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ORIGINAL ARTICLE

# Effect of multispecies probiotic supplements on serum minerals, liver enzymes and blood pressure in patients with type 2 diabetes

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**Abstract** Emerging evidence suggests that diabetes is associated with altering serum minerals, elevated liver enzymatic activity and blood pressures. This study was designed to determine the effects of multispecies probiotic supplements on serum minerals, liver enzymes and blood pressure in diabetic patients. This randomized double-blinded controlled clinical trial was performed among 58 diabetic patients aged 35–70 y. Subjects were randomly assigned to consume either multispecies probiotic supplements ($N$=28) or the placebo group ($N$=30) for 8 weeks. The multispecies probiotic supplement was consisted of seven viable and freeze-dried strains: *Lactobacillus acidophilus* ($2\times10^9$ CFU), *Lactobacillus casei* ($7\times10^9$ CFU), *Lactobacillus rhamnosus* ($1.5\times10^9$ CFU), *Lactobacillus bulgaricus* ($2\times10^8$ CFU), *Bifidobacterium breve* ($2\times10^{10}$ CFU), *Bifidobacterium longum* ($7\times10^9$ CFU), *Streptococcus thermophilus* ($1.5\times10^9$ CFU) and 100 mg fructo-oligosaccharide with lactose as carrier substances. Fasting blood samples were taken at baseline and after 8-week intervention to measure serum minerals, liver enzymes and total bilirubin. Consumption of the probiotic supplements, compared to the placebo, resulted in an increased serum calcium concentrations (0.21 vs. −0.83 mg/dL, $P$=0.009) and a decreased serum alanine aminotransferase (ALT) levels (−2.46 vs. 4.62 mg/dL, $P$=0.02). We did not find a significant difference in terms of effect on serum magnesium, zinc, iron, alkaline phosphatase (ALP), aspartate aminotransferase (AST) levels and blood pressures comparing the two groups. In conclusion, multispecies probiotic supplementation among diabetic patients had beneficial effects on serum calcium and ALT concentrations.

**Keywords** Probiotics · Serum minerals · Liver enzymes · Blood pressure · Type 2 diabetes

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## Introduction

Type 2 diabetes mellitus (T2DM) is characterised by endothelial dysfunction and accelerated atherosclerosis, in combination with the adverse effects of advanced glycation end products resulting from hyperglycemia [1, 2]. It is also associated with central obesity, elevated blood pressures [3], increased activity of liver enzymes especially alanine aminotransferase (ALT) [4] and variations in serum levels and poor delivery of several mineral elements [5]. Elevated liver enzymes in patients with T2DM mostly attribute to fatty infiltration of the liver and can result in insulin resistance [6, 7]. In addition, increased blood pressure leads to thrombosis in vessels [8], stroke, cardiovascular diseases including heart failure, aortic aneurysms, diffuse atherosclerosis and pulmonary embolism [9].

A number of treatment options are available for T2DM including the use of food supplements [10, 11], micronutrients [12], lifestyle changes including dietary changes, physical exercise and weight loss [13], vitamin D [14] and resveratrol [15]. Recently, probiotics consumption were shown to increase mineral absorption [16], lower liver enzymes [17] or inhibited their increase [18] as well as reduce blood pressure in non-diabetic subjects [19]. Capcarova et al. [20] reported that consumption

of *Enterococcus faecium* M74 preparation ($2\times10^9$ CFU) in broiler chickens led to a significant increase of serum calcium and iron levels. Supplementation of *Bifidobacterium pseudocatenulatum*, *Bifidobacterium longum* and *Bifidobacterium longum* ($10^8$–$10^9$ CFU) decreased serum aspartate aminotransferase (AST) and ALT levels after 7 weeks in obese rats [21]. Decreased activity of liver enzymes by probiotics might be due to attenuated liver injury, reduced inflammation [22], decreased gut permeability and endotoxemia [23]. Furthermore, the beneficial effects of probiotics on blood pressure might be a result of bioactive peptides including the angiotensin-converting-enzyme (ACE) inhibitory peptides [19, 24].

