]. In addition, few studies have documented that

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there is a positive association between CIMT and systemic inflammation [6, 7].

The beneficial effects of synbiotics supplementation on metabolic profiles have previously reported in patients with T2DM [8], gestational diabetes mellitus (GDM) [9], non-alcoholic fatty liver disease [10], and non-obese T2DM [11]. Our previous study showed that taking synbiotic bread containing *Lactobacillus sporogenes* ($10^8$ CFU) plus 0.07 g of inulin/g for 8 weeks among patients with T2DM significantly increased plasma nitric oxide (NO) and malondialdehyde (MDA), but did not affect total antioxidant capacity (TAC) and total glutathione (GSH) levels [12]. Furthermore, Kooshki et al. [8] demonstrated that synbiotic supplementation for 8 weeks among T2DM patients could decrease circulating levels of inflammatory cytokines. In a meta-analysis study by Tabrizi et al. [13], it was also observed that synbiotic supplementation among patients with diabetes had beneficial effects on glycemic control, triglycerides, total- and VLDL-cholesterol levels. In addition, probiotic intake was benefit in control of streptozotocin-induced diabetes and its complications [14]. However, another study indicated that probiotic supplementation for 6 weeks among patients with T2DM did not affect high-sensitivity C-reactive protein (hs-CRP) levels [15].

Synbiotic intake might affect CIMT through amending markers of inflammation and oxidative stress. Synbiotics and probiotics might influence biomarkers of inflammation and oxidative stress through the effect on caveolin-1, endothelial nitric oxide synthase (NOS), neuronal NOS downregulation [16], synthesis of GSH, initiation of apoptosis, and the upregulation of oxidative pentose pathway activity [17]. To our knowledge, data on the effects of synbiotic supplementation on CIMT and biomarkers of inflammation and oxidative stress in people with overweight, diabetes, and CHD are scarce. Therefore, the purpose of this study was to evaluate the effects of synbiotic supplementation on CIMT and biomarkers of inflammation and oxidative stress in these patients.

## Materials and Methods

### Participants and Ethics Statements

The current study was a randomized, double-blind, placebo-controlled trial, registered in the Iranian registry of clinical trials (http://www.irct.ir: IRCT201503025623N37), conducted at a cardiology clinic affiliated to Kashan University of Medical Sciences (KUMS), Kashan, Iran, between March 2015 and March 2016. This study was done according to the principals of the Declaration of Helsinki, and the study protocol was approved by the ethics committee of KUMS. All patients were informed about the aims and protocol of the study. Written informed consent was obtained from all subjects prior to the intervention. Overweight (BMI = 25–29.9 kg/m$^2$) and obese individuals (obese BMI ≥ 30 kg/m$^2$) with T2DM, aged 40–85 years old with CHD, were included. Diagnosis of T2DM was conducted based on the criteria of the American Diabetes Association [18], and diagnosis of CHD was performed based on the American Heart Association [19]. Those consuming synbiotics and/or probiotics supplements within the past 3 months, changes in the dosage and kind of medications, taking anti-inflammatory drugs like corticosteroids, having a myocardial infarction within the past 3 months, having cardiac surgery within the past 3 months, and significant renal of hepatic failure were not included in this study.

### Study Design

At the onset of the study, all participants were categorized according to age (< 60 and ≥ 60 years), BMI (25–29.9 and ≥ 30 kg/m$^2$), gender (19 females and 11 males in each group), and the dosage and kind of medications. Then, participants in each block were randomly allocated into two treatment groups to take either synbiotic supplements containing three probiotic bacteria spices *Lactobacillus acidophilus* strain T16 (IBRC-M10785), *Lactobacillus casei* strain T2 (IBRC-M10783), and *Bifidobacterium bifidum* strain T1 (IBRC-M10771) ($2 \times 10^9$ CFU/g each) plus 800 mg inulin or placebo (n = 30 each group) daily for 12 weeks. Synbiotic and placebo capsules were made by Tak Gen Zist Pharmaceutical Company (Tehran, Iran). Placebos (starch) were similar in color, shape, size, and package to the synbiotic capsules. It is well known that it would be more appropriate if the strains used in probiotic supplements for human consumption derived from the human intestinal tract are well characterized, able to outlive the rigors of the digestive tract and possibly colonize, biologically active against the target, as well as to be stable and amenable to commercial production and distribution [20]. Due to the lack of evidence about the appropriate dosage of probiotics for people with overweight, diabetes, and CHD, we used the abovementioned doses based on few previous studies in healthy subjects [21, 22]. Compliance to the trial protocol was evaluated by unused containers of the synbiotic and placebo capsules which were returned to the investigators. Moreover, we sent a reminder on subjects' cell phones regarding consumption of supplements. Three dietary records and physical activity records as metabolic equivalents (METs) in hours per day at weeks 0, 3, 6, 9, and 12 of the trial were obtained from each participant. We used modified Nutritionist IV software (First Databank, San Bruno, CA) to establish average daily nutrient intakes of patients.

