The effects of probiotic supplementation on antioxidant status in type 2 diabetic patients: A double- blind randomized clinical trial

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Research

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Abstract

Background

Type 2 diabetes mellitus is one of the most common metabolic disorders worldwide. Oxidative stress plays a major role in the pathogenesis and progression of diabetes. This study wanted to evaluate the effects of probiotics on antioxidant status in type 2 diabetic patients.

Methods

A double blind, randomized, controlled clinical trial was conducted on 59 patients aged 25–65 years with type 2 diabetes mellitus. Subjects were randomly assigned into two groups to take either multispecies probiotic supplements (n = 30) or placebo (n = 29) twice a day for 6weeks. The multispecies probiotic supplement consisted of 7 viable and freeze-dried bacterial species: *lactobacillus acidophilus, L_casei, L_bulgaricus, L_rhamnosus, Bifidobacterium breve, B_ longum, Streptococcus thermophilus*, and 100 mg fructo-oligosaccharide. Fasting blood samples, 24-h dietary recalls and anthropometric measurements were collected at baseline and at the end of the intervention.

Results

Consumption of probiotic supplements, compared to the placebo, resulted in a significant increase in total antioxidant capacity ($P < 0.001$). But the probiotics had no significant effect on total oxidant status and paraoxonase ($P > 0.05$). A significant reduction in fasting blood glucose (146.5 ± 44 vs. 132.7 ± 34, $p = 0.001$) was found due to the consumption of probiotic as compared with the placebo group and this reduction was 9 percent.

Conclusions

Our study showed that multispecies probiotic supplementation in diabetic patients for 6 weeks could increase total antioxidant capacity and decrease fasting blood glucose. Therefore, it is suggested that probiotics can serve as a complementary supplement to control diabetic complications.

Introduction

Type 2 diabetes mellitus is one of the most common metabolic disorders worldwide. the number of diabetic patients is expected to rise to 629 million people by 2045.[1]

Previous studies report that oxidative stress plays a major role in the pathogenesis and progression of diabetes[2].

Diabetic patient has increased free radical production and reduced the antioxidant defense [3, 4]. Hyperglycemia and insulin resistance are accompanied by the increase of advanced glycation end-products (AGEs) density[5]; in addition, hyperglycemia by several mechanisms such as glucose autoxidation, increased flux of glucose and other sugars through the polyol pathway, increased expression of the receptor for advanced glycation end products and its activating ligands, activation of protein kinase C (PKC) isoforms can increase vascular damage and induce oxidative stress[6, 7]. Probiotics are specified as live microorganisms that when administered in adequate amounts, confer a health benefit on the host[8]. Some health benefits consist of the prevention or management of diarrhea, constipation, lactose intolerance, diabetes mellitus,[9] inflammatory bowel disease, inflammatory bowel syndrome, crohn□s disease, colon cancer, atopic dermatitis, topical allergies,[10] hepatic encephalopathy, [11] allergy[12] and modulating intestinal microbiota.[13, 14]

Some studies reported that the consumption of probiotics improved antioxidant status.[15–17] Scavenging of reactive oxygen species, metal ion chelating ability, inhibition of ascorbate autoxidation, enzyme inhibition, inhibiting cytotoxic activity, capturing ROS,[18–20] regulating microbiota composition by inhibiting the excessive proliferation of noxious bacteria,[21] reducing intestinal PH and suppressing growth of harmful bacteria[22, 23] are underlying antioxidant mechanisms of probiotics.

Studies in diabetes conducted in animals have been demonstrated that some species of probiotics decrease oxidative stress.[22–26] In humans, the consumption of probiotic yogurt containing *L_acidophilus La5* and *B_ Lactis Bb12* has been shown to improve antioxidant status in diabetic patients. [16] Inline-with the antioxidant properties of probiotics, some of other human studies confirm this attribute.[27, 28] Most previous studies have been done in animal models. Although several studies in humans have been conducted, evidence of the effects of probiotics on some antioxidant biomarkers is rare.

Probiotics could improve metabolic disease like diabetes through modulating intestinal microorganism. [29, 30] So alteration in gut microbiota by probiotics may be regarded as a novel approach to improving antioxidant status. This study aimed to investigate the effect of consumption of probiotic supplements on antioxidant biomarkers, including total antioxidant capacity (TAC), total oxidant status (TOS) and paraoxonase (PON) in type 2 diabetic patients.

