Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world. Oral administration of synbiotic has been proposed as an effective treatment of NAFLD because of its modulating effect on the gut flora, which can influence the gut-liver axis.

Objective: The objective was to evaluate the effects of supplementation with synbiotic on hepatic fibrosis, liver enzymes, and inflammatory markers in patients with NAFLD.

Design: In a randomized, double-blind, placebo-controlled clinical trial conducted as a pilot study, 52 patients with NAFLD were supplemented twice daily for 28 wk with either a synbiotic or a placebo capsule. Both groups were advised to follow an energy-balanced diet and physical activity recommendations.

Results: At the end of the study, the alanine aminotransferase (ALT) concentration decreased in both groups; this reduction was significantly greater in the synbiotic group. At the end of the study, the following significant differences [means (95% CIs)] were seen between the synbiotic and placebo groups, respectively: ALT [-25.1 (-26.2, -24) compared with -7.29 (-9.5, -5.1) IU/L; P < 0.001], aspartate aminotransferase [-31.33 (-32.1, -30.5) compared with -7.94 (-11.1, -4.8) IU/L; P < 0.001], γ-glutamyltransferase [-15.08 (-15.5, -14.7) compared with -5.21 (-6.6, -3.9) IU/L; P < 0.001], high-sensitivity C-reactive protein [-2.3 (-3.3, -1.5) compared with -1.04 (-1.5, -0.6) mmol/L; P < 0.05], tumor necrosis factor-α [-1.4 (-1.7, -1.1) compared with -0.59 (-0.8, -0.3) mmol/L; P < 0.001], total nuclear factor κ-B p65 [-0.016 (-0.022, -0.011) compared with 0.001 (-0.004, -0.007) mmol/L; P < 0.001], and fibrosis score as determined by transient elastography [-2.98 (-3.6, -2.37) compared with -0.77 (-1.32, -0.22) kPa; P < 0.001].

Conclusions: Synbiotic supplementation in addition to lifestyle modification is superior to lifestyle modification alone for the treatment of NAFLD, at least partially through attenuation of inflammatory markers in the body. Whether these effects will be sustained with longer treatment durations remains to be determined. This trial was registered at clinicaltrials.gov as NCT01791959. Am J Clin Nutr 2014;99:535–42.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world and may lead to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (1). No treatment has yet been approved for NAFLD, and the only recognized management strategies include lifestyle modifications (2).

The concept that gut microbiota, including the billions of bacteria resident within the human gastrointestinal tract, can be involved in the pathogenesis of liver disorders has been proposed. The gut consists of a complex of microorganism species, the concentration and type of which are mostly influenced by host genotype and nutrient availability (3). It is known that the liver is susceptible to the exposure of intestine-derived bacterial products because of a close anatomic and functional connection between the intestinal lumen and the liver through the portal system (4, 5). The gut-liver axis is an important pathway in the development of NAFLD, which is associated with small intestinal bacterial overgrowth and increased intestinal permeability (6–9). The contribution of microflora in the progression of NAFLD is mainly based on increased hepatic oxidative stress due to the increased production of ethanol, and lipopolysaccharides in the intestinal lumen, and subsequent release of inflammatory cytokines in some inflammatory cells (5, 9). Of these inflammatory cytokines, TNF-α appears to play a crucial role in both insulin resistance and metabolic disorders (10, 11).

Background: Tumor necrosis factor-α (TNF-α) has been implicated in the pathogenesis of a variety of diseases, including chronic liver disease (12). Activation of nuclear factor-kappa B (NF-κB) is a key step in the induction of cytokines (13) and other proinflammatory mediators, including TNF-α (14). Several studies have shown that TNF-α is an important inflammatory mediator in liver disease (15, 16). Dietary patterns that are rich in saturated fat (17) and high in n-6 polyunsaturated fatty acids (18) can increase the risk of NAFLD.

Objective: The objective of this study was to evaluate the effects of synbiotic supplementation on hepatic fibrosis, liver enzymes, and inflammatory markers in patients with NAFLD.