## Assessment of Anthropometric Measures

Weight and height (Seca, Hamburg, Germany) were measured without shoes in light clothing in the cardiology clinic by a trained nutritionist, at baseline and at the end of the study. BMI was calculated as weight (kg) divided by height squared ($m^2$).

## Clinical Assessment

Measurements of the CIMT (maximum and mean of left and right CIMT) were carried out in the patients at the 2-cm distance of the common carotid bifurcation, by the same sonographist, at baseline and after the 12-week treatment using a Doppler ultrasonography device (Samsung Medison V20, Korea) with linear multifrequencies of 7.5- to 10-MHz probe. The physician was blinded to any clinical information of the participants. Systolic (SBP) and diastolic blood pressure (DBP) was quantified via a sphygmomanometer (ALPK2, Zhejiang, China).

## Biochemical Assessment

Ten milliliters fasting blood samples were taken at baseline and after the 12-week intervention at Kashan Reference Laboratory, Kashan, Iran. Serum hs-CRP values were assessed by an ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay coefficient variances (CVs) of 4.6 to 7.0%, respectively. The plasma NO using Griess method [23], TAC by the method of ferric reducing antioxidant power developed by Benzie and Strain [24], total GSH using the method of Beutler et al. [25], and MDA concentrations by the thiobarbituric acid reactive substances spectrophotometric test [26] were determined. CVs for plasma TAC, GSH, and MDA were lower than 5%.

## Randomization

Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation concealments were done from the researchers and participants and were carried out by a trained staff member at the cardiology clinic.

## Statistical Methods

The Shapiro-Wilk test was applied to control the normal distribution of variables. The intention-to-treat (ITT) analysis of the primary study end-point was conducted for all of the randomly allocated subjects. For non-normally distributed variables (hs-CRP, GSH, MDA, and SBP), we applied Log transformation. Independent sample $t$ test was used to establish changes in anthropometric measures and dietary intakes

between the two groups. Differences in proportions were evaluated by Fisher's exact test. To compare within-group differences (before and after treatment), we used paired-samples $t$ tests. To determine the effects of synbiotic supplementation on CIMT, biomarkers of inflammation, and oxidative stress, we used one-way repeated measures ANOVA. Adjustment for changes in baseline values of biochemical parameters was performed by analysis of covariance (ANCOVA) using general linear models. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

To calculate sample size, we used the standard formula suggested for clinical trials by considering type one error ($\alpha$) of 0.05 and type two error ($\beta$) of 0.20 (power = 80%). Based on a previous study [8], we used 1.96 mg/L as SD and 1.57 mg/L as the difference in mean ($d$) of hs-CRP as key variable. Based on this, we needed 25 persons in each group. Assuming five dropouts in each group, the final sample size was determined to be 30 persons per group.

## Results

During the intervention phase of the study, two participants were excluded from the synbiotic group [withdrawn due to personal reasons ($n = 2$)] and three [withdrawn due to personal reasons ($n = 3$)] from the placebo group (Fig. 1). However, all 60 participants were included in the final analysis using ITT principle. Overall, the compliance rate was high, such that higher than 90% of capsules were taken throughout the study in both groups. No side effects were reported following the supplementation of synbiotics in people with diabetes and CHD throughout the study.

