Iran Red Crescent Med J. 2017 April; 19(4):e42902. doi: 10.5812/ircmj.42902.

Published online 2017 February 13. **Research Article**

The Effect of Synbiotic Supplementation on Body Composition and Lipid Profile in Patients with NAFLD: A Randomized, Double Blind, Placebo-Controlled Clinical Trial Study

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Received 2016 October 09; **Revised** 2016 December 17; **Accepted** 2017 February 05.

Abstract

Background: Regarding the growing prevalence of non-alcoholic fatty liver disease, concentrating on various strategies for its prevention and management seems necessary.
Objectives: This study aimed to assess the effects of synbiotic administration on body composition and lipid profile in patients with NAFLD.
Methods: Eighty patients with NAFLD participated in this randomized, double-blind, placebo-controlled clinical trial (from March to July 2014) in Iran. Based on AST and ALT as main variables of the study, 34 patients were required in each group (power 80% and $\alpha = 5\%$). Considering a 20% sample loss, 80 patients were enrolled. Synbiotic supplement in form of a 500 mg capsule (containing 7 species of probiotic bacteria and Fructooligosaccharides) was administrated to patients in the intervention group and those in the placebo group received 1 placebo capsule daily for 8 weeks. At the baseline and the end of the study, body composition and lipid profile were evaluated.
Results: A significant reduction was observed in weight (P = 0.001), body fat (P = 0.02), and total cholesterol (P = 0.04) within the synbiotic group. On the other hand, WC (P = 0.02), total cholesterol, and LDL-c (P = 0.04 and P = 0.001, respectively) were significantly increased in the placebo group. TG, HDL-c, and FBG levels remained statistically unchanged in both groups. Significance between-group differences were seen in total cholesterol (P = 0.01), LDL (P = 0.01), weight, WC, and body fat after adjustment for energy intake (P = 0.05).
Conclusions: Synbiotic supplementation may improve lipid profile and body composition in patients with NAFLD and might be useful in prevention of the disease progression.

Keywords: Nonalcoholic Fatty Liver Disease, Symbiotic, Body Composition, Total Cholesterol, LDL- Cholesterol, HDL- Cholesterol, Lipid Profile

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a health problem in the world and is the most common chronic liver disease that has spread parallel to the epidemic obesity (1, 2). The prevalence of NAFLD in the general population has been reported between 2.8 to 30% in different countries (3, 4), with Iran being an estimated 7% for children and 35% for adults (5, 6). Changes in intestinal bacterial flora due to stress or poor nutritional habits are important in the pathogenesis or progression of NAFLD (7).

On the other hand, most of studies on the relationship between microbial flora and obesity observed significant changes in composition and metabolic function of gut microbiome in obese individuals (8, 9). It seems that obesity is associated with certain intestinal microbiome, which have the ability to have more energy extraction from diet and cause more fatty acids uptake in the liver and peripheral tissues (10).

Several studies have supported the hypothesis that small intestinal bacterial over growth and qualitative changes in microbiom may contribute to obesity and NAFLD progression. Based on beneficial effects of probiotics and prebiotics on the human intestinal microbial ecosystem, they have been suggested as a complementary therapeutic approach in NAFLD (11-13).

Most investigations regarding NAFLD treatment have been conducted on overweight and obese patients, and to

our knowledge, there is no study that has investigated the effect of symbiotic alone on body composition and lipid profile in lean and normal weight NAFLD patients. Accordingly, with respect to the high prevalence of NAFLD in our country, more researches on treatment of these patients seem necessary. Therefore, in the present clinical trial we evaluated the effect of short-term synbiotic supplementation, as a simple, low cost, and without side effect treatment component on lipid profile and body composition in NAFLD patients while addressing some of the mechanisms of action by which synbiotic may function.

2. Methods

The protocol of this randomized, double blind, placebo-controlled clinical trial was approved by the Research Ethics Committee of Isfahan University of Medical Sciences and registered in the Iranian registry of clinical trial (IRCT2013122811763N15) (www.irct.ir).