Design: A randomized, double-blind, placebo-controlled clinical trial was conducted at the Digestive Diseases Research Institute, Tehran University of Medical Sciences, Tehran, Iran (FZ). The study was registered at clinicaltrials.gov as NCT01791959.

Results: At the end of the study, the alanine aminotransferase (ALT) concentration decreased in both the synbiotic and placebo groups; this reduction was significantly greater in the synbiotic group. At the end of the study, the following significant differences were seen between the synbiotic and placebo groups, respectively: ALT [-25.1 (-26.2, -24) compared with -7.29 (-9.5, -5.1) IU/L; P < 0.001], aspartate aminotransferase [-31.33 (-32.1, -30.5) compared with -7.94 (-11.1, -4.8) IU/L; P < 0.001], γ-glutamyltransferase [-15.08 (-15.5, -14.7) compared with -5.21 (-6.6, -3.9) IU/L; P < 0.001], high-sensitivity C-reactive protein [-2.3 (-3.3, -1.5) compared with -1.04 (-1.5, -0.6) mmol/L; P < 0.05], tumor necrosis factor-α [-1.4 (-1.7, -1.1) compared with -0.59 (-0.8, -0.3) mmol/L; P < 0.001], total nuclear factor κ-B p65 [-0.016 (-0.022, -0.011) compared with 0.001 (-0.004, -0.007) mmol/L; P < 0.001], and fibrosis score as determined by transient elastography [-2.98 (-3.6, -2.37) compared with -0.77 (-1.32, -0.22) kPa; P < 0.001].

Conclusions: Synbiotic supplementation in addition to lifestyle modification is superior to lifestyle modification alone for the treatment of NAFLD, at least partially through attenuation of inflammatory markers in the body. Whether these effects will be sustained with longer treatment durations remains to be determined. This trial was registered at clinicaltrials.gov as NCT01791959.

References:


resistance and hepatic inflammatory cell recruitment in NAFLD/
NASH (10–12).

Probiotics are live microorganisms that are beneficial to human
health when ingested (13). On the basis of NAFLD characteristics,
including steatosis and liver inflammation, followed by fibrosis,
and the proven immunomodulatory, antifibrotic and antiinflam-
matory properties of probiotics and/or prebiotics in animals (14–
17) and limited human studies (18, 19), we hypothesized that,
in addition to lifestyle modifications (diet and exercise), manipulation
of enteric flora by consumption of symbiotic may act as a
novel adjunctive therapeutic strategy in patients with NAFLD.

To evaluate this hypothesis, a double-blind, randomized, placebo-
controlled clinical trial was designed to determine whether sup-
plementation with symbiotic will further improve the efficacy of
lifestyle modifications on NAFLD management while addressing
some of the mechanisms of action by which symbiotic may
function.

SUBJECTS AND METHODS

Recruitment and eligibility screening

Patients were identified and recruited from the Haraz Clinic in
Amol, Iran. The diagnosis of NAFLD was made on the basis of
the presence of steatosis, on ultrasound examination, associated
with a persistently elevated alanine aminotransferase (ALT)
concentration of ≥60 U/L for 6 mo before the study and at the
time of randomization. Exclusion criteria included anyone with
viral hepatitis, alcohol use, other causes of chronic liver disease,
diabetes mellitus, untreated hypothyroidism, clinically or bio-
chemically recognized systemic diseases, psychiatric disorders
impairing the patient’s ability to provide written informed
consent, and pregnancy, lactation, and lack of effective birth
control in women of childbearing age. Men and women ≥18 y
of age were recruited.

Study design

All eligible patients with NAFLD were recruited in March 2012.
Interviews and questionnaires were all administered at the
Haraz Clinic. Patients signed an informed consent form after
a full review of the inclusion and exclusion criteria and an ex-
planation of the risks and benefits of the study, which were
approved by the ethics committee of the National Nutrition and
Food Technology Research Institute and the Digestive Diseases
Research Institute of Tehran University of Medical Sciences. At
week 0, patients were randomly assigned to receive either syn-
biotic supplementation or the identical-appearing placebo cap-
sule (maltodextrin) twice daily for 28 wk, and baseline data were
gathered. Follow-up assessments were performed every 7 wk at
weeks 7, 14, 21, and 28 after randomization.