Previous studies have generally assessed the effect of single strain probiotic mostly in animal models. Therefore, the aim of this study was to investigate the effects of multispecies probiotic supplementation on serum minerals (serum calcium, magnesium, zinc and iron), liver enzymes including serum alkaline phosphatase (ALP), ALT and AST and blood pressures among type 2 diabetic patients.

## Subjects and methods

*Participants* This randomized double-blinded controlled clinical trial was carried out in Kashan, Iran, during November 2011 to February 2012. On the basis of sample size formula suggested for randomized clinical trials [21], we considered the type I error of 5 % ($\alpha$=0.05) and type II error of 20 % ($\beta$=0.2; Power=80 %) and serum ALT levels as a key variable and we reached the sample size of 32 patients for each group. Patients with T2DM [fasting plasma glucose (FPG)≥126 mg/dL] aged 35 to 70 y were recruited in this study. Individuals who met inclusion criteria were called for participation in the study from those that attended Golabchi Diabetes Clinic affiliated to Kashan University of Medical Sciences, Kashan, Iran. Exclusion criteria were pregnant, using insulin or vitamin supplements, or had chronic kidney disease, liver, lung and chronic or acute inflammatory disease, heart valve disease, short bowel syndrome and allergies. 64 patients with T2DM were recruited. After matching for age, sex, BMI, type and dosage of oral hypoglycemic medications they were randomly assigned to receive either multispecies probiotic supplements ($N$=32) or the placebo ($N$=32) for 8 weeks. The study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethical committee of Kashan University of Medical Sciences approved the study and informed written consent was obtained from all participants.

*Study design* To obtain information about dietary intake they entered into a 2-week run-in period, when they refrained from taking any other probiotic food. During the run-in period, they recorded their dietary intakes for three non-consecutive days. At the end of run-in period, subjects were randomly assigned to receive either the placebo or multispecies probiotic supplement on every day for 8 weeks. Participants were asked not to alter their routine physical activity or usual diets and not to consume any probiotic capsules other than the one provided to them by the investigators. They were also asked to avoid consuming any fermented products. Placebo or multispecies probiotic supplements were provided for participants every month. Compliance with consumption of capsules was monitored once a week through phone interviews. The compliance was also checked by the use of three day dietary records completed throughout the study. To obtain nutrient intakes of participants based on these three-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods.

*Assessment of variables* Anthropometric measurements were assessed at baseline and after 8 weeks of intervention. Body weight was measured without shoes and in a minimal clothing by a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kg divided by height in meters squared. Fasting blood samples (10 mL) were taken at baseline and after eight-week intervention at Kashan reference laboratory after an overnight fast. Samples were analyzed for serum calcium, magnesium, zinc, iron, ALP, AST, ALT and total bilirubin. Serum Calcium, magnesium, iron, ALP, AST, ALT and bilirubin concentrations were assayed using mentioned kits (Pars Azmun Inc, Tehran, Iran). A serum zinc concentration was assayed using zinc kit (Elitec, Italy).

*Characteristics of supplements* The multispecies probiotic supplements (FamiLact Co, Tehran, Iran) were consisted of seven viable and freeze-dried strains: *Lactobacillus acidophilus* ($2\times10^9$ CFU), *Lactobacillus casei* ($7\times10^9$ CFU), *Lactobacillus rhamnosus* ($1.5\times10^9$ CFU), *Lactobacillus bulgaricus* ($2\times10^8$ CFU), *Bifidobacterium breve* ($2\times10^{10}$ CFU), *Bifidobacterium longum* ($7\times10^9$ CFU), *Streptococcus thermophilus* ($1.5\times10^9$ CFU) and 100 mg fructo-oligosaccharide with lactose as carrier substances. Placebo (the same substance without bacteria) was packed in identical capsules and coded by the producer to guarantee blinding.