There was no statistically significant difference in mean age, height, weight, BMI, and METs at pre- and post-intervention between the two groups (Table 1). Mean smoking, consumption of antidiabetic and antilipidemic drugs, hypertension rate, consumption of angiotensin-converting enzymes inhibitors, aldosterone receptor blockers drugs, and blocker drugs (β-blocker and calcium channel blocker) of study participants were not statistically different between the two groups.

Considering the 3-day dietary records obtained during the intervention, there was no statistically significant difference in terms of daily dietary macro- and micro-nutrient intakes between synbiotic and placebo groups (Table 2).

After the 12-week intervention, compared with the placebo, synbiotic supplementation significantly decreased serum hs-CRP ($- 3101.7 \pm 5109.1$ vs. $- 6.2 \pm 3163.6$ ng/mL, $P = 0.02$), plasma MDA ($- 0.6 \pm 1.0$ vs. $- 0.1 \pm 0.3$ μmol/L, $P = 0.01$), and significantly increased NO levels ($+ 7.8 \pm 10.3$ vs. $- 3.6 \pm 6.9$ μmol/L, $P < 0.001$) (Table 3). We did not observe

**Fig. 1** Summary of patient flow diagram
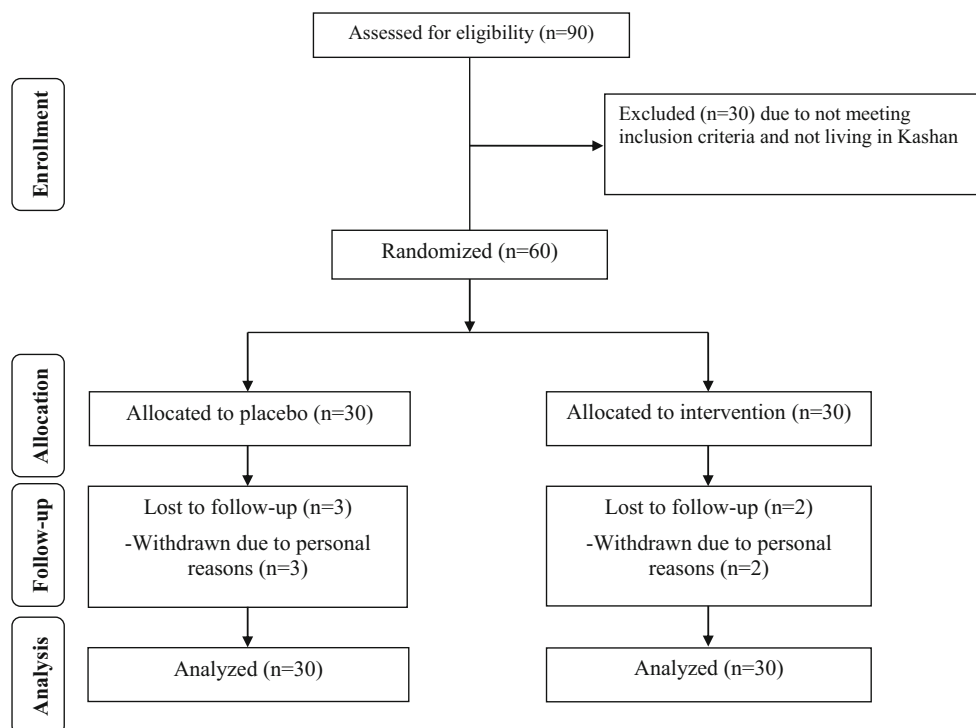


any significant changes of synbiotic supplementation on other biomarkers of oxidative stress and CIMT levels.

Baseline levels of plasma NO ($P = 0.003$), GSH ($P = 0.01$), and SBP ($P = 0.02$) were significantly different between the two groups. Therefore, we controlled the analyses for the baseline levels. When we adjusted the analyses for baseline values of biochemical variables, our findings did not alter (Table 4).

## Discussion

To the best of our knowledge, this study is the first report of synbiotic supplementation on CIMT, biomarkers of inflammation, and oxidative stress in people with overweight, diabetes, and CHD. We found that synbiotic supplementation for 12 weeks among people with overweight, diabetes, and CHD had beneficial effects on serum hs-CRP, plasma NO, and MDA levels; however, it did not have any effect on other biomarkers of oxidative stress and CIMT levels.