According to a multistage, cluster, random-sampling method,
6140 subjects were selected for the NAFLD screening study in
Amol, of whom 2460 had a diagnosis of NAFLD based on ul-
trasound and liver enzyme results (Figure 1). Those with the
highest fibrosis grades, determined by transient elastography
(FibroScan; echosens), who agreed to participate were randomly
assigned by age and sex into the symbiotic or placebo group.
Randomization lists were computer-generated by a statistician
and given to the interviewer. Subjects, investigators, and staff
were blind to the treatment assignment until the end of the study.

At the first visit (week 0), baseline data were gathered and
patients were provided with a 7-wk supply of capsules. At each
follow-up visit (every 7 wk), patients were given another set
of capsules. Each symbiotic capsule (Protexin) contained 200
million of 7 strains of friendly bacteria (Lactobacillus casei,
Lactobacillus rhamnosus, Streptococcus thermophilus, Bifid-
obacterium breve, Lactobacillus acidophilus, Bifidobacterium
longum, and Lactobacillus bulgaricus) and prebiotic (fructooli-
gosaccharide) and probiotic cultures [magnesium stearate (source:
mineral and vegetable) and a vegetable capsule (hydroxypropyl
methyl cellulose)]. Adherence was assessed by capsule counts
confirmed at each visit.

Both groups were advised to follow an energy-balanced diet
and physical activity recommendations according to the Clinical
Guidelines on the Identification, Evaluation, and Treatment of
Overweight and Obesity in Adults from the NIH and the North
American Association for the Study of Obesity (20). The distri-
bution of nutrients in relation to the total caloric value was
as follows: ≤30% of total energy as fat (10% as SFAs, 15% as
MUFAs, and 5% as PUFAs), 15–18% as protein, 52–55% as car-
bohydrate, <300 mg/d as dietary cholesterol, and 20–30 g fiber/d.
Patients were also advised to exercise ≥30 min, 3 times/wk.

Clinical, paraclinical, and dietary intake assessments

All patients underwent measurements of weight, height, and
waist and hip circumferences. Each individual’s BMI was cal-
culated by using the following formula: BMI = weight (in kg)/
height (in m)². Waist-to-hip ratio (WHR) was measured ac-
cording to WHO recommendation (21).

Biochemical testing was performed on each patient at weeks 0,
7, 14, 21, and 28 after they had fasted for 12 h. All biochemical
assessments were performed in the same laboratory using standard
laboratory methods. Total bilirubin and γ-glutamyltransferase
(GGT) were measured by enzymatic colorimetric assay (Parsazmoun).
ALT, aspartate aminotransferase (AST), and alkaline phospha-
tase (ALP) concentrations were measured by photometric assay
(Reckon). Inflammatory factors were assessed at baseline and at
the end of the study. Fasting high-sensitivity C-reactive protein
concentrations were measured by ELISA (Diagnostics Biochem
Canada Inc). Plasma TNF-α concentrations were measured by
using a commercial ELISA kit (KOMA BIOTECH Inc) with a
lower sensitivity limit of 10 pg/mL. Nuclear factor-κ-B (NF-κB)
p65 was measured in peripheral blood mononuclear cell (PBMC)
nuclear extracts by using an ELISA kit (Cell Signaling) according
to the manufacturer’s protocol.

Fasting glucose concentrations were measured by using the
GOD/POD method. Fasting insulin concentrations were mea-
sured by ELISA (Mercodia AB). HOME-IR was used to
determine the degree of insulin resistance by using the following
formula (22): HOME-IR = [fasting insulin (mU/L) × fasting
blood glucose (mg/dL)]/405.

