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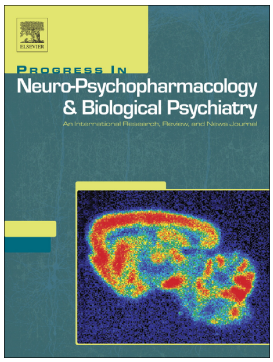

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The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in type 2 diabetic patients with coronary heart disease: a randomized, double-blind, placebo-controlled trial

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Running Title: Vitamin D and probiotic administration and type 2 diabetes mellitus

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ABSTRACT

Background: This study was carried out to evaluate the effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in diabetic people with coronary heart disease (CHD).

Methods: This randomized, double-blind, placebo-controlled trial was carried out among 60 diabetic people with CHD, aged 45-85 years old. Subjects were randomly allocated into two groups to receive either 50,000 IU vitamin D every 2 weeks plus 8×10^9 CFU/g probiotic of Lactocare Zisttakhmir Co (n=30) or placebo (n=30) for 12 weeks. Fasting blood samples were obtained at baseline and after the 12-week intervention to determine metabolic profiles.

Results: After the 12-week intervention, compared with the placebo, vitamin D and probiotic co-supplementation resulted in significant improvements in beck depression inventory total score (-2.8±3.8 vs. -0.9±2.1, P=0.01), beck anxiety inventory scores (-2.1±2.3 vs. -0.8±1.4, P=0.009) and general health questionnaire scores (-3.9±4.1 vs. -1.1±3.4, P=0.005). Compared with the placebo, vitamin D and probiotic co-supplementation resulted in significant reductions in serum insulin levels (-2.8±3.8 vs. +0.2±4.9 µIU/mL, P=0.009), homeostasis model of assessment-estimated insulin resistance (-1.0±1.6 vs. -0.1±1.5, P=0.02), and a significant increase in serum 25-OH-vitamin D (+11.8±5.9 vs. +0.1±1.4 ng/mL, P<0.001), the quantitative insulin sensitivity check index (+0.03±0.04 vs. -0.001±0.01, P=0.003) and serum HDL-cholesterol levels (+2.3±3.5 vs. -0.5±3.8 mg/dL, P=0.004). In addition, changes in serum high sensitivity C-reactive protein (hs-CRP) (-950.0±1811.2 vs. +260.5±2298.2 ng/mL, P=0.02), plasma nitric oxide (NO) (+1.7±4.0 vs. -1.4±6.7 µmol/L, P=0.03) and plasma total antioxidant capacity (TAC) (+12.6±41.6 vs. -116.9±324.2 mmol/L, P=0.03) in the supplemented group were significantly different from the changes in these indicators in the placebo group.

Conclusions: Overall, vitamin D and probiotic co-supplementation after 12 weeks among diabetic people with CHD had beneficial effects on mental health parameters, serum hs-CRP, plasma NO, TAC, glycemic control and HDL-cholesterol levels.

Keywords: Vitamin D, probiotic, mental health, inflammation, oxidative stress, type 2 diabetes mellitus, coronary heart disease

1. Introduction

Previous studies suggest that type 2 diabetes mellitus (T2DM) and coronary heart diseases (CHD) are comorbidities and the major risk factors of mortality (Gaiz et al. , 2017, Gress et al. , 2000). CHD is responsible for more than 80% of death in people with T2DM (Rathmann and Giani, 2004). Cognitive impairment, depression, and functional disability are involved in the course history of the disease (Gregg et al. , 2002). Insulin resistance and hyperinsulinemia in people with T2DM cause metabolic disorders; leading to decreased nitric oxide (NO) synthesis in blood vessel walls (Keymel et al. , 2011). Furthermore, inflammation and oxidative stress play important roles in the development of T2DM and CHD (Chehaibi et al. , 2016, Tatsch et al. , 2015), and the close relationship between increased inflammation and oxidative stress in both diseases is now well defined.