Materials And Methods

Subjects

A randomized double-blinded controlled clinical trial was conducted in Taleghani hospital, Tehran, Iran for 6 weeks. The study included a total of 68 patients aged 25–65 years with type 2 diabetes mellitus (T2DM) after obtaining the study participant written informed consent.

The exclusion criteria were as follows: required insulin injections; any changes in using medication; the presence of kidney, liver or inflammatory intestinal disease, cardiovascular diseases; pulmonary disease;

immunodeficiency disorders; allergy disease; short bowel syndrome; use of nutritional supplements; pregnancy or breast_ feeding; consumption of probiotics and placebo capsules less than %90 during the study.

The sample size was determined based on glycemic profile variables. For an expected change of 15 mg/dl between intervention and control groups and by considering α value equal to 0.05 and a power of 80%, the required sample size per group was 30. This number increased to 34 per group to accommodate the anticipated dropout rate.

Study design

In this double-blinded, randomized controlled clinical trial, patients with T2DM were randomly allocated to either the probiotic (intervention) or placebo (control) groups using a block randomization with matched subjects in each block based on sex.

Probiotic and placebo tablets had the same appearance and neither the patients nor researchers were aware of the treatment in the double- blind study. A total of 68 patients with T2DM were randomly assigned into two groups to receive either probiotic supplements (n=34) or placebo (n=34) for 6 weeks. Each group consumed either two placebo capsules or two probiotic capsules for 6 weeks. Participants were asked not to alter their usual dietary habits and routine physical activity and to avoid consuming any other synbiotic and probiotic products and other supplements during the study. In addition, the patients were instructed to avoid any changes in medication. Compliance was monitored by telephone interview once a week. Demographic information, food consumption and fasting blood samples were collected at the baseline and at the end of the study.

Data Collection

Macronutrient and micronutrient intake were estimated using 24- h dietary recall in three days and data were analyzed using Nutritionist 4 software (First Databank, Hearst Corp, San Bruno, CA, USA)

Physical activity level was assessed by modifiable physical activity questionnaire (MAQ). Reliability and validity of the questionnaire were confirmed in Iranian urban adult population.[31]

Weight of the participants was measured by a weight scale (Seca, Hamburg, Germany) with 0.1- kg accuracy with minimal clothing and no shoes. Height of the participants was measured using a stadiometer (Seca) with 0.1-cm accuracy without shoes. Body mass index (BMI) was calculated as a person's weight in kilograms divided by height in meters squared.

Laboratory Data

Blood samples were collected from the antecubital vein of the subjects' arm after a 12-h overnight fast. The serum samples were separated from whole blood by centrifugation at 3500 rpm for 10 min (Hettich

D-78532; Tuttlingen, Germany). The samples were frozen immediately and stored at -70̊ C until analyzed in research institute for endocrine sciences.

Fasting blood glucose was measured by an enzymatic method (Parsazmun Co. Kit). Serum total antioxidant capacity (TAC) and total oxidant status (TOS) were measured by chemical colorimetric technique Zell bio kit (Germany). Paraoxonase (PON) was measured by enzymatic colorimetric method using Zell bio kit (Germany).

Ethical approval

The study was conducted in accordance with the guidelines laid down in the declaration of Helsinki and approved by the ethics committee at Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.ENDOCRINE.REC.1395.250).

At the baseline, all participants signed an informed consent form. The trial has been registered in the Iranian registry of clinical trials, IRCT 2013100714925N1.

Intervention

Patients in the intervention group received 2 Familact probiotic capsules twice aday, one after lunch and one after the evening meal for 6 weeks. They were asked to keep the capsules in refrigerator during the study. Each capsule contained 500 mg probiotics which precisely consisted of the following species: The multispecies probiotic supplement consisted of 7 viable and freeze-dried bacterial species*: lactobacillus acidophilus [2□10^{9}*colony forming units (*CFU]), L_casei (7□10^{9} CFU), L_bulgaricus (2□10^{8} CFU), L_rhamnosus (1.5□10^{9} CFU), Bifidobacterium breve (3□10^{10} CFU), B_ longum (7□10^{9} CFU), Streptococcus thermophilus (1.5□10^{9} CFU)*, and 100 mg fructo-oligosaccharide with lactose as carrier substances*.* The Patients in the placebo group received 2 placebo capsules (the same substance with magnesium stearate and without bacteria) twice a day, one after lunch and one after evening meal.