NAFLD volunteers (by ultrasound and high levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were referred to the Isfahan endocrine and metabolism research center and enrolled into this study (from March to July 2014). Based on AST and ALT as main variables of the study, 34 patients were required in each group (power 80% and α = 5%). Considering a 20% sample loss, 80 patients were enrolled. Subjects were selected by systematic random sampling. We first randomly picked the first subject from a list of 330 patients referred to the endocrine and metabolism research center. Then, we selected each fourth subject from the list. The inclusion criteria included individuals between the ages of 18 - 60 years, no other liver disease, no inflammatory bowel disease, no self-reported specific disease and malignancies, no pregnancy and lactation, no vitamin-mineral, antioxidant, and omega-3 supplementation. Study volunteers were excluded for failure to follow trial guidelines (< 90% compliance, subject's compliance was evaluated by counting the remaining capsules at the end of the fourth and eight weeks).

Random assignment was done by block randomization. After block size has been determined (4 subjects), all possible balanced combinations of assignment within the block was calculated. Blocks were then randomly chosen to determine the patients' assignment into the groups. Participants were randomly allocated to 2 numerically equal groups from a double-blind, 80-person list, using a table of random digits and given either a synbiotic in form of a 500 mg capsule (Familact, produced by Zisttakhmir company) containing 7 species of probiotic bacteria (*Lactobacillus casei*, *Lactobacillus acidopholus*, *Lactobacillus rhamnosus*, *Lactobacillus bulgaricus*, *Bifidobacteriumbreve*, *Bifidobacteriumlongum*, *Streptococcus thermophiles*) and fructooligosaccharides or a placebo capsule (containing 120 mg starch, similar in shape and appearance).

The boxes containing the symbiotic and placebo capsules were coded (A and B) by an individual who was not aware of research objectives and investigators were blinded for the entire duration of the study. Study participants, as well as the placebo group, ingested those capsules once daily after dinner for 8 weeks. Six participants were excluded during the study (because of < 90% compliance, personal reasons and antibiotic therapy), which left 38 volunteers in the synbiotic group and 36 in the placebo group (Figure 1).

At the beginning of the study, enough information regarding the study and its process was given to all participants and written informed consent was obtained. All tests and evaluations were free of charge. General information was collected using an interview. Body weight was measured to the nearest 0.1 kg with minimal clothing by means of a calibrated digital seca balance (Seca, Hamburg, Germany). Height was measured to the nearest 0.5 cm without shoes by means of a calibrated seca stadiometer (Seca, Hamburg, Germany). Body mass index was calculated (BMI = weight in kg/height^2 in m). Waist and hip circumference were measured on a horizontal plane at the level of the iliac crest by an Ergonomic Circumference Measuring Tape. Body fat, lean body mass (LBM), and total body water (TBW) were measured using a body composition analyzer (Jawon Medical Company, Korea) while subjects were wearing light clothes and had bare feet and hands after 5 minutes resting. All measurements were performed by 1 person.

After a 12-hour overnight fast, venous blood samples were collected. After centrifugation, the serum samples were frozen and stored at -70°C. Fasting blood glucose (FBG) was measured by means of glucose hexokinase method; serum triglyceride (TG), high density lipoprotein (HDL), and total cholestrole were measured with enzymatic method (using commercial kits, Pars Azmoon Company, Tehran, Iran), and low density lipoprotein (LDL) was calculated using Friedwald formula.

Physical activity levels and dietary intake (3-day food record in gram before, and the same after intervention) were recorded at the baseline and the end of the study. Metabolic equivalent of task (MET) was used as a means of expressing intensity of recorded activities (14). Dietary data were analyzed using the Nutritionist IV software adjusted for Iranian foods (Version 4.1, First Databank Division, The Hearst Corporation, San Bruno, CA, USA).

Software package statistical analysis (SPSS, version 20; SPSS Inc, Chicago, IL, USA) was used for all statistical analyses. Descriptive statistics are presented as mean ± stan-