Earlier, some studies have reported the beneficial effects of vitamin D and probiotic in people with T2DM and CHD. We have previously showed that vitamin D supplementation (50,000 IU/2 week) for 6 months to diabetic patients with CHD had beneficial effects on glycemic status and high-sensitivity C-reactive protein (hs-CRP), NO, glutathione (GSH), and malondialdehyde (MDA) levels, but did not influence other metabolic profiles (Farrokhian et al. , 2017). Vitamin D supplementation (50,000 IU/week) for 10 weeks to people with non-alcoholic fatty liver disease decreased insulin resistance and fasting glucose (Foroughi et al. , 2016). Also, in a meta-analysis study, probiotic supplementation in patients with T2DM could improve fasting glucose, HDL-cholesterol, but did not affect other markers of glycemic control and lipid profiles (Li et al., 2016). However, no beneficial effects on cardiovascular risk were seen following the supplementation of 2,000 IU/day vitamin D for 24 weeks to people T2DM (Ryu et al. , 2014). There is evidence that suggest the importance of vitamin D and probiotic co-supplementation on

metabolic profiles. In a study by Jones et al.(2013), it was seen that circulating vitamin D in response to oral probiotic supplementation had significantly increased.

Given the antioxidant and anti-inflammatory effects of vitamin D and probiotic, we hypothesized that vitamin D and probiotic co-supplementation might be beneficial in diabetic people with CHD. The present study was, therefore, conducted to evaluate the effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in diabetic people with CHD.

2. Methods

2.1. Trial design and participants

This study was a 12-week randomized double-blinded placebo-controlled clinical trial was registered with the website for registration of clinical trials in Iran (http://www.irct.ir: IRCT2017073033941N4). People with T2DM diagnosed based on the criteria of the American Diabetes Association (2014), aged 45-85 years with CHD diagnosed based on the American Heart Association (Welles et al. , 2014) with 2- and 3-vessel CHD were included. The study was conducted from August 2017 and November 2017. Study protocol was approved by the research ethics committee of Kashan University of Medical Sciences (KAUMS) and informed consent was taken from all participants. Those taking vitamin D, probiotic and/or synbiotic within the last 3 months, and patients with thyroid disorders were not included in this study.

2.2. Study procedures

Firstly, all people were randomized into two groups to intake either 50,000 IU vitamin D3 every 2 weeks plus 8×10^9 CFU/g probiotic, containing *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus reuteri,* and *Lactobacillus fermentum* (each 2×10^9) (n=30) or placebo

(n=30) for 12 weeks. In addition, all people were matched according to age, BMI, gender and the dosage and type of medications. Vitamin D, probiotic and its placebos (paraffin and starch, respectively) were produced by Zahravi Pharmaceutical Company (Tabriz, Iran), Lactocare Zisttakhmir Company (Tehran, Iran) and Barij Essence Pharmaceutical Company (Kashan, Iran), respectively. They were completely identical in terms of their appearance, color, shape, size, smell and taste and packaging. Randomization process was conducted using computer-generated random numbers by a trained staff at the clinic, blinded to both participants and researchers. Compliance was evaluated by counting the remaining supplements and subtracting from the number of supplements provided to the participants and the measurement of serum 25-hydroxyvitamin D levels with the enzyme-linked immunosorbent assay (ELISA) method. A 3-day food record was obtained at week 1, 5, 9 and 12 of the intervention and macro- and micro-nutrients intakes were determined using the Modified Nutritionist-4 software program (First Databank, San Bruno, CA) adapted for the Iranian food pattern (Azar and Sarkisian, 1980).

2.3. Assessment of outcomes

Glycemic control was considered as the primary outcomes, and parameters of mental health and other metabolic profiles were defined as the secondary outcomes. Ten milliliters fasting blood samples were drown from antecubital vein at the beginning and after the 12-week intervention at Kashan reference laboratory, Kashan, Iran. Blood was collected in 2 separate tubes: 1) one without ethylenediaminetetraacetic acid (EDTA) to separate the serum, in order to determine serum insulin, lipid profiles and hs-CRP concentrations and 2) another one containing EDTA to assess plasma NO and biomarkers of oxidative stress. Blood samples were immediately centrifuged (Hettich D-78532, Tuttlingen, Germany) at 3500 rpm for 10 min to separate the serum, and were then stored at -80$^{\circ C}$ until being analyzed at the KAUMS reference laboratory.