Hypertension, inflammation, and oxidative stress increase the risk of atherosclerosis and CHD in patients with 2DM [27]. Our data supported that synbiotic supplementation for 12 weeks to people with overweight, diabetes, and CHD significantly decreased serum hs-CRP and significantly increased plasma NO concentrations, but did not affect CIMT levels. Earlier, some studies have assessed the effects of synbiotic supplementation on inflammatory cytokines. Supporting our study, synbiotic supplementation for 8 weeks

among T2DM patients could reduce serum hs-CRP concentration [8]. Cheng et al. [28] also demonstrated that soy milk containing *Streptococcus thermophiles* and *Lactobacillus plantarum* instigated NO production and endothelial NO synthase activity in umbilical vein endothelial cells. In addition, we have previously indicated that intake of a synbiotic food containing *Lactobacillus sporogenes* ($27 \times 10^7$ CFU) plus 1.1 g inulin for 6 weeks in diabetic patients resulted in a significant reduction in serum hs-CRP levels [29]. In contrast, a 3-month intake of synbiotic supplements did not affect inflammatory factors in healthy elderly individuals [30]. In addition, probiotic supplementation for 6 weeks in T2DM patients did not influence serum hs-CRP concentrations [15]. Diabetes and CVD are associated with increased concentrations of biomarkers of inflammation and oxidative stress [31]. Previous studies have documented that endothelium in individuals with diabetes might not able to produce sufficient amount of NO, which in turn blood vessels fail to relax in response to endothelium-dependent vasorelaxants [32]. Endothelial dysfunction is predictive of future CVD events [33]. Therefore, decreasing levels of hs-CRP and increasing levels of NO by synbiotics and/or probiotics may decrease CVD events. Few mechanisms may explain the beneficial effects of synbiotic on inflammatory markers. The upregulation of interleukin-18 (IL-18) gene expression by produced short-chain fatty acids (SCFA) [34] and increased production of methylketones family in gut following the intake of synbiotic [35] might result in its anti-inflammatory effects. In addition, probiotic intake may decrease inflammatory

**Table 1** General characteristics of study participants

|  | Placebo group ($n = 30$) | Synbiotic group ($n = 30$) | $P^1$ |
|---|---|---|---|
| Age (year) | $64.0 \pm 11.7$ | $64.2 \pm 12.0$ | 0.94 |
| Gender |  |  |  |
| Female | 19 (63.3%) | 19 (63.3%) | $1^†$ |
| Male | 11 (36.7%) | 11 (36.7%) |  |
| Duration of diabetes mellitus (year) | $6.9 \pm 1.8$ | $7.0 \pm 1.7$ | 0.88 |
| Height (cm) | $158.5 \pm 10.8$ | $156.4 \pm 6.8$ | 0.39 |
| BMI at study baseline (kg/m$^2$) | $29.6 \pm 4.6$ | $32.3 \pm 6.0$ | 0.05 |
| BMI change (kg/m$^2$) | $0.1 \pm 0.6$ | $-0.01 \pm 0.5$ | 0.51 |
| MET-h/day at study baseline | $26.7 \pm 1.9$ | $26.4 \pm 1.9$ | 0.53 |
| MET-h/day change | $-0.01 \pm 1.0$ | $0.05 \pm 0.8$ | 0.76 |
| Smoking (%) | 3 (10.0) | 3 (10.0) | $1.00^†$ |
| Aspirin 80 mg (%) | 30 (100) | 30 (100) | $1.00^†$ |
| Statin (%) | 30 (100) | 30 (100) | $1.00^†$ |
| Insulin therapy (%) | 9 (30.0) | 8 (26.7) | $1.00^†$ |
| Antidiabetic drugs (%) |  |  |  |
| Monotherapy | 16 (66.7) | 16 (69.6) |  |
| Combination therapy | 8 (33.3) | 7 (30.4) | $1.00^†$ |
| Hypertension (%) | 23 (76.7) | 22 (73.3) | $1.00^†$ |
| ACEI/ARB drugs (%) | 30 (100) | 30 (100) | $1.00^†$ |
| Blocker drugs (%) |  |  |  |
| β-blocker | 27 (90.0) | 28 (93.3) |  |
| Calcium channel blocker | 3 (10.0) | 2 (6.7) | $1.00^†$ |