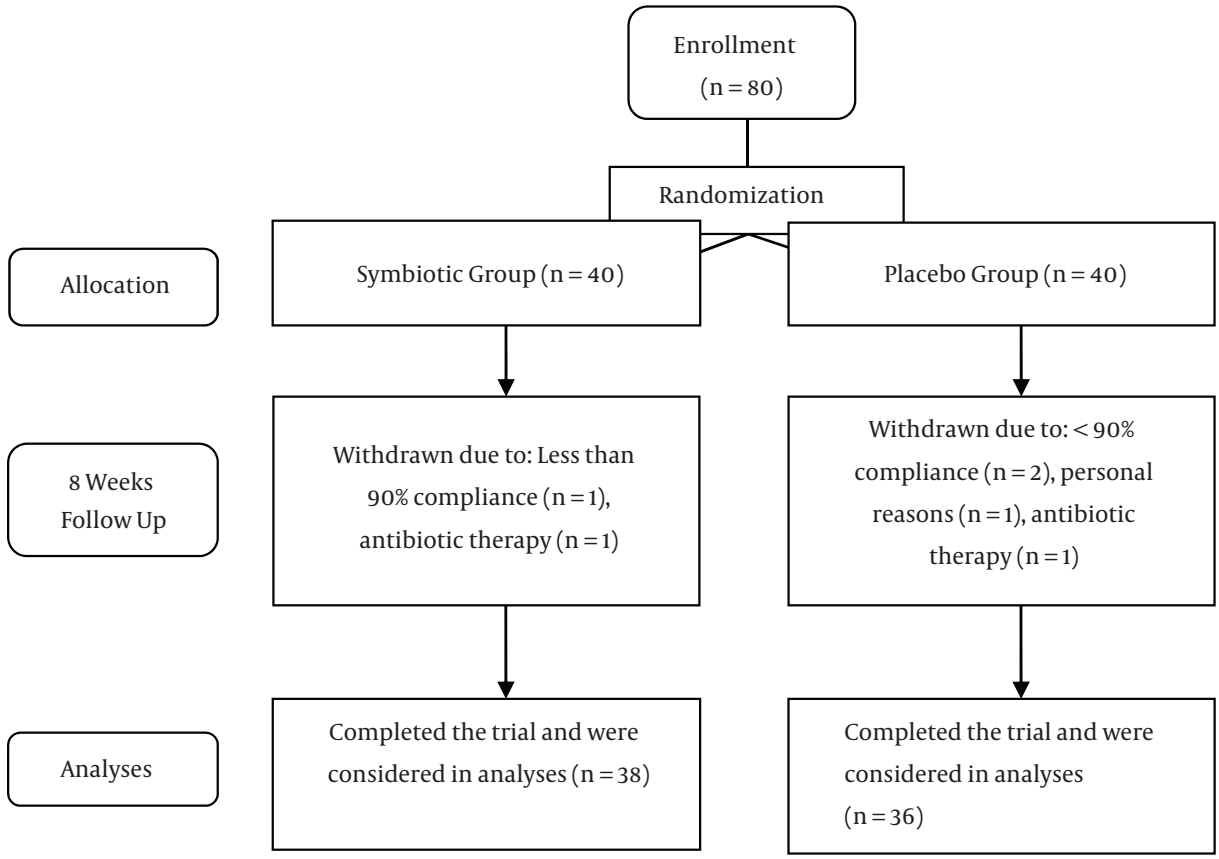

Figure 1. Follow Chart of Participants Throughout the Study

dard deviation (SD). The normality of data was checked by the Shapiro-Wilk test. Paired t-test (in case of normal distribution) or nonparametric statistical test, Wilcoxon signed rank test (in case of non-normal distribution), was used for comparing data within groups. Independent t-test (in case of normal distribution) or nonparametric statistical test, Mann–Whitney (in case of non-normal distribution), was used for comparing data between two groups. Analysis of covariance (ANCOVA) was used for evaluating between group differences based on quantitative data; adjustment was made for differences in baseline covariates. Within and between groups, differences based on qualitative variables were assessed using McNemar and Chi-square tests, respectively. All tests were two-sided and P values < 0.05 were considered statistically significant.

3. Results

Baseline characteristics were similar in the synbiotic and placebo groups (Table 1). No significant differences were found between the 2 groups at the baseline.

FBG and lipid profile were reported in Figure 2. Total cholesterol was significantly decreased in the synbiotic group after the intervention (P = 0.04), total cholesterol and LDL (P = 0.04 and P = 0.001, respectively) were significantly increased in the placebo group. TG, HDL, and FBG showed no significant change in either group. ANCOVA shows significant differences in total cholesterol (P = 0.01) and LDL (P = 0.01) between groups but detected no differences in FBG, TG, and HDL between groups after adjustment for energy intake.