After patients were randomly assigned into the 2 groups, liver
fibrosis was also assessed by transient elastography at baseline
and at the end of the study. Transient elastography was performed
by using the same equipment and by the same operator who was
also blind to the study randomization and was unaware of the
clinical and laboratory results.
For the assessment of nutrient intakes, patients received food records at weeks 0, 7, 14, 21, and 28 and were instructed to record their daily dietary intake for 3 d, including a weekend day. Dietary intakes were then analyzed by using Nutritionist 4 (First DataBank), incorporating the use of food scales and models to enhance portion size accuracy. National food-composition tables were used as a reference (23). Physical activity was also assessed by using the metabolic equivalent of task (MET) questionnaire (24) at weeks 0, 7, 14, 21, and 28.

Follow-up

Each follow-up visit consisted of data collected through a standardized medical history, 3 food records, anthropometric measurements, and serum collection, and capsule counts were used to assess adherence to study treatment. An assessment of adverse events that may have occurred was also performed at each visit. The final patient follow-up was in October 2012 and included a transient elastography assay in addition to all of the follow-up assessments mentioned.

Primary and secondary outcomes

The primary outcome measure was a significant reduction in ALT concentration. Secondary outcome measures were transient elastography score, inflammatory factor concentrations in serum and PBMCs, anthropometric variables, and serum concentrations of AST, GGT, total bilirubin, and ALP.
Statistical analysis

Data were analyzed with the use of STATA software (version 11; StataCorp). For all analyses a P value <0.05 was considered statistically significant. Continuous and categorical data were presented as means ± SDs and frequency, respectively. Demographic variables were analyzed by using a chi-square or t test, as appropriate.

The data were analyzed according to the intention-to-treat principle. Patients missing laboratory measurements of the primary and secondary outcome measures were imputed. A multiple imputation procedure was used based on Multivariate Imputation by Chained Equations. In the multiple imputation procedure, 5 imputed data sets were generated. The results of the 5 imputed data sets were pooled to obtain data estimates.

Means and 95% CIs for changes from baseline in ALT and AST were compared at 7, 14, 21, and 28 wk by using ANCOVA models for ALT at each time point. ANCOVA models were also used to compare changes from baseline to the end of treatment in transient elastography score, GGT, ALP, and bilirubin. All ANCOVA models were adjusted for the baseline value of each outcome and mean change in BMI, WHR, MET, and energy. To determine the emergence of any time-related patterns of response to treatment, means and 95% CIs for changes relative to baseline in ALT, AST, and BMI were plotted against time from baseline until the end of treatment.

RESULTS

Characteristics of the patients

Fifty-two patients were included in the study and were randomly assigned into the 2 groups—synbiotic (n = 26) or placebo (n = 26). Patient screening, enrollment, and retention by treatment group are shown in Figure 1. The baseline clinical and demographic data of the 2 groups were similar with respect to anthropometric data, laboratory data, and liver echogenicity and elasticity (Table 1).

Primary outcome

Eighty-eight percent of patients completed 28 wk of treatment and had end-of-study clinical and laboratory variables obtained as well as transient elastography results. All enrolled patients were included in the analysis of the primary outcome—reduction