Statistical analysis

Statistical analyses were performed using SPSS version 21. Continuous variables were expressed as mean ± standard deviation and descriptive statistics were presented as frequencies. Chi-square test was used to compare the qualitative variable between two groups. The normality of the distribution of variables was checked by the Kolmogorov-Smirnov test. The log-transformation was used for the variables normally distributed.

The paired samples t-tests was applied to compare the mean values of variables before and after the intervention in each group. The independent samples t-test was used to detect differences between the two groups, adjusted as confounding factors in this study. Age, vitamin E, saturated fatty acid and poly unsaturated fatty acid intakes were different between the two groups, adjusted as confounding factors in this study. Differences between the two groups after the intervention were specified by analysis of covariance, adjusting for baseline measurements and confounding factors.

Results

The study included a total of 68 patients with type 2 diabetes 9 participants were excluded from the statistical analysis (4 individuals in probiotic group and 5 in placebo group) as they needed to have insulin (n = 4) or supplement therapy (n = 1) or they did not complete their interventions in the expected time (n = 4). So, data for 59 patient (n=30 in probiotic group and n=29 in placebo group) were analyzed. Patients did not report any adverse effects related to probiotic consumption during the study.

Table 1 shows the characteristics of the participants in each group at baseline.

There was a statistically significant difference between the two groups with respect to the mean age of diabetic patients (P= 0.018). But other baseline characteristics of the patients did not differ between the two groups (P> 0.05). The analysis of dietary intakes is shown in table 2. At the beginning of the study, no statistically significant differences were found between two groups with respect to energy, carbohydrate, protein, saturated fatty acid, monounsaturated fatty acid, cholesterol, dietary fiber and vitamin C intakes (P> 0.05). But there were statistically significant differences between the two groups with respect to the intake of polyunsaturated fatty acid, vitamin E and total fat at baseline the study (P< 0.05). At the end of study, there were statistically significant differences in dietary intakes of energy, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid and dietary fiber between the two groups (P< 0.05). The intake of polyunsaturated fatty acid and dietary fiber was significantly decreased in placebo group before and after the intervention (P< 0.05). At the baseline, no significant differences were found between the two groups in terms of antioxidant biomarkers, including TAC, TOS, PON (P> 0.05).

Result of the ANCOVA (analysis of covariance) indicated that there were statistically significant differences between the two groups in TAC at the end of the study after adjusting for age, total fat, polyunsaturated fatty acid and vitamin E intake (P= 0.001). There were no significant differences in the TOS and PON levels between the two groups at the end of the study.

As shown in table 3, TAC level was significantly increased compared with the probiotic group at the baseline (P= 0.003). No significant statictically differences were found between the two groups with respect to TOS and PON level (P> 0.05).

Discussion

Type 2 diabetes mellitus is a metabolic disorder, characterized by heperglycemia and elevated levels of free radicals that can cause lipid peroxidation.[4] In addition, antioxidant defense is disturbed in diabetic patients leading to the expansion of diabetes complications, such as retinopathy, nephropathy and neuropathy[32]. So the promotion in antioxidant status can contribute to diabetes management.[4]

The aim of our study was to investigate the effect of consumption of probiotic supplements on antioxidant biomarkers, including total antioxidant capacity (TAC), total oxidant status (TOS) and PON in type 2 diabetic patients. The results of this study showed that consumption of multispecies probiotic

supplement for 6 weeks among subjects with type 2 diabetic patients improved antioxidant status and TAC level and significantly decreased fasting blood glucose; however, MDA and PON levels remained unchanged in the intervention group.

In the present study, the intake of polyunsaturated fatty acid, vitamin E and total fat were significantly different between probiotic and placebo groups at baseline so we adjust them. There were no statistically significant differences in energy, carbohydrate, protein, saturated fatty acid, monounsaturated fatty acid, cholesterol, dietary fiber and vitamin C intakes between groups. However, at the end of study, there were statistically significant difference between the two groups with respect to dietary intakes of energy, saturated fatty acid, monounsaturated fatty acid, polyunsaturated fatty acid and dietary fiber.