Enzymatic kits (Pars Azmun, Tehran, Iran) were used to evaluate fasting plasma glucose (FPG) and lipid profiles with inter- and intra-assay coefficient of variations (CVs) less than 5%. Insulin values were assessed by the use of ELISA kit (DiaMetra, Milano, Italy) with inter- and intra-assay CVs of 3.4 to 4.9%, respectively. The homeostasis model of assessment-insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI) were determined according to the standard formula (Pisprasert et al. , 2013). Hs-CRP values were determined by an ELISA kit (LDN, Nordhorn, Germany) with inter- and intra-assay CVs of lower than 7.0%. Plasma total antioxidant capacity (TAC) by the method of Benzie and Strain (Benzie and Strain, 1996), GSH using the method of Beutler et al.(1985), MDA by the method of spectrophotometric test (Janero, 1990), and NO levels using Griess method (Tatsch et al. , 2011) were determined with inter- and intra-assay CVs of lower than 5%. Systolic (SBP) and diastolic blood pressure (DBP) was determined via a sphygmomanometer (ALPK2, Zhejiang, China).

2.4. Clinical assessment

Beck Depression Inventory (BDI) was assessed using a self-compiled questionnaire (Beck et al. , 1961). Anxiety evaluated by Beck Anxiety Inventory (BAI) developed by Beck et al. (1988). The general health questionnaire-28 (GHQ-28) comprises 28-item consisting of 4 subscales (Goldberg and Hillier, 1979).

2.5. Statistical methods

The normality of study variables was determined using the Kolmogorov-Smirnov test. Outcome log-transformation was used if model residual has non-normal distribution (MDA, SBP and DBP). The intention-to-treat (ITT) analysis was applied for all randomly allocated subjects. Anthropometric measures as well as macro- and micro-nutrient dietary intakes were compared

between the two groups, using independent samples *t*-test. The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic profiles were assessed using repeated measures analysis of variance. The P-value of <0.05 was considered statistically significant. All statistical analyses in this study were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

3. Results

Four subjects in the supplements and placebo groups were dropped out for personal reasons (Fig.1). However, all 60 people were included in the final analysis based on the ITT principle. Overall, the compliance rate was high, because of more than 90% of capsules were consumed throughout the study in both groups.

Mean age, height, weight, BMI and METs at baseline and after the 12-week treatment were not statistically different between treatment and placebo groups (Table 1).

We observed no significant changes in macro- and micronutrients between the two groups (Data not shown).

Compared with the placebo, vitamin D and probiotic co-supplementation led to significant improvements in BDI score (-2.8±3.8 vs. -0.9±2.1, P=0.01), BAI scores (-2.1±2.3 vs. -0.8±1.4, P=0.009) and GHQ scores (-3.9±4.1 vs. -1.1±3.4, P=0.005) (Table 2). Compared with the placebo, vitamin D and probiotic co-supplementation resulted in a significant reduction in insulin values (-2.8±3.8 vs. +0.2±4.9 μIU/mL, P=0.009), HOMA-IR (-1.0±1.6 vs. -0.1±1.5, P=0.02), and a significant elevation in 25-OH-vitamin D (+11.8±5.9 vs. +0.1±1.4 ng/mL, P<0.001), QUICKI

(+0.03±0.04 vs. -0.001±0.01, P=0.003) and serum HDL-cholesterol levels (+2.3±3.5 vs. -0.5±3.8 mg/dL, P=0.004). In addition, changes in hs-CRP (-950.0±1811.2 vs. +260.5±2298.2 ng/mL, P=0.02), plasma NO (+1.7±4.0 vs. -1.4±6.7 μmol/L, P=0.03) and plasma TAC (+12.6±41.6 vs. -116.9±324.2 mmol/L, P=0.03) in the supplemented group were significantly different from the placebo group.