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 52)</th>
<th>Synbiotic group (n = 26)</th>
<th>Placebo group (n = 26)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>46.0 ± 9.2</td>
<td>46.35 ± 8.8</td>
<td>45.69 ± 9.5</td>
<td>0.801</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>25/27</td>
<td>14/12</td>
<td>11/15</td>
<td>0.405</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Yes</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>48</td>
<td>24</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Metabolic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.0 ± 9.7</td>
<td>163.4 ± 8.0</td>
<td>160.5 ± 10.9</td>
<td>0.297</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>83.6 ± 11.8</td>
<td>85.7 ± 10.0</td>
<td>81.5 ± 13.2</td>
<td>0.199</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>102.6 ± 6.8</td>
<td>102.4 ± 6.8</td>
<td>102.8 ± 6.2</td>
<td>0.152</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.7 ± 2.4</td>
<td>32.1 ± 2.4</td>
<td>31.3 ± 2.3</td>
<td>0.247</td>
</tr>
<tr>
<td>WHR</td>
<td>—</td>
<td>0.94 ± 0.1</td>
<td>0.97 ± 0.1</td>
<td>0.842</td>
</tr>
<tr>
<td>MET (h/d)</td>
<td>31.7 ± 4.2</td>
<td>31.39 ± 4.1</td>
<td>32.00 ± 4.2</td>
<td>0.598</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>2271.0 ± 452.2</td>
<td>2351.0 ± 515.1</td>
<td>2190.9 ± 372.2</td>
<td>0.205</td>
</tr>
<tr>
<td>Serum biochemistry tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>70.4 ± 6.6</td>
<td>69.3 ± 2.3</td>
<td>71.5 ± 9.1</td>
<td>0.246</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>67.3 ± 6.9</td>
<td>66.4 ± 2.6</td>
<td>68.3 ± 9.4</td>
<td>0.323</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>230.6 ± 11.2</td>
<td>231.4 ± 10.4</td>
<td>229.8 ± 12.1</td>
<td>0.603</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>89.2 ± 2.0</td>
<td>89.5 ± 1.5</td>
<td>89.0 ± 2.5</td>
<td>0.370</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.96 ± 0.2</td>
<td>0.96 ± 0.3</td>
<td>0.95 ± 0.2</td>
<td>0.831</td>
</tr>
<tr>
<td>FBS (mg/dL)</td>
<td>98.2 ± 18.7</td>
<td>99.6 ± 24.2</td>
<td>98.9 ± 21.4</td>
<td>0.217</td>
</tr>
<tr>
<td>Insulin (mU/L)</td>
<td>11.1 ± 4.8</td>
<td>11.2 ± 3.4</td>
<td>11.1 ± 4.1</td>
<td>0.967</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.7 ± 1.4</td>
<td>2.8 ± 1.0</td>
<td>2.7 ± 1.2</td>
<td>0.877</td>
</tr>
<tr>
<td>Inflammatory factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP (ng/mL)</td>
<td>4.25 ± 2.7</td>
<td>4.23 ± 2.7</td>
<td>4.27 ± 2.8</td>
<td>0.956</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>11.79 ± 3.2</td>
<td>12.39 ± 3.9</td>
<td>11.20 ± 2.3</td>
<td>0.181</td>
</tr>
<tr>
<td>NF-κB p65</td>
<td>0.072 ± 0.02</td>
<td>0.076 ± 0.01</td>
<td>0.065 ± 0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Liver histology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transient elastography (kPa)</td>
<td>8.6 ± 2.1</td>
<td>9.4 ± 1.9</td>
<td>7.9 ± 2.1</td>
<td>0.012</td>
</tr>
<tr>
<td>Ultrasound [n (%)]</td>
<td></td>
<td></td>
<td></td>
<td>0.108</td>
</tr>
<tr>
<td>Grade 2</td>
<td>37 (71.2)</td>
<td>17 (65.4)</td>
<td>20 (76.9)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>15 (28.8)</td>
<td>9 (34.6)</td>
<td>6 (23.1)</td>
<td></td>
</tr>
</tbody>
</table>

1ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBS, fasting blood sugar; GGT, γ-glutamyltransferase; hs-CRP, high-sensitivity C-reactive protein; MET, metabolic equivalent of task; NF-κB, nuclear factor κ-B; WHR, waist-to-hip ratio.

2Mean ± SD (all such values).

3FibroScan (echosens).
in ALT concentration—which decreased significantly in both groups \((P < 0.01)\); however, the mean reduction in the symbiotic group was greater than that in the placebo group \((P < 0.001)\). The change in ALT concentration at the 21st, and 28th weeks (Table 2) showed a significant difference between those treated with symbiotic or placebo \((P < 0.001)\).

