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Effects of Probiotics on Nonalcoholic Fatty Liver Disease in Obese Children and Adolescents

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See ''The Use of Probiotics in Pediatric Nonalcoholic Fatty Liver Disease: Teachable Moment or Missed Opportunity?'' by Nobili and Cucchiara on page 336.

ABSTRACT

Objectives: This study aims to evaluate the effects of some probiotics on sonographic and biochemical nonalcoholic fatty liver disease (NAFLD).

Methods: This randomized triple-blind trial was conducted among 64 obese children with sonographic NAFLD. They were randomly allocated to receive probiotic capsule (containing *Lactobacillus acidophilus* ATCC B3208, 3×10^9 colony forming units [CFU]; *Bifidobacterium lactis* DSMZ 32269, 6×10^9 CFU; *Bifidobacterium bifidum* ATCC SD6576, 2×10^9 CFU; *Lactobacillus rhamnosus* DSMZ 21690, 2×10^9 CFU) or placebo for 12 weeks.

Results: After intervention, in the probiotic group the mean levels of alanine aminotransferase decreased from 32.8 (19.6) to 23.1 (9.9) U/L ($P = 0.02$) and mean aspartate aminotransferase decreased from 32.2 (15.7) to 24.3 (7.7) U/L ($P = 0.02$). Likewise the mean cholesterol, low-density lipoprotein-C, and triglycerides as well as waist circumference decreased in the intervention group, without significant change in weight, body mass index, and body mass index *z* score. After the trial, normal liver sonography was reported in 17 (53.1%) and 5 (16.5%) of patients in the intervention and placebo groups, respectively.

Conclusions: The present findings suggest that a course of the abovementioned probiotic compound can be effective in improving pediatric NAFLD.

Key Words: adolescents, children, nonalcoholic fatty liver disease, obesity, probiotics

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What Is Known

- Nonalcoholic fatty liver disease is a growing disorder in children and adolescents.
- Low compliance of children for lifestyle change necessitates other treatments.
- No medical treatment is approved for pediatric nonalcoholic fatty liver disease.

What Is New

- A course of probiotics studied had significant effect on improving the level of liver enzymes and sonographic fatty liver.
- A 12-week course of probiotics studied was effective in improving nonalcoholic fatty liver disease and lipid profile of obese children.
- The effects of the probiotics studied on improving pediatric nonalcoholic fatty liver disease were independent of the weight status.

Nonalcoholic fatty liver disease (NAFLD) is considered as the most common chronic liver disease among children and adolescents (1). The condition is the hepatic manifestation of metabolic syndrome (2). NAFLD is a general term that refers to a spectrum of liver disease ranging from steatosis to nonalcoholic steatohepatitis and fibrosis (3).

During last decades, the prevalence of NAFLD had a considerable escalating trend in the pediatric population; this increasing prevalence is mainly because of the nutritional transition and the rapid increase in the prevalence of childhood obesity (4). Epidemiological studies, using noninvasive diagnostic methods, have reported an estimated prevalence rate of NAFLD from 3% to 10% among general pediatric population (5) to 70% to 80% among obese children and adolescents (6).

The pathogenesis of pediatric NAFLD remains to be determined, and the condition is considered a challenging issue for both pediatricians and health care providers. It is documented that the complex interaction between genetic, epigenetic, and environmental factors results in the development and progression of NAFLD in children and adolescents (7).

It is also suggested that as an environmental factor, the gut microbiota have a significant impact on intrahepatic fat accumulation; however, the mechanisms are not yet fully determined (8). The interaction between the liver and gut, that is, the gut-liver axis, could explain the beneficial role of gut microbiota composition for pediatric NAFLD (9). Therefore, recent evidence suggests that targeting the axis using probiotics may be an appropriate approach for treating NAFLD (10,11).