4. Discussion

We found that vitamin D and probiotic co-supplementation for 12 weeks to diabetic people with CHD had beneficial effects on mental health parameters, glycemic control, HDL-cholesterol levels, hs-CRP, NO and TAC levels. To our knowledge, this research is the first study conducted to determine the effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic profiles among diabetic people with CHD.

4.1. Effect on clinical symptoms

We proved that vitamin D and probiotic co-supplementation for 12 weeks to diabetic people with CHD significantly improved mental health parameters. In agreement with our study, after the 9-week vitamin D supplementation (50,000 IU/week) among adolescent girls, there was a significant reduction on mild, moderate, and severe depression score (Bahrami et al. , 2017). We have previously reported that taking 50,000 IU/2 week vitamin D supplement for 12 weeks by maintenance methadone treatment subjects had favorable effects on psychological symptoms (Ghaderi et al. , 2017). In addition, probiotic supplementation for 12 weeks to people with multiple sclerosis was benefit in improving parameters of mental health (Kouchaki et al. , 2017). However, high-dose VD3 supplementation (50,000 IU/week) for 52 weeks to dialysis population did not improve the depressive symptoms, but had a beneficial effect on vascular depression

(Wang et al. , 2016). In a meta-analysis study, probiotic supplementation had an insignificant effect on mood (Ng et al. , 2017). Increased gene expression of tyrosine hydroxylase and enhancement of the bioavailability of multiple neurotransmitters like dopamine and noradrenalin by vitamin D may improve mental health parameters (Humble, 2010, Khoraminya et al. , 2013). Furthermore, probiotic might improve symptoms of mental health through elevated tryptophan concentrations and reduced serotonin levels (Desbonnet et al. , 2008). The synergism between the immunomodulatory and anti-inflammatory effects of both vitamin D and probiotic might boost their effect on clinical symptoms of psychiatric illnesses.

4.2. Effect on metabolic abnormalities

We found that 12-week vitamin D plus probiotic administration to diabetic people with CHD was associated with significant reductions in insulin, HOMA-IR, and a significant rise in QUICKI and serum HDL-cholesterol levels, but unchanged FPG and other lipid profiles. Mirhosseini et al.(2017) found that vitamin D supplementation, a minimum dose of 4000 IU/day, significantly decreased FPG, HbA1c, and HOMA-IR, and improved insulin sensitivity in people with T2DM. Consuming vitamin D by women with gestational diabetes mellitus resulted in a significant decrease in HOMA-IR, QUICKI, and LDL-cholesterol concentrations, but did not useful for improving other metabolic profiles (Akbari et al. , 2017). In addition, another meta-analysis study demonstrated that probiotic supplementation decreased HbA1c, insulin levels and HOMA-IR in patients with T2DM, but did not affect lipid profiles (Yao et al. , 2017). Probiotic supplementation also improved lipid metabolism through decreasing total- and LDL-cholesterol values, but unchanged other lipid profiles (Cho and Kim, 2015). Insulin resistance results in peripheral arterial disorder, hypertension, and the endothelial cell dysfunctions, thus increasing risk factors for stroke and heart failure (Patel et al. , 2016). Vitamin

D intake plays a key role in the regulation of cellular calcium signaling along with an indirect effect on regulating insulin secretion (Bergsten, 1995). Furthermore, vitamin D may affect C-peptide production (Marques et al. , 2004), and suppress renin-angiotensin activity (Takiishi et al. , 2014). Probiotic intake may have anti-diabetic effects due to facilitating production of SCFA (Belenguer et al. , 2006). In addition, probiotic may improve insulin sensitivity by increased anti-inflammatory factors production and decreased oxidative stress (Ma et al. , 2004, Paszti-Gere et al. , 2012).