Table 2 shows a comparison of body composition changes between groups. Weight and body fat decreased in the synbiotic group significantly (P = 0.001 and P = 0.02, respectively) and waste circumference (WC) increased in the placebo group significantly (P = 0.02). Other variables, including body mass index (BMI), waist/hip ratio (WHR), lean body mass (LBM), slim lean mass (SLM), protein (Pr), mineral (Min), and total body water (TBW) remain unchanged in both groups. Comparison of body composition variables between groups through multivariable analysis of covariance with adjustment for energy intake and base-

Table 1. Baseline Characteristics of the Study Participants[a]

General Characteristics	Symbiotic (n = 38)	Placebo (n = 36)	P Value
Age, y[b]	46.57 ± 1.7	47.78 ± 1.7	0.62
Women[c]	33 (82.5)	22 (64.7)	0.11
Men [c]	7 (17.5)	12 (35.3)	0.11
Weight, kg[b]	75.21 ± 2.14	73.90 ± 2.20	0.67
Height, cm[b]	159.25 ± 1.51	162.66 ± 1.66	0.13
BMI, kg/m^2[b]	29.58 ± 0.76	28.18 ± 0.68	0.18
WC, cm[b]	90.82 ± 1.74	88.91 ± 1.57	0.42
WHR[b]	0.910 ± 0.01	0.895 ± 0.01	0.47
Body fat[b]	26.56 ± 1.08	24.54 ± 1.25	0.22
LBM[d]	48.93 ± 1.37	50.15 ± 1.66	0.88
TBW[c]	34.75 ± 1.18	36.25 ± 1.24	0.73
Physical activity, m[c]	33.42 ± 0.47	34.75 ± 1.05	0.49
Energy intake, kcal/d[b]	2116.62 ±95.48	2200.28 ± 109.33	0.56
Carbohydrate intake g/d[d]	306.99 ± 17.93	307.00 ± 14.36	0.74
Protein intake, g/d[b]	67.01 ± 3.84	72.15 ± 4.05	0.36
Fat intake, g/d[b]	72.28 ± 5.08	78.87 ± 6.72	0.43
Vitamin C intake, mg/day[d]	114.79 ± 20.83	80.78 ± 10.86	0.40
Vitamin E intake, mg/d[d]	37.00 ± 4.03	44.89 ± 5.36	0.23
FBG[d]	97.48 ± 1.49	101.46 ± 2.40	0.30
TG[d]	162.61 ± 11.86	174.71 ± 20.49	0.96
Total Chol[d]	203.05 ± 8.04	187.41 ± 6.64	0.35
LDL[d]	121.55 ± 6.81	104.73 ± 6.12	0.22
HDL[d]	45.36 ± 1.94	45.25 ± 1.80	0.80

Abbreviations: BMI, body mass index; FBG, fasting blood glucose; HDL, high density lipoprotein; LBM, lean body mass; LDL, low density lipoprotein; TBW, total body water; TG, triglyceride; total chol, total cholesterol; WC, waist circumference; WHR, waste/hip ratio.
[a]Values are expressed as mean ± SE or No. (%).
[b]Independent T test.
[c]Chi square.
[d]Mann-whitney.