### Secondary outcomes

We found a significant decrease in the BMI and WHR within both groups; however, no significant differences were observed between the 2 groups in these variables (Figure 2). At the end of the 28-wk treatment period, a significant improvement in several liver variables was seen within and between both groups and before and after the adjustment for MET and energy intake. Compared with the placebo group, patients taking symbiotic capsules had a significantly greater decrease in the following liver enzymes: ALT \((69.30 \pm 2.3\) to \(44.20 \pm 3.8\) in the symbiotic group and \(71.46 \pm 9.1\) to \(64.17 \pm 11.1\) in the placebo group; \(P < 0.001)\), AST \((66.38 \pm 2.6\) to \(35.05 \pm 2.7\) in the symbiotic group and \(68.29 \pm 9.41\) to \(60.34 \pm 13.1\) in the placebo group; \(P < 0.001)\), and GGT \((89.51 \pm 1.5\) to \(74.42 \pm 1.8\) in the symbiotic group and \(88.99 \pm 2.5\) to \(83.78 \pm 3.1\) in the placebo group; \(P < 0.001)\), whereas no significant difference was found within and between the 2 groups in total bilirubin and ALP (Table 2). All of these reductions were significantly different from those at 14 wk.

Fasting blood sugar (FBS) and insulin concentrations also decreased significantly in both groups and between groups before and after adjustments. The reductions in insulin resistance variables of the symbiotic group compared with the placebo group were as follows: FBS decreased as much as \(-7.96\) compared with \(-2.82\) mg/dL \((P < 0.001)\), \(-2.02\) compared with \(-0.92\) mU/L \((P < 0.05)\), and HOMA-IR \(-0.68\) compared with \(-0.39\) \((P < 0.001)\) (Table 2).

### DISCUSSION

At the end of the treatment, the transient elastography results also showed a significant improvement within and between both groups; the mean reduction in the fibrosis score in the symbiotic group was significantly greater than that in the placebo group \((9.36 \pm 1.9\) to \(6.38 \pm 1.5\) in the symbiotic group compared with \(7.92 \pm 2.1\) to \(7.16 \pm 2.0\) in placebo group; \(P < 0.001)\). In the symbiotic group, 95% of patients showed some improvement in their fibrosis score, whereas the scores of 5% remained unchanged. Of those with improvements, 8% had a 1-level reduction in their fibrosis score, whereas 36% and 56% had 2- and 3-level reductions, respectively. Fibrosis scores also decreased in the placebo group, but less than in the symbiotic group. Only 36% of the patients in this group had one or more levels of reduction in their fibrosis scores.

All of the inflammatory markers decreased after treatment in both groups, although the mean decrease in the symbiotic group was greater than in the placebo group (Table 3). None of the patients completing the study had any serious adverse events, which indicated tolerance to the treatment. Two minor adverse events were reported; one patient complained of moderate headaches and one of abdominal pain in the symbiotic and placebo groups, respectively, both of which were resolved without reoccurrence.

### TABLE 2

<table>
<thead>
<tr>
<th>Change from baseline</th>
<th>Symbiotic group ((n = 26))</th>
<th>Placebo group ((n = 26))</th>
<th>(P) value(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 7</td>
<td>(-8.0 ) ((-10.7, -5.3))</td>
<td>(-4.7 ) ((-6.4, -3.1))</td>
<td>0.123</td>
</tr>
<tr>
<td>Week 14</td>
<td>(-11.3 ) ((-13.4, -9.1))</td>
<td>(-4.4 ) ((-5.5, -3.4))</td>
<td>0.02</td>
</tr>
<tr>
<td>Week 21</td>
<td>(-15.3 ) ((-17.6, -13.1))</td>
<td>(-2.5 ) ((-4.4, -0.5))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Week 28</td>
<td>(-25.1 ) ((-26.2, -24.0))</td>
<td>(-7.3 ) ((-9.5, -5.1))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 7</td>
<td>(-14.4 ) ((-17.3, -11.5))</td>
<td>(-4.9 ) ((-6.6, -3.2))</td>
<td>0.01</td>
</tr>
<tr>
<td>Week 14</td>
<td>(-19.1 ) ((-21.6, -16.6))</td>
<td>(-7.7 ) ((-11.6, -3.8))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Week 21</td>
<td>(-22.5 ) ((-24.8, -20.2))</td>
<td>(-5.0 ) ((-8.1, -1.8))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Week 28</td>
<td>(-31.3 ) ((-32.1, -30.5))</td>
<td>(-7.9 ) ((-11.4, -4.8))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 7</td>
<td>(-0.23 ) ((-0.3, -0.2))</td>
<td>(-0.11 ) ((-0.2, -0.02))</td>
<td>0.033</td>
</tr>
<tr>
<td>Week 14</td>
<td>(-0.35 ) ((-0.4, -0.2))</td>
<td>(-0.12 ) ((-0.2, -0.04))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Week 21</td>
<td>(-0.43 ) ((-0.5, -0.3))</td>
<td>(-0.13 ) ((-0.2, -0.04))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Week 28</td>
<td>(-0.68 ) ((-0.8, -0.5))</td>
<td>(-0.39 ) ((-0.5, -0.3))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GGT concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 14</td>
<td>(-7.54 ) ((-7.7, -7.3))</td>
<td>(-2.61 ) ((-3.3, -1.9))</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Week 28</td>
<td>(-15.08 ) ((-15.5, -14.7))</td>
<td>(-5.21 ) ((-6.6, -3.9))</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\(^1\) ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, \(\gamma\)-glutamyltransferase.