*Statistical analysis* To ensure the normal distribution of variables, Histogram and Kolmogrov-Smirnov test were applied. We used paired-samples t-tests to identify within group differences (before and after intervention). Student's $t$ test was used to detect differences between groups. $P$<0.05 was considered as statistically significant. All statistical analyses were done using the Statistical Package for Social Science version 17 (SPSS Inc., Chicago, Illinois, USA).

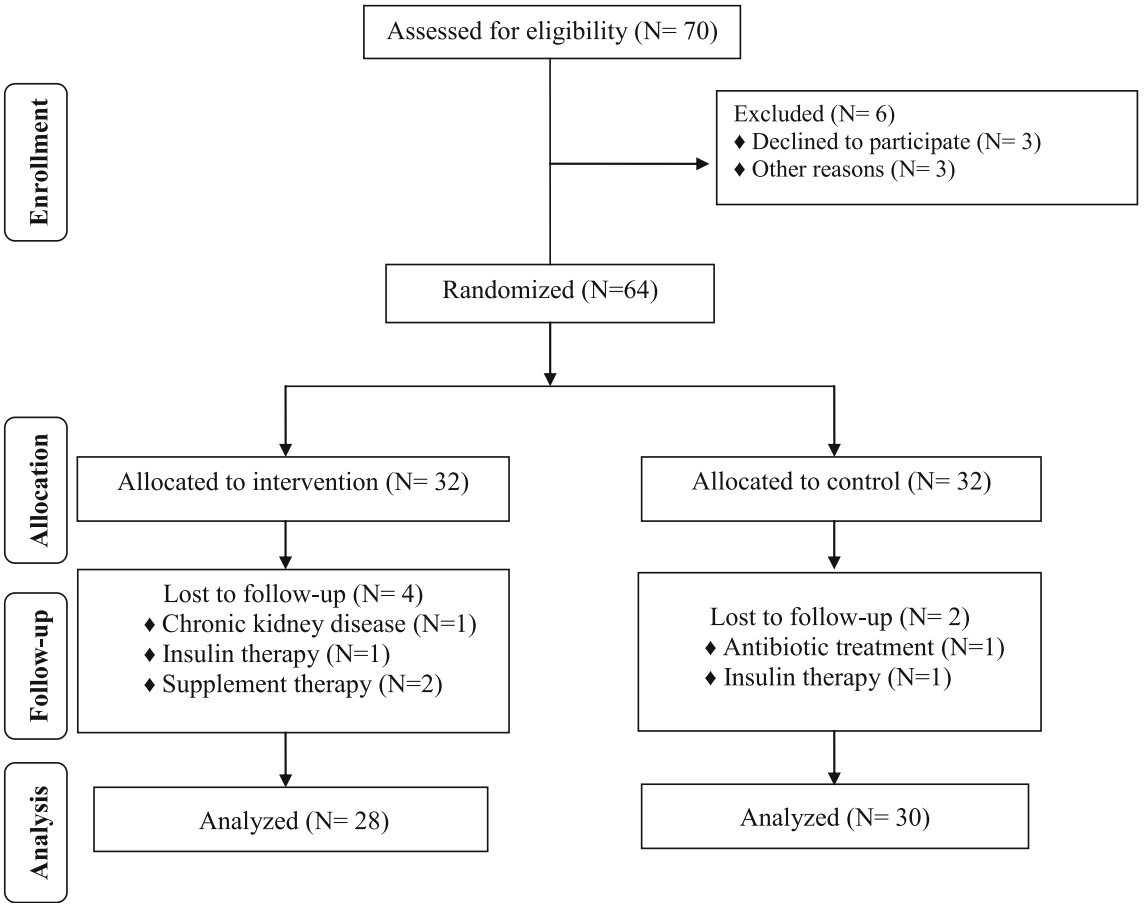


**Fig. 1** Summary of patient flow

## Results

Among individuals in the placebo group, 2 patients [need for antibiotic treatment (*N*=1) and need for insulin therapy (*N*=1)] were excluded. The exclusions in the multispecies probiotic group was 4 persons [chronic kidney disease (*N*=1), supplement therapy (*N*=2) and need for insulin therapy (*N*=1)]. Finally, 58 participants [placebo group (*N*=30) and probiotic group (*N*=28)] completed the trial (Fig. 1).