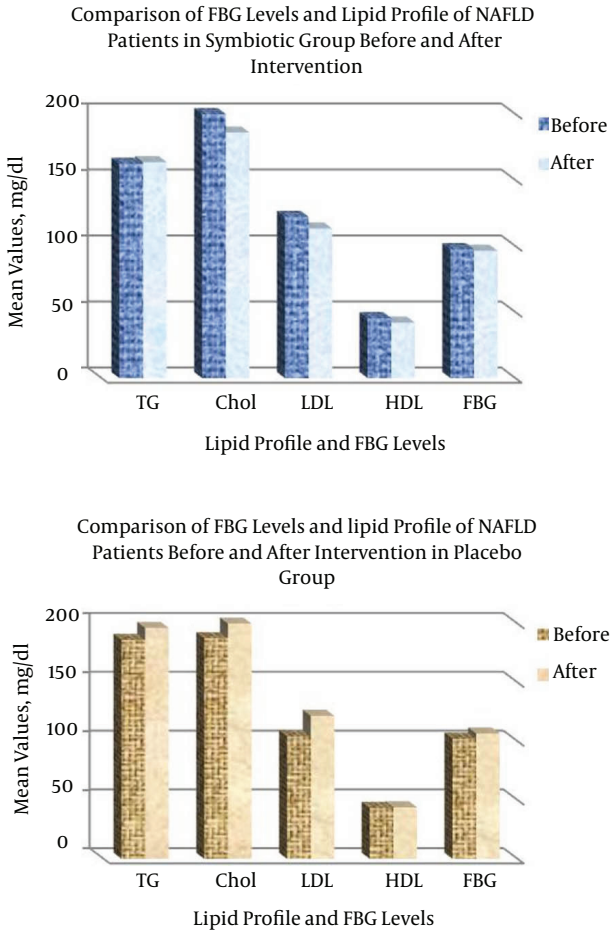

Figure 2. Lipid Profile and FBG of Participants Before and After Intervention 1

line values showed no significant difference.

4. Discussion

Increasing prevalence of NAFLD in general population of different countries call for natural and safe strategies for management of this disease. Already, there is no registered drug for the treatment of NAFLD (15, 16). NAFLD treatment specially focuses on life-style modifications (17). Results emerging from the present study demonstrate that synbiotic supplementation in NAFLD patients significantly improved body composition and lipid profile.

Our results are consistent with results from different studies. Lee et al. in an experimental study found that Lactobacillus rhamnosus produce conjugated linoleic acid and indicate anti-obesity effects in diet-induced obese mice (18). Sato et al. evaluated the effects of milk fermented by *Lactobacillus gasseri* on adipocyte size in rats and achieved similar results (19). Ley RE et al. evaluated 16SrRNA gene sequences from the gutmicrobiota of genetically obese ob/ob mice. They reported specific changes in gut microbiota composition, which could result in the increased short chain fatty acids (SCFAs) production and energy harvest (20). Festi et al. in a review, confirmed the pathogenic role exerted by gut microbiota on the development of metabolic disorders. They concluded that prebiotics or probiotics administration could improve gut barrier integrity, thus, ameliorating metabolic balance and

Table 2. Body Composition of Participants Before and After the Intervention

		Symbiotic Group (n = 38)		Placebo Group (n = 36)		P Value[a]	P Value[b]
		Mean ± SE	P Value[c]	Mean ± SE	P Value[c]		
Weight	Before	74.53 ± 2.08	0.001	73.66 ± 2.31	0.16	0.001	0.05
	After	73.76 ± 2.07		74.99 ± 2.39			
	Dif	-0.76 ± 0.22		1.33 ± 0.93			
BMI	Before	29.16 ± 0.70	0.46	28.00 ± 0.70	0.27	0.06	0.33
	After	29.08 ± 0.70		28.51 ± 0.85			
	Dif	-0.07 ± 0.10		0.51 ± 0.46			
WC	Before	90.07 ± 1.75	0.14	88.84 ± 1.67	0.02	0.001	0.05
	After	89.25 ± 1.58		90.32 ±1.76			
	Dif	-0.82 ± 0.54		1.48 ± 0.60			
WHR	Before	0.90 ± 0.01	0.44	0.89 ± 0.01	0.59	0.85	0.34
	After	0.89 ± 0.01		0.88 ± 0.03			
	Dif	-0.01 ± 0.00		3.55 ± 3.57			
Body fat	Before	26.56 ± 1.08	0.02	24.54 ± 1.25	0.26	0.001	0.05
	After	25.19 ± 1.15		25.43 ± 1.43			
	Dif	-0.91 ± 0.39		-0.01 ± 0.68			
LBM	Before	26.10 ± 1.06	0.24[d]	24.24 ± 1.31	0.45[d]	0.97	0.59
	After	25.19 ± 1.15		25.01 ± 1.39			
	Dif	-0.91 ± 0.39		0.77 ± 0.68			
TBW	Before	34.47 ± 1.22	0.60[d]	36.33 ± 1.31	0.42[d]	0.69	0.17
	After	34.87 ± 1.04		35.98 ± 1.22			
	Dif	0.40 ± 0.48		-0.35 ± 0.37			

[a] Mann-whitney (comparison of different between groups).
[b] ANCOVA (adjusted for energy intake).
[c] Paired T test.
[d] Wilcoxon.

promoting weight loss (21).