Lifestyle interventions are the first-line treatment and prevention strategies for management of obesity and NAFLD. Given

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the poor compliance with lifestyle modification, some studies indicate that combination of these interventions with pharmacological interventions and dietary supplementation would have more favorable effect (12). It seems that among a recommended series of therapeutic dietary supplements, probiotics may be the most promising agents because of their safety, tolerability, and efficacy (12).

Given the increasing trend of NAFLD and its related morbidities, inappropriate compliance of children for lifestyle interventions, lack of effective and safe medication, as well as growing recommendations to evaluate the effectiveness of probiotics through interventional studies, this study aims to evaluate the effects of probiotics on NAFLD in obese children and adolescents.

METHODS

This randomized triple-blind placebo-controlled trial was conducted in 2014 in Isfahan, Iran. The eligibility criteria consisted of being age 10 to 18 years and having age- and sex-specific body mass index (BMI) of $\geq$85th percentile (13), along with sonographic findings of NAFLD; those with any evidence of other chronic liver diseases, history of alcohol drinking, or chronic medication use were not included. For all participants, we excluded other causes of chronic hepatic disease including Wilson disease, autoimmune hepatitis, viral hepatitis (B and C), HIV, α-1-anti-trypsin deficiency, hemochromatosis, and other metabolic diseases. Exclusion criteria were considered as allergy and intolerance to the medication, and or loss of follow-up.

Participants were randomly selected from children and adolescents referred to the pediatric clinics affiliated to Isfahan University of Medical Sciences (IUMS). Considering a power of 80% and type 1 error of 5%, we required a sample size of 32 participants in each group of medication and placebo to detect significant differences in liver ultrasound findings between the 2 groups.

The study protocol was reviewed by the pediatrics review board of IUMS and approved by regional ethics committee of IUMS (research project number: 393891); it was registered at the Iranian Registry of Clinical Trials (*http://www.irct.ir*; identifier: IRCT2013100414882N1). Oral assent and written informed consents obtained from the participants and their parents.

All participants were referred to a radiologist for performing liver ultrasonography. Fatty liver was diagnosed based on the ultrasonographic diagnostic criteria (14–16).

The CONSORT diagram of the trial is presented in Supplemental Digital Content 1, Checklist, *http://links.lww.com/MPG/A820*. After including all eligible subjects to the study, by using computer-generated random numbers, the subjects were randomly assigned to one of the 2 groups receiving medication and placebo. Random allocation of patients to 2 groups was performed by sequentially numbered containers. An assistant performed randomization, so the group allocation was blinded for the investigators and participants.

For subjects in the medication group, 1 daily probiotic capsule was administered for 12 weeks. This medication (Prokid) was produced by Gostaresh Milad Pouya Company, Iran. The microbial strains were as follows: *Lactobacillus acidophilus* ATCC B3208, 3×10^9 colony forming units (CFU); *Bifidobacterium lactis* DSMZ 32269, 6×10^9 CFU; *Bifidobacterium bifidum* ATCC SD6576, 2×10^9 CFU; *Lactobacillus rhamnosus* DSMZ 21690, 2×10^9 CFU.

We used this medication because in our clinical experience it had beneficial effects for other gastrointestinal problems, and also because it was available in Iran. Those in the placebo group received a daily placebo capsule (supplied by Amin Pharmaceutical Company, Isfahan, Iran) for 12 weeks. The probiotic and placebo capsules were identical in shape, size, and color; they were provided to participants with similar boxes. Neither the investigators nor the participants could distinguish the boxes of placebo from medication.

Healthy lifestyle habits were recommended for participants of both groups; they were encouraged to increase their daily activity, to walk fast, and to decrease the screen time, as well as to improve their dietary habits by increasing the intake of fruits and vegetables, and by decreasing the consumption of fast foods, high-fat meals, and sweet snacks.

All participants were checked each month for their weight, height, and their compliance to drug ingestion and for prescribing the placebo or the medication.

At the beginning and that end of the trial, anthropometric examination, laboratory measurements, and ultrasonographic evaluation were performed for all participants. The within-group and between-group differences in the concentration of liver enzymes, grade of fatty liver, and anthropometric measures were compared before and after the trial.