4.3. Effect on inflammation and oxidative stress

This research proved that compared with the placebo, taking vitamin D plus probiotic for 12 weeks by diabetic people with CHD decreased hs-CRP, and increased NO and TAC levels, but unaltered GSH and MDA levels. We have previously showed that vitamin D supplementation (50,000 IU/2 week) for 12 weeks to women with PCOS had beneficial effects on hs-CRP and MDA levels, but did not affect other parameters of inflammation and oxidative stress (Maktabi et al. , 2017). In addition, vitamin D3 intake (200,000 IU) significantly decreased inflammatory markers and increased TAC after 4 weeks in elderly subjects (de Medeiros Cavalcante et al. , 2015). Supporting our findings, the consumption of 200 g/day yogurt containing probiotic (10^8 CFU/g each) for 8 weeks in overweight people decreased inflammatory factors (Zarrati et al. , 2014). However, in a meta-analysis study, vitamin D intake did affect no significant influence on inflammatory factors (Jamka et al. , 2016). Moreover, in a meta-analysis study conducted among people with T2DM, probiotic use did not have a significant effect on CRP levels (Kasinska and Drzewoski, 2015). T2DM is correlated with the elevated biomarkers of inflammation and oxidative stress (Styskal et al. , 2012). The different findings might be explained by different parameters like disease condition, vitamin D and probiotic dose, and different study designs. Less

production of parathyroid hormone (Struglia et al. , 2015), decreasing reactive oxygen species and pro-inflammatory factors by vitamin D (Jain and Micinski, 2013) might describe its beneficial effects on inflammation and oxidative stress. Furthermore, probiotic intake may useful in reducing inflammatory factors through producing SCFA in the gut (Sadrzadeh-Yeganeh et al. , 2010) and the decreasing production of hydrogen peroxide radicals (Komers and Anderson, 2003).

The current study had few limitations. We did not measure fecal bacteria loads before and after probiotic intake, and characterization of the microbiome at baseline, during and after therapy. In addition, we did not evaluate the effects of vitamin D and probiotic co-supplementation on gene expression related to inflammation and insulin resistance.

Overall, vitamin D and probiotic co-supplementation after 12 weeks among diabetic people with CHD had beneficial effects on mental health parameters, glycemic control, HDL-cholesterol levels, hs-CRP, NO and TAC, but did not affect other metabolic profiles and blood pressures.

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Authors' contributions

ZA contributed in conception, design, statistical analysis and drafting of the manuscript. FR, VO and FB. contributed in conception, data collection and manuscript drafting. The final version was confirmed by all authors for submission.

Conflicts of interest

None.

Clinical trial registration number

http://www.irct.ir: IRCT2017073033941N4.

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Table 1

General characteristics of study participants

	Placebo group (n=30)	Vitamin plus probiotic group (n=30)	P[1]
Age (y)	67.3±11.0	71.5±10.9	0.13
Gender			
Female	16 (53.3)	14 (46.7)	0.60†
Male	14 (46.7)	16 (53.3)	
Height (m)	159.7±9.4	156.1±11.0	0.17
Weight at study baseline (kg)	72.4±15.5	70.1±13.4	0.53
Weight at end-of-trial (kg)	72.5±15.4	70.0±13.4	0.51
Body weight change (kg)	0.1±1.5	-0.1±0.7	0.64
BMI at study baseline (kg/m^2)	28.2±4.9	29.0±6.2	0.59
BMI at end-of-trial (kg/m^2)	28.3±4.8	29.0±6.2	0.62
BMI change (kg/m^2)	0.1±0.6	-0.01±0.3	0.69
MET-h/day at study baseline	26.8±1.9	27.1±1.8	0.50
MET-h/day at end-of-trial	26.9±2.0	27.2±2.0	0.48
MET-h/day change	0.1±0.5	0.1±0.8	0.80

Data are means± SDs.

[1] Obtained from independent samples *t*-test.

† Obtained from Pearson Chi-square test.

BMI, body mass index; METs, metabolic equivalents.