Several studies showed the antioxidant and anti-diabetic properties of probiotics.[16, 24-27] The imbalance of microbiota in the gastrointestinal tract has been demonstrated in T2DM.[33]

According to Larsen et al. diabetic patients increased gram-negative bacteria in their intestinal tract. The gut microbiota in patient with T2DM can result in a decreased ratio of Firmicutes to Clostridia, an increased ratio of Betaproteobacteria and an increased ratio of Bacteroidetes to Firmicutes.It has been demonstrated that Probiotics may improve intestinal microbiota composition by increasing gram positive bacteria.[34]

In fact, probiotics can modulate the composition of microbiota and inhibit the excessive proliferation of harmful bacteria that can cause oxidative stress. Some species of probiotics can reduce intestinal PH and subjugate the growth of harmful bacteria through producing lactic acid, propionic acid and acetic acid.[22, 23]

Some of the similar studies have shown improved antioxidant status in animal models after consuming probiotics. For example, Yadav et al. demonstrated that treatment with probiotic dahi can suppress oxidative damage in the pancreatic tissues of diabetic rats by inhibiting lipid peroxidation, producing nitric oxide and improving the activities of SOD, CAT and GPX.[26]

The feeding of the probiotic dahi significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, and oxidative stress in high fructose induced diabetic rats.[25]

Harisa et al. reported that treatment with *L. acidophilus* improved the antioxidant potential activity of aryl esterase enzyme and decreased fasting blood sugar, HbA1c, and MDA concentrations in diabetic rats.[24]

Amar et al. found that treatment with *Bifidobacterium animalis* subsp. Lactis 420 reversed bacterial translocation to mesenteric adipose tissue, decreased the expression of pro-inflammatory cytokines in liver, muscle and adipose tissue and improved insulin sensitivity in HFD mice.[35]

In the present study, supplementation with multispecies probiotics led to an increase in TAC level. However, the lack of significant changes in plasma TOS and PON levels could be explained by the short duration of the study and dosage of bacteria used in the study.

The results of this study confirm previous reports on the antioxidant property of probiotics in human models. A clinical trial performed by Ejtahed et al. indicated that consumption of probiotic yogurt improved fasting blood glucose the erythrocyte superoxide dismutase and glutathione peroxidase activities, and total antioxidant status.[16] However, it should be noted that fermented milks because of the presence of antioxidant peptides have both radical scavenging activity and inhibited lipid peroxidation.[28]

Asemi et al. showed that multispecies probiotic supplementation for 8 weeks in diabetic patients decreased serum hs-CRP and increased plasma total GSH, but no significant changes were observed in TAC level.[27] In the present study, TAC level was increased in the probiotic group.

The lack of significant changes in TAC levels and differences in previous studies might be explained by the different characteristics of the subjects.

Several mechanisms are involved in antioxidant effects of probiotics. Some strains of probiotic bacteria have antioxidant activity through scavenging reactive oxygen species, metal ion chelating, and inhibition of ascorbate autoxidation, enzyme inhibition, inhibiting cytotoxic activity and capturing ROS.[18-20] Regulation of the level of antioxidant metabolites such as GSH,[36] butyrate[37] and folate[38] are other antioxidant mechanisms of probiotics.

In deed Probiotics by expressing antioxidant enzymes, up-regulate antioxidant activities of the host,[36, 39, 40] down-regulate activities of ROS-producing enzymes, and ameliorate antioxidant status.[41]

It is revealed that vitamin B12 and folate deficiency can raise oxidative stress in diabetic patient.[42] The consumption of probiotics could improve the level of vitamin B12 and folate and ameliorate antioxidant defense.[38]

Moreover, some strains of probiotic bacteria have been reported as mediator in antioxidant signaling pathway by inhibiting NFκB and ROS production,[43] decreasing ROS level, regulating Nrf2 expressions, [44] and inhibiting PKC[45]. This result showed that consumption of probiotic was effective in increasing antioxidant activity in diabetes.

The limitations of our study were as follows: a low sample size, short duration of intervention and alteration in dietary intake. This highlights that studies need to be conducted with a higher dosage at a longer duration. In addition, further studies are needed to assess the effect of probiotics lipid peroxidation. Further investigations are needed to survey the effects of probiotics on intestinal microbiota composition and free lipopolysaccharides and short chain fatty acids.

Conclusion

Inconclusion, the results indicated that consumption of multispecies probiotic supplement among Type 2 diabetic patients for 6 weeks improved antioxidant status, and TAC level and significantly decreased fasting blood glucose.

Abbreviations

AGEs
advanced glycation end-products
PKC
protein kinase C
TAC
total antioxidant capacity
TOS
total oxidant status
PON
paraoxonase
T2DM
type 2 diabetes mellitus
MAQ
modifiable physical activity questionnaire

Declarations

Ethics approval and consent to participate: The study was conducted in accordance with the guidelines laid down in the declaration of Helsinki and approved by the ethics committee at Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.ENDOCRINE.REC.1395.250). At the baseline, all participants signed an informed consent form. The trial has been registered in the Iranian registry of clinical trials, IRCT 2013100714925N1.