Anthropometric Measurements

Anthropometric measurements were performed by a trained nurse using standard protocols and calibrated instruments. Weight and height of participants were measured with light clothes and without shoes. BMI was calculated as weight (kilograms) divided by height squared (meter square). Waist circumference (WC) was measured by a nonelastic tape at a point midway between the lower border of the rib cage and the iliac crest at the end of normal expiration.

Laboratory Measurements

Fasting venous blood sample was taken from the antecubital vein after 12 hours of fasting in the referral laboratory. Serum alanine aminotransferease (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase were measured on fresh samples by standard kits (Pars Azmoun, Tehran, Iran) using auto-analyzer (Hitachi, Tokyo, Japan).

Ultrasongraphic Evaluation

An expert radiologist performed the ultrasonographic assessment before and after the trial using an ultrasound multifrequency curvilinear 3.5 to 5 MHz probe of Siemens Company (Munich, Germany, Sonoline G50 series, model number 7474922).

The ultrasonic detection of fat was used as a proxy measure. Grades I, II, and III were defined when the echogenicity of liver obscured the walls of portal vein branches, the diaphragmatic outline, and fatty infiltration. The following categories were used: mild (grade 1) minimal diffuse increase in hepatic echogenicity with normal visualization of diaphragm and intrahepatic vessel borders, moderate (grade 2) moderate diffuse increase in hepatic echogenicity with slightly impaired visualization of diaphragm and intrahepatic vessel borders, severe (grade 3) marked increase in hepatic echogenicity with poor penetration of posterior segment of right lobe of liver and poor or no visualization of hepatic vessels and diaphragm (17).

Statistical Analysis

Statistical analysis was performed by SPSS version 20 (SPSS Inc, Chicago, IL) using Student's *t* test, Wilcoxon signed-rank, and Mann-Whitney *U* tests. *P* value <0.05 was considered as statistically significant.

RESULTS

Initially, 68 patients were enrolled in the study; 64 patients with NAFLD were selected and allocated into 2 intervention

TABLE 1. Baseline demographic and anthropometric characteristics in patients with NAFLD

Variables	Probiotic group, n = 32	Placebo group n = 32	P
Age mean (SD)	12.7 (2.2)	12.6 (1.7)	0.80
Gender (boys), %	43.8	56.2	0.32
Weight, kg	61.6 (20)	59.1 (13.6)	0.56
BMI, kg/m^2	26.44 (4.3)	26.61 (2.26)	0.85
BMI z score	2.87	2.91	0.69
WC, cm	82.2 (14.7)	81.4 (6.8)	0.78
ALT, U/L	32.8 (19.6)	28.7 (13.7)	0.34
AST, U/L	32.2 (15.7)	30.2 (12.9)	0.57
Fatty liver (grade)			
Grade I n (%)	20 (62.5)	18 (56.2)	0.61
Grade II n (%)	12 (37.5)	14 (43.8)	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; NAFLD = nonalcoholic fatty liver disease; WC = waist circumference.

(n = 32) and placebo (n = 32) groups (Supplemental Digital Content 2, Fig. 1, *http://links.lww.com/MPG/A821*). Baseline characteristics of patients in the 2 study groups are presented in Table 1. The groups were similar regarding age, sex, anthropometric characteristics, biochemical measurements, and radiologic findings of NAFLD. The mean BMI level of all participants was greater than the 95th percentile, based on Center for Disease Control and Prevention curves (13).

The within-group and between-group changes after the trial are presented in Table 2. Within-group comparison showed significant decrease in mean levels of ALT, AST, and WC in the group receiving the probiotic medication. ALT and WC did not show significant changes in the placebo group. No significant change was seen for weight, BMI, and BMI z score at the end of trial.