Table 2 Mental health parameters, cardio-metabolic risk and biomarkers of oxidative stress at baseline and after the 12-week intervention in type 2 diabetic patients with coronary heart disease

	Placebo group (n=30)			Vitamin D plus probiotic group (n=30)			P[1]
	Baseline	End-of-trial	Change	Baseline	End-of-trial	Change	
Serum 25-OH-vitamin D (ng/mL)	13.8±3.0	13.9±3.4	0.1±1.4	14.7±2.9	26.5±6.1	11.8±5.9	<0.001
BDI score	21.5±3.9	20.6±3.7	-0.9±2.1	23.2±4.6	20.4±3.4	-2.8±3.8	0.01
BAI score	16.5±4.0	15.7±3.7	-0.8±1.4	17.9±4.7	15.8±4.0	-2.1±2.3	0.009
GHQ score	46.5±7.0	45.4±5.7	-1.1±3.4	49.1±7.7	45.1±5.7	-3.9±4.1	0.005
FPG (mg/dL)	126.8±34.9	124.3±34.8	-2.5±17.9	121.3±49.7	114.9±46.6	-6.3±26.5	0.51
Insulin (μIU/mL)	13.1±7.8	13.3±7.9	0.2±4.9	13.7±7.3	10.9±7.4	-2.8±3.8	0.009
HOMA-IR	4.0±2.5	3.9±2.5	-0.1±1.5	4.2±3.7	3.2±2.6	-1.0±1.6	0.02
QUICKI	0.32±0.02	0.32±0.02	-0.001±0.01	0.32±0.03	0.35±0.06	0.03±0.04	0.003
Triglycerides (mg/dL)	153.5±65.4	153.6±61.6	0.1±17.7	149.2±92.3	137.4±61.5	-11.8±69.7	0.37
VLDL-cholesterol (mg/dL)	30.7±13.1	30.7±12.3	0.01±3.5	29.8±18.5	27.5±12.3	-2.4±13.9	0.37
Total cholesterol (mg/dL)	146.7±29.1	146.9±33.9	0.3±18.0	138.8±41.7	144.2±33.3	5.4±30.5	0.43
LDL-cholesterol (mg/dL)	72.6±18.7	73.3±23.9	0.8±16.4	68.1±29.1	73.5±28.2	5.4±20.6	0.33
HDL-cholesterol (mg/dL)	43.4±8.2	42.8±7.4	-0.5±3.8	40.9±8.2	43.2±8.2	2.3±3.5	0.004

hs-CRP (ng/mL)	3430.9±2939.3	3691.4±3024.7	260.5±2298.2	3410.0±2384.9	2460.0±2135.8	-950.0±1811.2	0.02
NO (μmol/L)	37.7±8.2	36.3±8.7	-1.4±6.7	38.5±3.9	40.2±3.3	1.7±4.0	0.03
TAC (mmol/L)	1032.8±367.7	915.8±284.5	-116.9±324.2	987.9±91.7	1000.5±91.4	12.6±41.6	0.03
GSH (μmol/L)	510.6±123.0	498.4±158.5	-12.2±122.5	686.4±80.5	704.4±142.7	18.0±112.7	0.32
MDA (μmol/L)	3.3±1.3	3.4±1.0	0.1±0.7	2.8±0.2	2.7±0.3	-0.1±0.3	0.07
SBP (mmHg)	129.2±8.5	128.2±9.9	-1.0±6.1	132.7±7.9	132.0±8.4	-0.7±6.4	0.85
DBP (mmHg)	78.0±8.3	76.7±7.8	-1.3±6.1	76.8±7.2	76.1±7.0	-0.7±4.7	0.69