No serious adverse reactions were reported following the consumption of multispecies probiotic supplements. Mean age and height, weight and BMI were similar between the groups at baseline (Table 1).

Nor was there any difference in dietary intake (Table 2).

Consumption of the probiotic supplements, resulted in an increased serum calcium concentrations (0.21 vs. −0.83 mg/dL, *P*=0.009) and decreased serum ALT levels (−2.46 vs. 4.62 mg/dL, *P*=0.02) (Table 3). There was no significant difference on serum magnesium (*P*=0.28), zinc (*P*=0.3), iron (*P*=0.83), ALP (*P*=0.19), ALT (*P*=0.23) and total bilirubin (*P*=0.65) levels between the groups. Despite a significant decrease of probiotic supplements and the placebo consumption on systolic (−5.90; *P*=0.008, −5.56 mmHg; *P*=0.04, respectively) and diastolic blood pressures (−5.25; *P*=0.003, −7.03 mmHg; *P*=0.005, respectively), no significant differences were found between the two groups.

## Discussion

The study showed that consumption of multispecies probiotic supplements for 8 weeks by subjects with T2DM resulted in

**Table 1** General characteristics of the study participants[1]

| | Placebo (*N*=30) | Probiotic supplements (*N*=28) | *P*[2] |
|---|---|---|---|
| Age (y) | 52.1±6.9 | 49.6±9.9 | 0.26 |
| Height (cm) | 155.2±6.2 | 154.4±7.6 | 0.66 |
| Weight at study baseline (kg) | 74.2±12.2 | 75.6±13.3 | 0.66 |
| Weight at end-of-trial (kg) | 73.3±12.0 | 74.1±13.3 | 0.81 |
| BMI at study baseline (kg/m$^2$) | 30.7±4.1 | 31.9±6.4 | 0.39 |
| BMI at end-of-trial (kg/m$^2$) | 30.3±4.0 | 31.3±6.4 | 0.50 |

[1] Data are means ± standard deviation

[2] Obtained from independent *t* test

**Table 2** Dietary intakes of study participants at run-in period and throughout the study[1]

| | Run-in period | | | Throughout the study | | |
|---|---|---|---|---|---|---|
| | Placebo (*N*=30) | Probiotic supplements (*N*=28) | *P*[2] | Placebo (*N*=30) | Probiotic supplements (*N*=28) | *P*[2] |
| Energy (kcal/d) | 2197±344 | 2058±411 | 0.16 | 2154±225 | 2184±215 | 0.60 |
| Calcium (mg/d) | 951.3±183.8 | 871.4±308.2 | 0.22 | 1002.9±253.6 | 966.6±200.1 | 0.54 |
| Magnesium (mg/d) | 251.2±98.1 | 267.4±101.7 | 0.53 | 282.9±77.5 | 272.9±72 | 0.60 |
| Zinc (mg/d) | 8.1±2.3 | 9.0±2.3 | 0.14 | 8.7±1.4 | 9.3±1.7 | 0.12 |
| Iron (mg/d) | 14.6±3.3 | 13.4±3.6 | 0.10 | 14.6±3.3 | 13.6±2.7 | 0.20 |
| Phosphorus (mg/d) | 982.2±266.1 | 976.0±286.0 | 0.93 | 1058.7±187.3 | 1035.4±158.3 | 0.60 |

[1] Data are means ± standard deviation

[2] Obtained from independent *t* test

rise of serum calcium and lowering serum ALT. There was no effect on blood pressure, serum levels of magnesium, zinc, iron, ALP, AST and total bilirubin.