Probiotics can influence host metabolism in various ways. There is a specific microbiota in obese subject that extract energy from the diet more effectively. Microbial products, mainly SCFAs, influence on host's metabolism, intestinal transit time, energy absorption and appetite. Moreover, the complex interactions between gut microbiota and host's immune system may contribute to development of obesity (10, 21).

Our study demonstrated a reduction in total and LDL cholesterol that was statistically significant, whereas, in placebo group total and LDL cholesterol increased significantly. Change in cholesterol level was not unexpected as over several years, more and more experimental and clinical trials indicated that probiotics could lead to a decrease in serum cholesterol (22-26). Findings of a recent meta-analysis indicated that probiotics had a significantly good effect on normalizing total cholesterol, as we observed (27). Ghasemi findings also indicated that the addition of synbiotic to the diet decreased serum cholesterol and LDL cholesterol concentrations in broilers (28). In contrast with our results, Wong VW et al. did not find any significant effect for Lepicol probiotic and prebiotic formula on body mass index, waist circumference, glucose, and lipid levels (29).

Our results showed no significant change in TG and HDL cholesterol. In 2013, Bhathena et al. observed that the cholesterol fraction had more reduction compared to hepatic neutral lipids, whereas the proportion of TG remained unchanged. Free cholesterol accumulation in the mitochondria, but not TG, is known to sensitize hepatocytes to cytokine-induced apoptosis and disease progression. Probiotic is thought to reduce cholesterol augmentation in the mitochondria and restore antioxidant levels, offering

some protection from liver damage (7). Moreover, fermentation products of lactic acid bacteria inhibit cholesterol-synthesizing enzymes and thus reduce cholesterol production. The bacteria may facilitate elimination of cholesterol in feces and may inhibit its reabsorption into the body; in addition, these bacteria may interfere with the recycling of bile salt (a metabolic product of cholesterol) (22, 24). We observed no effect on HDL level, may be HDL requires long-term intervention to change or there are unknown mechanisms in this regard (27).

Our synbiotic capsules were containing Fructooligosaccharides, which are now becoming increasingly popular due to their prebiotic effects. They can enhance the growth of Bifidobacteria or Lactobacilli and may help control or reduce the growth of harmful bacteria (30). Moreover, they contribute to reduce body weight and body fat by modulating food intake and appetite, by promoting the production of glucagon-like peptide-1 (GLP-1), peptide YY, and the decrease of ghrelin, as well as, at the same time, by decreasing fatty acid storage (21). Few studies have used a combination of probiotics and prebiotics in the form of supplements to evaluate their exclusive effects on patients with NAFLD. To our knowledge, the present study is the first double blind placebo controlled clinical trial that evaluated the effect of symbiotic as a low cost therapeutic component without side effects on body composition and lipid profile in lean and normal weight NAFLD patients.

Several limitations must be considered in the interpretation of our findings, including limited duration of clinical trial and sample size. Ultrasound was used to approve NAFLD in participants. It is well known that liver histology is the gold standard for NAFLD/NASH diagnosis. Although an ultrasound is reasonably accurate, it cannot identify fatty infiltration of the liver below a threshold of 30% (27). Other possible probiotic sources, especially dairies were not controlled in this study since they are an important part of a usual diet in our population. Furthermore, due to budget limitation we were not able to evaluate other factors and focal microbiota change. More clinical trials, with longer intervention periods and higher dosage of symbiotic, may show better results.

In conclusion synbiotic supplementation could improve body composition and lipid profile in NAFLD patients and might be useful in management of NAFLD or be protective against progression of the disease.

Acknowledgments

This article was extracted from Msc. dissertation, which was approved by the School of Nutrition and Food Sciences, Isfahan University of Medical Sciences with code 392326. Authors thank all the participants and colleague of endocrine and metabolism research center and department of community nutrition for their cooperation during the study.

Footnotes

Funding/Support: Food security research centre, school of nutrition and food science, Isfahan University of Medical Sciences, Isfahan, Iran.

Conflicts of Interest: There are no conflicts of interest.

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