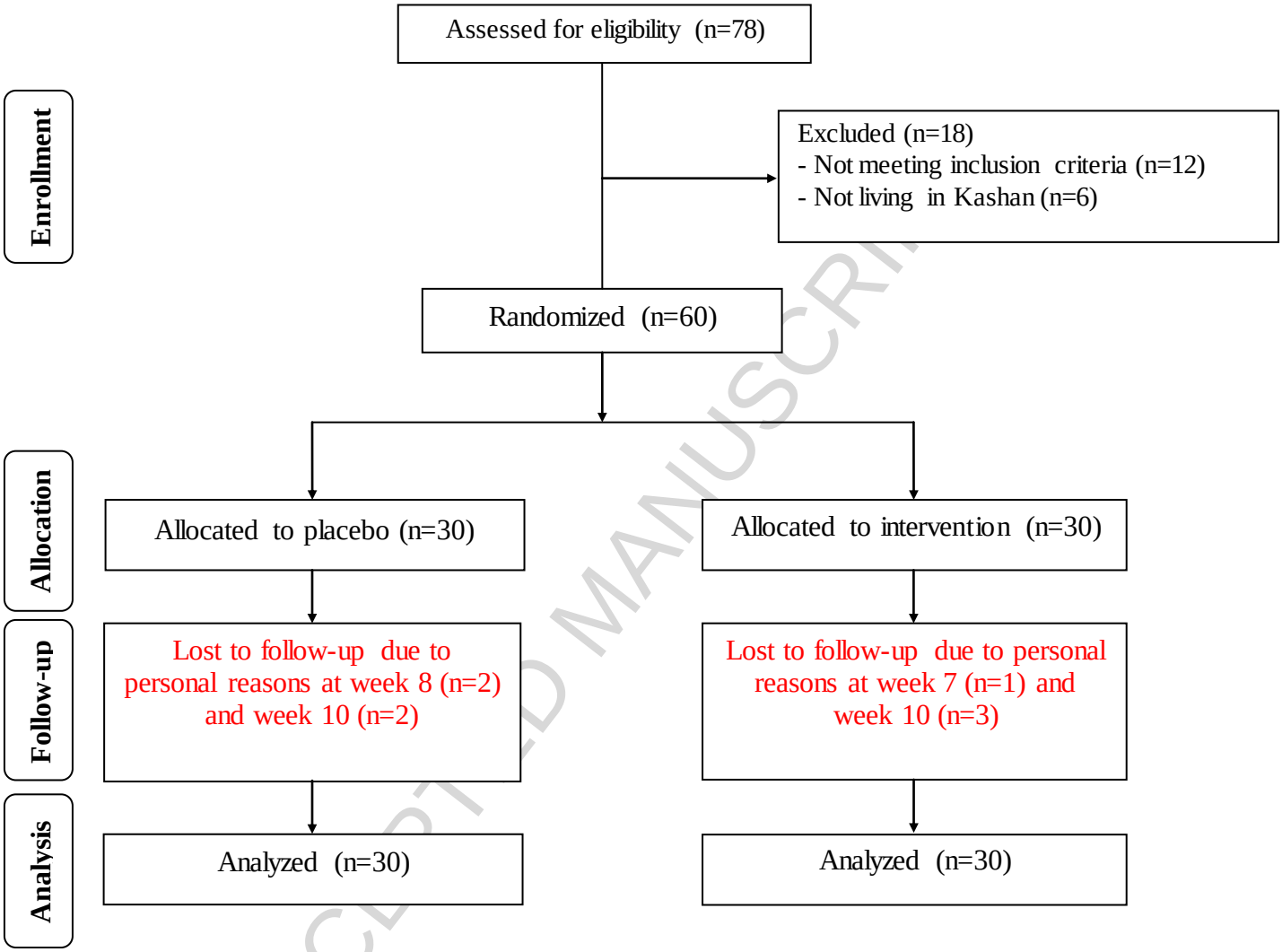
All values are means± SDs.

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

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; DBP, diastolic blood pressure; FPG, fasting plasma glucose; GHQ, General Health Questionnaire; GSH, total glutathione; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NO, nitric oxide; QUICKI, quantitative insulin sensitivity check index; SBP, systolic blood pressure; TAC, total antioxidant capacity.

Legend to figure:

Fig. 1. Summary of patient flow diagram.



Ethical statement

This randomized, double-blind, placebo-controlled trial, registered in the Iranian registry of clinical trials (http://www.irct.ir: IRCT2017073033941N4), was conducted at a cardiology clinic affiliated to Kashan University of Medical Sciences (KAUMS), Kashan, Iran, between August 2017 and November 2017.

This investigation was conducted according to the principals of the Declaration of Helsinki and the study protocol was approved by the research ethics committee of KAUMS. All people were informed about the aims and protocol of the study and written informed consent was obtained from all people prior to the intervention.

Highlights

- This study has evaluated the effects of vitamin D plus probiotic intake in CHD.
- Vitamin D plus probiotic in CHD patients improved psychological symptoms.
- Vitamin D plus probiotic in CHD patients improved metabolic status.