Elevated activities of liver enzymes and blood pressures among patients with T2DM are associated with adverse outcomes [6–8]. To the best of our knowledge, this study is the first examining the effect of multispecies probiotic supplements on serum minerals, liver enzymes and blood pressures among patients with T2DM.

Earlier studies mostly in animals reported beneficial effects of probiotics on serum calcium. Capcarova et al. [25] showed that consumption of a multi-strain probiotic preparation resulted in rise of serum calcium in broiler chickens, but did not affect serum iron levels. The use of fortified soya milk with *Lactobacillus acidophilus* also increased calcium absorption [26]. Consumption of *Enterococcus faecium* M74 preparation ($2\times10^9$ CFU) in broiler chickens also increased serum calcium and iron levels [20]. Similar findings were seen with consumption of strain *Enterococcus faecium* EK13 (dosage $10^9$ CFU/ml) in piglets for 14 days [16]. However, consumption of a probiotic preparation in chicks had not affect serum copper, zinc and manganese levels after 6 weeks [27].

Children fed with probiotic milk beverage containing *Lactobacillus acidophilus* were shown decreased significant serum hemoglobin levels after 101 days [28]. The exact mechanisms by which probiotics may be affect serum calcium are unknown. Panda et al. [29] showed that the use of *Lactobacillus sporogenes* had a positive effect on bone breaking

**Table 3** Means (±standard deviation) of serum minerals, liver enzymes and blood pressure at baseline and after the intervention

| | Placebo (*N*=30) | | | | Probiotic supplements (*N*=28) | | | | *P*[2] |
|---|---|---|---|---|---|---|---|---|---|
| | Wk0 | Wk8 | Change | *P*[1] | Wk0 | Wk8 | change | *P*[1] | |
| Calcium (mg/dL) | 10.21±0.99 | 9.38±0.70 | −0.83±1.07 | <0.0001 | 9.35±1.59 | 9.56±0.40 | 0.21±1.74 | 0.52 | 0.009 |
| Magnesium (mg/dL) | 2.06±0.43 | 1.99 ±0.46 | −0.07±0.66 | 0.57 | 1.81±0.45 | 1.55±0.59 | −0.26±0.71 | 0.06 | 0.28 |
| Zinc (mg/dL) | 65.55±39.24 | 92.96±13.86 | 27.41±40.98 | 0.001 | 73.87±27.45 | 113.38±35.04 | 39.51±47.57 | <0.0001 | 0.30 |
| Iron (mg/dL) | 66.13±32.98 | 76±43.35 | 9.87±40.19 | 0.18 | 69.53±62.94 | 76.21±41.61 | 6.68±69.58 | 0.61 | 0.83 |
| ALP[3] (mg/dL) | 174.30±58.77 | 179.23±51.97 | 4.93±35.91 | 0.45 | 134.14±40.98 | 152.39±41.38 | 18.25±40.67 | 0.02 | 0.19 |
| AST[4] (mg/dL) | 24.86±14.82 | 28.96 ±10.19 | 4.11±15.11 | 0.14 | 21.14±17.01 | 30.00±10.45 | 8.86±15.11 | 0.004 | 0.23 |
| ALT[5] (mg/dL) | 23.16±8.32 | 27.8±10.85 | 4.62±10.81 | 0.02 | 24.71±11.37 | 22.25±13.12 | −2.46±13.10 | 0.32 | 0.02 |
| Bilirubin (mg/dL) | 0.85±0.52 | 0.81±0.50 | −0.04±0.22 | 0.26 | 0.86±0.77 | 0.80±0.31 | −0.06±0.71 | 0.65 | 0.91 |
| SBP[6] (mmHg) | 142.36±16.57 | 136.80±13.12 | −5.56±14.82 | 0.04 | 139.00±14.76 | 133.10±14.56 | −5.90±10.96 | 0.008 | 0.92 |
| DBP[7] (mmHg) | 90.86±12.50 | 83.83 ±11.76 | −7.03±12.62 | 0.005 | 87.25±8.37 | 82.00±9.14 | −5.25±8.49 | 0.003 | 0.52 |