In the intervention group, 17 (53.1%) patients had normal liver sonography after the trial ($P < 0.001$), whereas this frequency was 5 (16.5%) in the placebo group ($P = 0.008$). At the end of the trial, the intervention group had significantly lower mean level of liver enzymes than the interventional group. After the trial, compared with the placebo group, the frequency of normal liver ultrasonography was significantly higher, and the frequency of high-grade fatty liver was significantly lower than the placebo group ($P < 0.05$). Reduction in sonographic grading of NAFLD was in parallel to the decrease in mean level of liver enzymes (Table 2).

At the beginning of study, in the intervention group, 14 patients had abnormal ALT and AST; at the end of the trial, 5 patients had abnormal ALT and AST (78% improved, $P = 0.004$). ALT became normal in 9 participants; 7 of them also had decreased sonographic grading of NAFLD (77.8%).

At baseline, the triglyceride and low-density lipoprotein (LDL) levels had no significant difference in the intervention and placebo groups, but the cholesterol level was significantly higher in the intervention group. At the end of the trial, the mean serum level of cholesterol, triglyceride, and LDL decreased significantly in the intervention group ($P < 0.001$), whereas in the placebo group, the mean triglyceride level had significant decrease ($P < 0.001$).

DISCUSSION

In this randomized controlled trial, we investigated the effect of kind of probiotics on weight status, ultrasonographic, and biochemical variables in obese children and adolescents with NAFLD. Our findings indicated that this supplement had some improving effect on the study variables, notably on liver enzymes and NAFLD sonographic grades.

Recent evidences have demonstrated that gut microbiota contribute to the development of NAFLD through the gut-liver axis. Our findings are consistent with some review studies, which evaluated the effect of probiotics on treatment of NAFLD through interventional studies, and have confirmed their beneficial effects (18,19).

Although several animal and experimental studies have demonstrated improving effects of probiotics on different metabolic components of NAFLD, available clinical trials on human

TABLE 2. Mean level of waist circumference, liver enzymes and frequency of different grades of NAFLD according to ultrasonographic findings, before and after trial in intervention and placebo groups

	Probiotic			Placebo		
Variables	Before	After	P^*	Before	After	P^*
WC, cm	82.2 (14.7)	80.3 (15.1)	0.001	81.4 (6.8)	80 (7.2)	0.06
AST, U/L	32.2 (15.7)	24.3 (7.7)‡	0.002	30.2 (12.9)	26.6 (11.8)‡	0.006
ALT, U/L	32.8 (19.6)	23.1 (9.6)‡	0.002	28.9 (13.7)	26.2 (12.9)‡	0.20
Lipid profile						
Cholesterol	157.31 (57.11)†	145.06 (47.87)‡	<0.001	108.00 (24.33)†	105.43 (23.56)‡	0.07
HDL-C	46.25 (12.08)†	46.75 (11.32)	0.18	34.53 (4.64)†	36.53 (10.21)‡	0.25
LDL-C	87.93 (28.74)	81.65 (23.49)	<0.001	79.25 (14.18)	78.31 (12.93)	<0.001
Triglyceride	112.53 (50.46)	100.56 (44.80)	<0.001	96.03 (20.65)	91.87 (19.14)	<0.001
Fatty liver (grade) [n (%)]						
Normal	0 (0%)	17 (53.1%)‡	<0.001	0 (0%)	5 (16.5%)‡	
Grade I	20 (62.5%)	8 (25%)		18 (56.2%)	15 (46.9%)	0.008
Grade II	12 (37.5%)	7 (21.9%)		14 (43.8%)	12 (37.5%)	

ALT = alanine aminotransferase; AST = aspartate aminotransferase; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NAFLD = nonalcoholic fatty liver disease; WC = waist circumference.

$^*P$ value for difference within groups throughout the study.

$^†P$ value <0.05 for difference between probiotic and placebo groups before intervention.

$^‡P$ value <0.05 for difference between probiotic and placebo groups after intervention.

population, especially among pediatric population, are scarce (19). In this regard, 2 previous clinical trials have investigated different strains of probiotics among children and adolescents (20,21).