Consent for publication: Not applicable

Availability of data and material: All data generated or analyzed during this study are included in this published article.

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Tables

Table 1. Baseline characteristics of the study participants

	Probiotic group (n = 30)	Placebo group (n = 29)	P-value**
Age (y)	58.5 (52-64)	62 (57-65)	0.018
Sex			
Men	13 (43.3%)	16 (55.2%)	0.36
Weight (kg)	75.24 ± 16	74.34 ± 9	0.79
BMI (Kg/m^2)	27.67 ± 4	27.16 ± 4	0.63
Physical activity†			
Low	11 (36.7%)	13 (44.8%)	0.52
Moderate	19 (63.3%)	16 (55.2%)	
Consumption of drug			
Yes	14 (46.7%)	14 (48.3%)	0.9
No	16 (53.3%)	15 (51.7%)	
Duration of diabetes (y)	6.16 ± 3	5.89 ± 3	0.72

Age is presented as Median and interquartile range

Data are presented as Mean ± SD for continuous and percent for categorical variables

** P value obtained from either one sample T-test or Chi-square test

Table 2. Dietary intakes of subjects throughout the study

Variables	Probiotic group (n = 30)	Placebo group (n = 29)	P-value
Energy (Kcal)			
Baseline	1966 ± 454	2009 ± 415	0.7
After intervention	1851 ± 309	2044 ± 378	0.03‡
P†	0.12	0.54	
Carbohydrate (g)			
Baseline	242 (192-295)	246 (176-277)	0.9
After intervention	223 (189-253)	240 (194-290)	0.3
P†	0.28	0.95	
Protein (g)			
Baseline	80 ± 37	73 ± 25	0.4
After intervention	74 ± 26	83 ± 36	0.2
P†	0.47	0.19	
Total fat (g)			
Baseline	72 ± 36	93 ± 30	0.01*
After intervention	71 ± 30	82 ± 22	0.1
P†	0.85	0.12	
Saturated fat (g)			
Baseline	20 ± 8	21 ± 7	0.6
After intervention	18 ± 5	25 ±16	0.02‡
P†	0.13	0.18	
Monounsaturated fat (g)			
Baseline			
After intervention	17.32 ± 8.95	20 ± 8	0.1
P†	15.86 ± 6.35	20 ± 7	0.01‡
	0.35	0.81	
Polyunsaturated fat(g)			
Baseline	24 ± 24	43 ± 23	0.003*
After intervention	30 ± 20	33 ± 14	0.05
P†	0.21	0.04†	
Cholestrol			
Baseline	208 ± 145	195 ± 108	0.7
After intervention	209 ± 141	208 ± 139	0.9
P†	0.95	0.72	
Dietary fiber (g)			
Baseline	11 ± 7	9 ± 5	0.2
After intervention	11 ± 6	7 ± 4	0.006‡
P†	0.94	0.004†	
Vitamin E			
Baseline	12 ± 12	19 ± 13	0.03*
After intervention	16 ± 10	15 ± 7	0.9
P†	0.12	0.16	
Vitamin C			
Baseline	59 ± 50	56 ± 50	0.8
After intervention	82 ± 97	83 ± 93	0.9
P†	0.18	0.12	

Data are presented as mean ± SD

* P value obtained from Independent Samples t test.

†, P value obtained from paired sample t test.

‡, analysis of covariance, adjusted for age, difference intake of total fat, polyunsaturated fatty acid and vitamin E, and baseline values .

Table 3. Effects of 6 weeks of the consumption of probiotic or placebo capsules on antioxidant factors

Variables	Probiotic group (n = 30)	Placebo group (n = 29)	P-value
TAC (mmol/l)			
Baseline	0.27 ± 0.06	0.27 ± 0.05	0.84
After intervention	0.3 ± 0.07	0.27 ± 0.06	0.001‡
P†	0.003†	0.39	
TOS (µmol/l)			
Baseline	3.08 ± 1	2.68 ± 1	0.22
After intervention	2.83 ± 2	2.44 ± 1	0.59
P†	0.27	0.26	
PON			
Baseline	122.9 ± 92	132.5 ± 96	0.57
After intervention	127.53 ± 100	138 ± 104	0.94
P†	0.44	0.41	

Data are presented as mean ± SD

* P value obtained from Independent Samples t test).