[1] Indicates within-group differences (paired samples *t* test)

[2] Indicates between group differences (Independent samples *t* test)

[3] *ALP* alkalin phosphatase

[4] *AST* aspartate aminotransferase

[5] *ALT* alanine aminotransferase

[6] *SBP* systolic blood pressure

[7] *DBP* diastolic blood pressure

strength and bone ash content that result from favorable environment in intestinal tract. Other effects may involve production of organic acids short chain fatty acid (SCFA) in gut, which in turn could improve protein digestibility and increased calcium release from organic compositions and absorption [28].

Our findings shown that probiotic supplements lowered serum ALT levels for 8 weeks among patients with T2DM, but did not affect serum ALP, AST and bilirubin levels. Consistent with our study, a decrease in serum ALT levels was seen with *Bifidobacterium pseudocatenulatum*, *Bifidobacterium longum* and *Bifidobacterium longum* ($10^8$–$10^9$ CFU) after 7 weeks in high fat diet-induced obese rats [21]. Similar findings were reported with consumption of *Bifidobacterium Catenulatum* and *Lactobacillus Fermentum* [30] and use of *Lactobacillus plantarum* and *Bifidobacterium infantis* in rats [31]. Administration of heat-killed *Lactobacillus brevis* (dosage of 100 or 500 mg/kg once a day) after 35 days was inhibited an increase in serum ALT and AST levels in alcoholic liver disease using ethanol-containing diet-fed mice [18]. The exact mechanisms by which probiotics may be affect serum ALT are unknown. Segawa et al. [18] showed the inhibition of TNF-α and sterol regulatory element-binding protein (SREBPs) up-regulation by *Lactobacillus brevis*, which in turn may decrease serum ALT levels. Furthermore, elevated ALT, a sign of hepatocyte damage results from damaged biological membranes [32], inhibiting insulin signaling and increased insulin resistance [33] and/or promote liver damage in concert with pro-inflammatory cytokines [32] result from accumulation of fatty acids in the liver may led to increased liver enzyme of ALT. Improving insulin resistance as a result of usage probiotic [34] might resulted in decreased ALT levels.

We did not find any significant effect of the consumption of probiotic supplements on blood pressure which is inconsistent to previous studies [19, 24]. The beneficial effect of probiotics on blood pressure was attributed to release of bioactive peptides, such as the ACE inhibitory peptides [19, 24]. Furthermore, ACE-inhibitory peptides are present in dairy preparation and milk fermented with *Lactobacillus casei*, *L. acidophilus* and Bifidobacteria strains [35]. These peptides are enzyme and acid-resistant in the stomach and the antihypertensive capacity of these peptides has been demonstrated in human studies [36–38]. The beneficial effects of probiotics on blood pressure are highly strain specific, and the absent effect on blood pressures in the present study might be due to the choice, dosage of bacterial strain and the intervention time.

Several limitations must be considered in the interpretation of our findings. The study period was only 8 weeks. Due to budget limitations, we were unable to assessed inflammatory markers including TNF-α and measures of oxidative stress.

In conclusion, multispecies probiotic supplementation among diabetic patients had beneficial effects on serum calcium and ALT concentrations; however, it could not affect serum magnesium, zinc, iron, ALP, AST and total bilirubin levels as well as systolic and diastolic blood pressures.

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**Conflicts of interest** None

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## Authors' contribution

ZA conducted the study, carried out the statistical analysis, wrote the manuscript and contributed in the interpretation of the findings. SB, HS, AJ and A-MF contributed in drafting the manuscript and assisted in interpretation of the findings. All authors approved the final version of the manuscript.