In a double-blind clinical trial, Vajro et al (20) studied the effect of *Lactobacillus rhamnosus* strain GG in 20 obesity-related cases of pediatric NAFLD. They found that after 8 weeks, this strain resulted in normalization of fatty liver in 80% of cases and reduction in serum ALT level. They concluded that this probiotic could be used as an appropriate therapeutic tool for the treatment of children with NAFLD who were noncompliant with the recommended lifestyle modifications. Another clinical trial was conducted by Alisi et al (21) among 44 obese children with biopsy-proven NAFLD, they investigated the impact of VSL#3 versus placebo in a 4-month trial. VSL#3 containing 8 probiotic strains including *Streptococcus thermophilus*, *Bifidobacteria* (*B breve*, *B infantis*, and *B longum*), *Lactobacillus acidophilus*, *L plantarum*, *L paracasei*, and *L delbrueckii* subsp. *Bulgaricus* had significant improving effects on fatty liver and BMI.

Some review studies have reported that probiotics could have a proper effect on improving fatty liver and reducing its grade from severe stage to mild or normal (20–22). In this trial, although we observed some improvement both in the intervention and placebo groups regarding the ultrasonographic grading of NAFLD, the effects of the administered probiotic were better than placebo. The rate of improvement was 53% and 16.5% in the intervention and placebo groups, respectively. Our findings are consistent with the abovementioned trials conducted in the pediatric age group (20,21).

It is well established that serum AST and ALT levels are clinical markers of liver damage (23). Some clinical trials that used probiotics have demonstrated that these agents may decrease the level of at least 1 of these markers (22,24). The probiotic used in the present study reduced the concentrations of AST and ALT. After the trial, the levels of both markers were significantly lower in the probiotic group than in the placebo group. Improving ultrasonographic grading of NAFLD was also in parallel to the decreased liver enzymes.

Controversial results exist regarding the impact of probiotics on BMI and WC; some studies indicated beneficial effect, whereas others did not confirm it (20,21,25,26). The probiotic compound used in the present study had significant effect on WC, but not on weight and BMI. Lipid-lowering effect of probiotics is reported both in animal and human studies (27–29).

The probiotic used in the present trial had some favorable effects on reducing the concentrations of cholesterol, LDL, and triglyceride. Our finding on HDL is in line with some previous studies that did not, however, document beneficial effects of probiotics in increasing this lipoprotein with useful functions. It is suggested that HDL level may increase after long-term treatment of probiotics (21).

The probiotic used in this trial resulted in some improvement in the lipid profile of participants. At baseline, triglycerides and LDL concentrations were not significantly different between the intervention and placebo groups, but total cholesterol level was significantly higher in the intervention group. After the trial, total cholesterol, triglycerides, and LDL had mild to moderate decrease in the group receiving the probiotic compound. Triglycerides level had significant decrease in the placebo group; this change may be because of recommendations on lifestyle change.

Study Limitations and Strengths

Small sample size, short duration of trial and follow-up, and lack of assessment of inflammatory factors are the main limitations of our study. As the sample size was not large enough, we could not evaluate sex differences regarding the impact of probiotic. In addition, the probiotic used in this trial was a mixture of four probiotic strains. For obtaining more accurate results, we suggest to design further studies in a way to evaluate a single probiotic agent and to conduct a cross-over trial (including a washout period) to assess the gut microbiota. Another limitation of the study is that we did not consider liver biopsy because of its invasive nature; as the main problem of the study participants was only excess weigh, and they had no serious hepatic problem, we limited the examinations to sonography and biochemical tests. The main strength of our study was its novelty in the pediatric age group.

CONCLUSIONS

A limited course of the probiotic administered in this trial could be effective in improving surrogate markers of NAFLD and the lipid profile. Further studies on various probiotic strains are recommended to be conducted in larger sample size and longer duration.

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