Data are means ± SDs

[1] Obtained from independent $t$ test

[†] Obtained from Fisher's exact test

*ACEI*, angiotensin-converting enzymes inhibitors; ARB, aldosterone receptor blockers; *METs*, metabolic equivalents

factors through modulating the toll-like receptors (TLRs)-mitogen-activated protein kinase-peroxisome proliferator-activated receptor gamma (PPAR-γ) signaling pathways and intestinal microbiota [36]. Data have already showed that PPAR-γ activation may inhibit the activation of TLR4 signaling cascade [37]. Moreover, the activation of PPAR-γ has been demonstrated to decrease inflammation by suppressing the activation of nuclear factor-κB (NF-κB) [38]. Another

**Table 2** Dietary intakes of study participants throughout the study

|  | Placebo group ($n = 30$) | Synbiotic group ($n = 30$) | $P^1$ |
|---|---|---|---|
| Energy (kcal/day) | $2159 \pm 230$ | $2171 \pm 244$ | 0.85 |
| SFAs (g/day) | $23.7 \pm 5.2$ | $23.7 \pm 5.9$ | 0.97 |
| Omega-3 fatty acids (g/day) | $1.2 \pm 0.4$ | $1.2 \pm 0.5$ | 0.77 |
| Omega-6 fatty acids (g/day) | $18.0 \pm 8.7$ | $18.3 \pm 9.3$ | 0.88 |
| Magnesium (mg/day) | $238.2 \pm 50.2$ | $259.6 \pm 59.8$ | 0.13 |
| Selenium (μg/day) | $49.9 \pm 13.0$ | $47.8 \pm 12.1$ | 0.51 |
| Manganese (mg/day) | $1.8 \pm 0.8$ | $2.0 \pm 0.8$ | 0.34 |
| Vitamin C (mg/day) | $138.2 \pm 58.9$ | $149.1 \pm 39.6$ | 0.40 |
| Vitamin A (μg/day) | $571.0 \pm 142.3$ | $604.8 \pm 124.2$ | 0.33 |
| Vitamin E (mg/day) | $10.9 \pm 1.9$ | $11.5 \pm 2.1$ | 0.27 |

Data are means ± SDs

[1] Obtained from independent $t$ test

*SFAs*, saturated fatty acids

**Table 3** Carotid intima-media thickness, biomarkers of inflammation, and oxidative stress at baseline and 12 weeks after the intervention in people with overweight, diabetes, and coronary heart disease

| | Placebo group (n = 30) | | | | Synbiotic group (n = 30) | | | | $P^2$ |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | End-of-trial | Change | $P^1$ | Baseline | End-of-trial | Change | $P^1$ | |
| Maximum left CIMT (mm) | 0.87 ± 0.22 | 0.87 ± 0.20 | − 0.005 ± 0.14 | 0.84 | 0.88 ± 0.23 | 0.86 ± 0.22 | − 0.02 ± 0.13 | 0.61 | 0.85 |
| Mean left CIMT (mm) | 0.71 ± 0.14 | 0.72 ± 0.14 | 0.005 ± 0.07 | 0.67 | 0.72 ± 0.19 | 0.72 ± 0.17 | − 0.003 ± 0.12 | 0.88 | 0.73 |
| Maximum right CIMT (mm) | 0.79 ± 0.16 | 0.78 ± 0.15 | − 0.01 ± 0.13 | 0.44 | 0.80 ± 0.16 | 0.78 ± 0.14 | − 0.02 ± 0.10 | 0.40 | 0.95 |
| Mean right CIMT (mm) | 0.66 ± 0.10 | 0.65 ± 0.09 | − 0.01 ± 0.08 | 0.26 | 0.65 ± 0.11 | 0.63 ± 0.12 | − 0.02 ± 0.05 | 0.01 | 0.70 |
| hs-CRP (ng/mL) | 7367.5 ± 4722.4 | 7361.3 ± 5294.6 | − 6.2 ± 3163.6 | 0.99 | 8628.9 ± 6172.2 | 5527.2 ± 5282.6 | − 3101.7 ± 5109.1 | 0.002 | 0.02 |
| NO (µmol/L) | 46.5 ± 9.9 | 42.9 ± 12.9 | − 3.6 ± 6.9 | 0.009 | 39.9 ± 6.1 | 47.7 ± 10.4 | 7.8 ± 10.3 | < 0.001 | < 0.001 |
| TAC (mmol/L) | 961.4 ± 148.6 | 985.7 ± 224.5 | 24.2 ± 236.8 | 0.57 | 936.7 ± 169.5 | 992.3 ± 174.1 | 55.6 ± 145.5 | 0.04 | 0.53 |
| GSH (µmol/L) | 595.3 ± 124.8 | 613.2 ± 140.7 | 17.9 ± 60.9 | 0.11 | 680.3 ± 129.1 | 698.3 ± 144.3 | 18.0 ± 115.0 | 0.39 | 0.91 |
| MDA (µmol/L) | 3.1 ± 0.5 | 3.0 ± 0.5 | − 0.1 ± 0.3 | 0.02 | 2.9 ± 0.4 | 2.3 ± 0.9 | − 0.6 ± 1.0 | 0.005 | 0.01 |
| SBP (mmHg) | 129.3 ± 20.8 | 128.4 ± 17.8 | − 0.9 ± 15.5 | 0.73 | 141.3 ± 20.2 | 137.9 ± 21.5 | − 3.4 ± 17.4 | 0.29 | 0.49 |
| DBP (mmHg) | 80.5 ± 12.2 | 82.1 ± 10.5 | 1.6 ± 11.0 | 0.42 | 83.5 ± 7.8 | 81.8 ± 8.7 | − 1.7 ± 7.9 | 0.25 | 0.18 |