\(^2\) Based on an ANCOVA model that regressed changes from baseline on treatment group, baseline value of the outcome, and mean change in BMI, waist-to-hip ratio, metabolic equivalent tasks, and energy.
to the mesenteric lymphatic circulation activates the immune system, which induces the regional and systemic production of proinflammatory cytokines and enhances the production of free radical species in the abdominal area, which might contribute to the evolution of NAFLD and steatohepatitis (25). Of these inflammatory cytokines, TNF-α appears to play a critical role in both insulin resistance and hepatic inflammatory cell recruitment in NAFLD/NASH (10–12). The production of TNF-α occurs as a result of NF-κB activation, which is a master regulator of inflammation (10, 26).

Most of the previous animal studies that evaluated the effect of probiotics on NAFLD have shown that probiotics can ameliorate NAFLD characteristics and inflammatory factors such as NF-κB activation and TNF-α secretion (15, 17); however, the findings regarding the expression of TNF-α in hepatic tissue are controversial (14, 16). Li et al (16) investigated the effect of VSL#3 on an experimental model of high-fat diet–induced fatty liver and reported that VSL#3 improved liver histologic results, reduced the content of hepatic total fatty acids, and decreased serum ALT concentrations similarly to the anti-TNF-α antibodies. These effects were associated with a reduction in Jun N-terminal kinase, a TNF-regulated stress kinase that promotes hepatic insulin resistance, and NF-κB activity, fatty acid β-oxidation, and mitochondrial uncoupling protein-2 expression, all being markers and factors characterizing insulin resistance; however they did not observe a reduction in hepatic TNF-α gene expression. They concluded that because VSL#3 therapy reduced hepatic activity of Jun N-terminal kinase, it is conceivable that it inhibited the production of TNF-α by some extrahepatic sources or operated at posttranscriptional levels to block the actions of TNF-α within the liver. Our data support their conclusion because we have observed that symbiotic supplementation reduced NF-κB activation in PBMCs and total TNF-α in serum, which indicates that probiotics can act as immunomodulatory agents in some extrahepatic organs.

Insulin resistance is one of the characteristics of NAFLD and of the metabolic syndrome; however, only a small number of studies have investigated the effects of symbiotic therapy on insulin resistance. The results of our study showed a significant reduction in FBS and insulin concentrations and an improvement in HOMA-IR. Malaguarnera et al (19) also showed a significant reduction in serum FBS, insulin, and HOMA-IR after treatment with *B. longum* plus fructooligosaccharide, which is consistent with the results of our study. These results are also in line with those of other previous studies (17, 27). It has been found that lipopolysaccharides are present at higher concentrations in the blood of subjects with type 2 diabetes mellitus or insulin resistance than in healthy subjects. Circulating lipopolysaccharides have also been shown to correlate with insulin and glucose concentrations and with HOMA-IR (28, 29). In response to

### TABLE 3

Mean changes (95% CIs) from baseline to end of treatment in inflammatory factors by treatment group