† Statistically Significant difference between the groups throughout the study ($p < 0.01$, Paired- Samples t test).

‡ Statistically Significant difference between the groups after the intervention ($p < 0.05$, ANCOVA, adjusted for age, difference intake of total fat, polyunsaturated fatty acid and vitamin E, and baseline values).

Figures

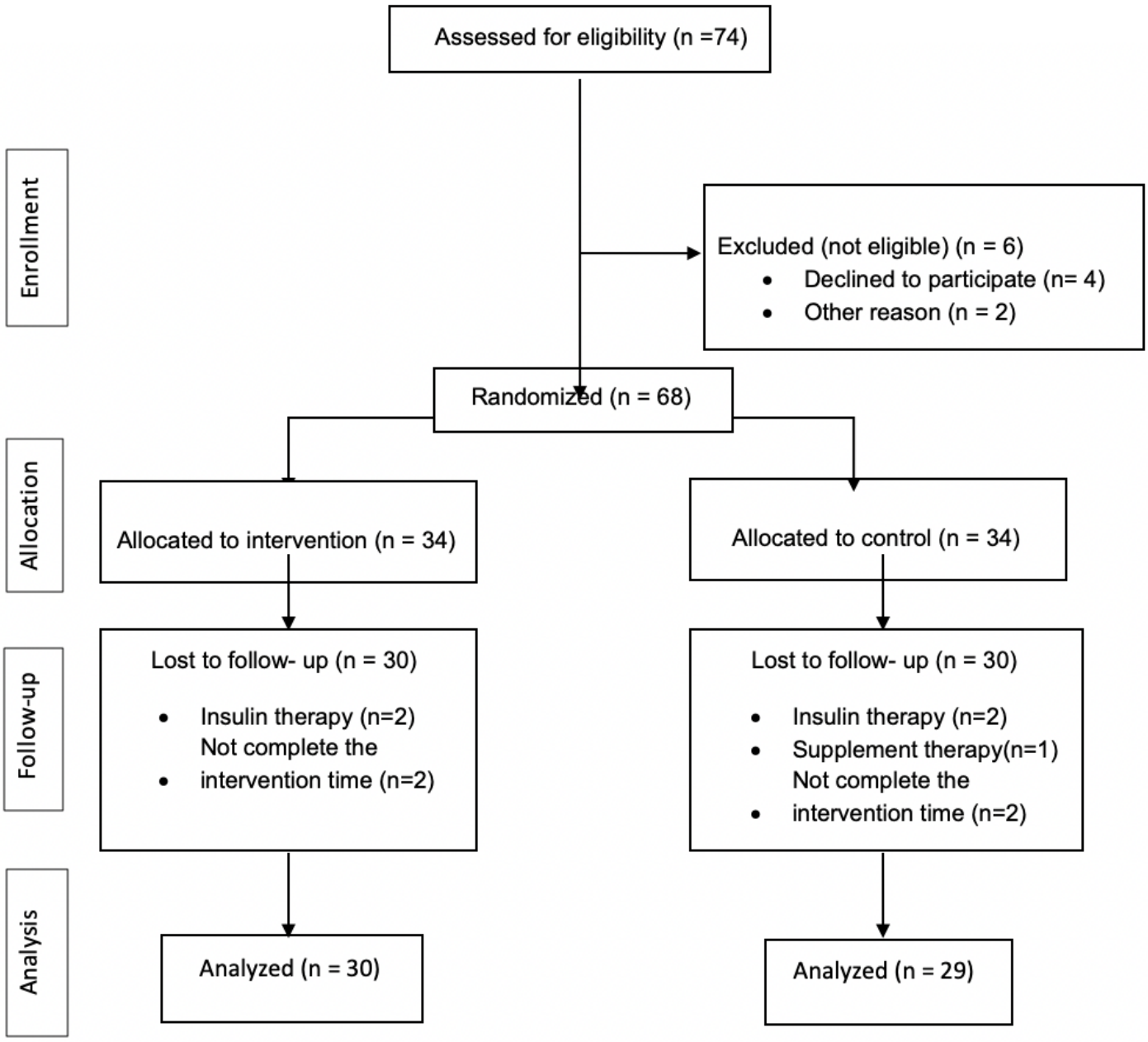

Figure 1

CONSORT flowchart of trial