All values are means ± SDs

[1] $P$ values represent paired-samples $t$ test

[2] $P$ values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA)

CIMT, carotid intima-media thickness; CHD, coronary heart disease; DBP, diastolic blood pressure; GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; TAC, total antioxidant capacity; T2DM, type 2 diabetes mellitus; SBP, systolic blood pressure

**Table 4** Adjusted changes in carotid intima-media thickness, biomarkers of inflammation, and oxidative stress in people with overweight, diabetes, and coronary heart disease

|  | Placebo group ($n = 30$) | Synbiotic group ($n = 30$) | $P^1$ |
|---|---|---|---|
| Mean left CIMT (mm) | $0.004 \pm 0.01$ | $-0.002 \pm 0.01$ | 0.80 |
| Maximum left CIMT (mm) | $-0.006 \pm 0.02$ | $-0.01 \pm 0.02$ | 0.86 |
| Mean right CIMT (mm) | $-0.01 \pm 0.01$ | $-0.02 \pm 0.01$ | 0.63 |
| Maximum right CIMT (mm) | $-0.02 \pm 0.01$ | $-0.01 \pm 0.01$ | 0.83 |
| hs-CRP (ng/mL) | $-433.3 \pm 743.2$ | $-2632.3 \pm 743.2$ | 0.04 |
| NO (μmol/L) | $-3.4 \pm 1.7$ | $7.6 \pm 1.7$ | $< 0.001$ |
| TAC (mmol/L) | $30.0 \pm 33.6$ | $49.8 \pm 33.6$ | 0.67 |
| GSH (μmol/L) | $12.2 \pm 17.1$ | $23.6 \pm 17.1$ | 0.64 |
| MDA (μmol/L) | $-0.1 \pm 0.1$ | $-0.6 \pm 0.1$ | 0.004 |
| SBP (mmHg) | $-3.1 \pm 2.8$ | $-1.2 \pm 2.8$ | 0.63 |
| DBP (mmHg) | $0.9 \pm 1.5$ | $-0.9 \pm 1.5$ | 0.40 |

All values are means ± SEs. Values are adjusted for baseline values

[1] Obtained from ANCOVA

*CIMT*, carotid intima-media thickness; *CHD*, coronary heart disease; *DBP*, diastolic blood pressure; *GSH*, total glutathione; *hs-CRP*, high-sensitivity C-reactive protein; *MDA*, malondialdehyde; *NO*, nitric oxide; *TAC*, total antioxidant capacity, *T2DM*, type 2 diabetes mellitus; *SBP*, systolic blood pressure

mechanism for anti-inflammatory effect of probiotics is modulating signaling pathway of tumor necrosis factor-alpha (TNF-$\alpha$) and NF-$\kappa$B. CRP is synthesized by the liver in response to releasing factors by fat cells such as interleukin 6 (IL-6) [39]. In a study by Hegazy et al. [40], it was observed that the consumption of probiotic in patients with ulcerative colitis for 8 weeks significantly ameliorated the inflammation by decreasing concentrations of IL-6, expression of TNF-$\alpha$, and NF-$\kappa$B. Furthermore, decreased superoxide anion, and hydroperoxides, such as MDA by synbiotics [41], may result in increased NO levels.