<table>
<thead>
<tr>
<th>Change from baseline to end of treatment</th>
<th>Symbiotic group (<em>n</em> = 26)</th>
<th>Placebo group (<em>n</em> = 26)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (ng/mL)</td>
<td>−2.30 (−3.0, −1.5)</td>
<td>−1.04 (−1.5, −0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>−1.40 (−1.7, −1.1)</td>
<td>−0.59 (−0.8, −0.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NF-κB p65</td>
<td>−0.016 (−0.022, −0.011)</td>
<td>0.001 (−0.004, −0.007)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

1 hs-CRP, high-sensitivity C-reactive protein; NF-κB, nuclear factor κ-B.

2 Based on an ANCOVA model that regressed changes from baseline on treatment group, baseline value of the outcome, and mean change in BMI, waist-to-hip ratio, metabolic equivalent tasks, and energy.

FIGURE 2. Changes in BMI and WHR during treatment and follow-up. The number of patients at each visit and within each treatment group was 26. Error bars indicate 95% CIs. BMI and WHR decreased significantly in both groups (*P* < 0.05); no significant differences were observed between the 2 groups. WHR, waist-to-hip ratio.
systemic insulin resistance, pancreatic β cells increase insulin hypersecretion and accelerate liver fat accumulation, which leads to NAFLD. Symbiotic could improve insulin resistance through mechanisms such as modifications to the gut flora, reductions in endotoxin concentrations, increases in fecal pH, and reductions in the production and absorption of intestinal toxins (7, 25).

One limitation of our study was the lack of liver biopsy results from which to derive a pathology score of disease; however, we did use transient elastography, which provides a quantitative, noninvasive evaluation of NAFLD by measuring hepatic fibrosis (30, 31). This technique has proven to be a reliable, noninvasive method for identifying patients with significant hepatic fibrosis. It is readily reproducible, and its score has low inter- and intra-observer variability (30, 31). In addition, because patients were evaluated with FibroScan after being randomly assigned, the transient elastography scores of the 2 groups were different at baseline: the placebo group had less fibrosis (average score: 7.92 ± 2.1) than did the symbiotic group (average score: 9.36 ± 1.9). This was an unintentional occurrence, which definitely contributed to the significant difference seen in the fibrosis scores of the 2 groups. Regardless of this, however, the transient elastography scores still decreased significantly—by almost 3 points (from 9.36 ± 1.9 to 6.38 ± 1.5)—in the symbiotic group before and after treatment, which indicated an influence of supplementation on liver fibrosis. Another limitation of this study was that we did not evaluate the gut microbiome, which could have indicated the effects of symbiotic consumption on gut microflora and confirmed our suggested mechanism of action.

The most important strengths of the current study were the relatively low evaluation period of NF-κB activity in PBMCs, the stratified blocked randomization design, and the inclusion of patients with newly diagnosed NAFLD who had not yet received treatment; all of these strengths are unique in comparison with the few other clinical trials that have evaluated the effects of probiotics alone or in combination with prebiotics on NAFLD (18, 19).

In conclusion, this randomized, double-blind, placebo-controlled trial found some evidence that symbiotic supplementation in addition to lifestyle modification is superior to lifestyle modification alone for the treatment of NAFLD, at least partially through attenuation of inflammatory markers in the body. This effect was seen beginning at week 14, and this trend was sustained until the end of the study. Whether these effects will be sustained with longer treatment durations remains to be determined.

We are indebted to the 17-Shahrivar Hospital in Amol for providing the equipment used in this study and to M. Fallah, the laboratory specialist who performed all laboratory procedures.

The authors’ responsibilities were as follows—AH: had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis; AH; HP, FZ, RM, and TE: conceived and designed the study and provided administrative, technical, or material support; MS, TE, and AH: analyzed and interpreted the data; TE and AH: drafted the manuscript; AH, HP, FZ, RM, TE, and MS: critically revised the manuscript for important intellectual content; and MS and TE: conducted the statistical analysis; and AH, HP, FZ, and RM: obtained funding and supervised the study. None of the authors had a conflict of interest.

REFERENCES