Our study demonstrated that synbiotic supplementation for 12 weeks to people with overweight, diabetes, and CHD resulted in a significant reduction in plasma MDA concentrations, but did not influence TAC and GSH levels. We have previously shown that probiotic supplementation for 6 weeks among women with GDM had beneficial effects on plasma TAC, MDA, and oxidative stress index, but did not affect plasma GSH levels [42]. In line with our findings, in an animal study, the administration of oligofructose, inulin, and the two *Bifidobacterium infantis* for 7 days was associated with a significant reduction in MDA levels [43]. In addition, a significant decrease in MDA levels was observed following the supplementation of probiotic for 30 days in rabbits [44]. Supplementation with synbiotic for 30 days to lactating mothers had positive effects on MDA concentrations in human breastmilk [45]. However, some investigators did not observe the beneficial effects of synbiotic supplementation on biomarkers of oxidative stress. For example, taking synbiotic diet containing *Lactobacillus plantarum* and *Bacillus coagulans* plus inulin for 6 weeks in an animal study did not affect MDA levels [46]. Furthermore, probiotic supplementation for 7 days among critically ill patients [47] and

taking multispecies probiotics supplements ($10^{10}$ CFU/day) for 14 weeks among trained men did not influence MDA levels [48]. The different findings might be explained by different study designs, different dosages of probiotics and inulin used, the lack of considering baseline levels of dependent variables, as well as different individuals of the study. Increased lipid peroxidation especially MDA maybe is associated with many diseases, including cancer, CVD, and diabetes mellitus [49]. In addition, lipid peroxidation has a key role in the pathogenesis and the complications of diabetes [49]. Therefore, synbiotics due to their anti-oxidative actions may be useful to decrease metabolic complications result from increased oxidative stress. Synbiotic intake may decrease MDA levels through reduced inflammatory markers result from produced SCFA in the gut [50], and its effect in decreased oxidized LDL and 8-isoprostanes [51]. In addition, antioxidant power of tissues can be accomplished by several mechanisms among which activities of antioxidant enzymes as superoxide dismutase (SOD) and glutathione peroxidase (GPx). In a study by Ghoneim et al. [44], probiotic supplementation increased activities of SOD and GPx in all studied organs although some of which was not significant. Furthermore, the antioxidant effects of probiotics may be due to activating nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 serves as a master regulator of a cellular defense system against oxidative stress [52]. In a study by Gao et al. [53], *Lactobacillus plantarum* markedly increased the gene expression of Nrf2.

Our study had a number of limitations. We did not assess the compliance to synbiotic supplementation by the use of a biomarker, and SCFA and fecal bacterial loads. In addition, duration of the study was brief. Longer duration of the intervention may have better effects on CIMT. In the current study, we used a

synbiotic containing mixed bacterial species plus inulin. In addition, one can conclude if the treatment effects observed in the current study was due to the effect of which component of the supplements. Therefore, further studies are needed with single supplements used in the current study in order to assess beneficial effects on biomarkers of inflammation and oxidative stress. Moreover, we did not collect stool samples from participants, so we did not know whether any of the probiotics survived in the colon.

Overall, synbiotic supplementation for 12 weeks among people with overweight, diabetes, and CHD had beneficial effects on serum hs-CRP, plasma NO, and MDA levels; however, it did not have any effect on other biomarkers of oxidative stress and CIMT levels. This suggests synbiotic supplementation may confer advantageous therapeutic potential for people with overweight, diabetes, and CHD management. Further research is needed in other patients with longer periods to determine the safety of this supplemental approach.

**Compliance with Ethical Standards**

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

**For Studies with Human Subjects** All procedures followed in the paper were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

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