

Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study

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ABSTRACT

Background. Probiotics have profound effect on nonalcoholic steatohepatitis (NASH) in animal models. We aimed to test the hypothesis that probiotics treatment was superior to usual care in reducing liver fat in NASH patients. **Material and methods.** Patients with histology-proven NASH were randomized to receive probiotics (n = 10) or usual care (n = 10) for 6 months. The Lepicol probiotic formula contained *Lactobacillus plantarum*, *Lactobacillus deslbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum*. The primary endpoint was change in intrahepatic triglyceride content (IHTG), as measured by proton-magnetic resonance spectroscopy, from baseline to month 6. Secondary endpoints included changes in liver biochemistry and metabolic profile. **Results.** IHTG decreased from $22.6 \pm 8.2\%$ to $14.9 \pm 7.0\%$ in the probiotic group (P = 0.034) but remained static in the usual care group ($16.9 \pm 6.1\%$ to $16.0 \pm 6.6\%$; P = 0.55). Six subjects in the probiotic group had IHTG reduced by more than 30% from baseline, compared to 2 subjects in the usual care group (P = 0.17). The probiotic group also had greater reduction in serum aspartate aminotransferase level (P = 0.008). On the other hand, the use of probiotics was not associated with changes in body mass index, waist circumference, glucose and lipid levels. **Conclusions.** Probiotics treatment may reduce liver fat and AST level in NASH patients. The therapeutic potential of probiotics in NASH should be tested in larger studies.

Key words. Fatty liver. Obesity. Randomized controlled trial. Prebiotics.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases worldwide.¹ Its active form, nonalcoholic steatohepatitis (NASH), may progress to cirrhosis and hepatocellular carcinoma.²⁻⁵ At present, there is no registered drug for the treatment of NASH. While lifestyle management is often advocated, it is difficult to maintain.⁶ It is important to explore new treatment strategies.

The human body contains up to 100 trillion bacterial cells, most of which reside in the gut. Gut microbes interact closely with the human body, and

have substantial effect on energy harvest from food, systemic inflammation and innate immunity.^{7,8} In animal and human studies, altered gut microbiota is associated with obesity.^{9,10} In addition, NASH patients have increased gut permeability and high blood levels of bacterial endotoxin, which results in liver injury.¹¹ In a recent report, changes in the fecal composition of Gamma proteobacteria and *Erysipelotrichi* were observed in 15 women with fatty liver induced by choline-deficient diet.¹² These data suggest that gut microbiota may be involved in the pathogenesis of NASH.

In animal studies, probiotics have profound effect on NASH.¹³⁻¹⁵ Probiotics lead to reduced activity of Jun N-terminal kinase and nuclear factor κ B.¹³ It also increases the number of natural killer T cells in the liver.¹⁴ Although another animal study failed to demonstrate an effect on hepatic steatosis and necroinflammation, probiotics prevented liver fibrosis through changes in the expression of transforming growth factor beta.¹⁶ It is unclear if probiotics are efficacious in human NASH patients.

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Furthermore, prebiotics are non-digestible carbohydrates that alter the composition and activity of the gut microbiota.¹⁷ It reduces de novo fatty acid synthesis, insulin resistance and metabolic endotoxemia.¹⁸⁻²⁰ Although there has been no trials on NASH, prebiotics lower cholesterol and triglyceride levels in humans.²¹

In this study, we aimed to test the hypothesis that probiotics treatment was superior to usual care in reducing liver fat in patients with NASH.

MATERIAL AND METHODS

Study design

This was an open-label, randomized controlled trial in patients with histology-proven NASH. Consecutive patients attending the hepatology clinic of the Prince of Wales Hospital, Hong Kong were screened. Eligible patients were randomized in 1:1 ratio to receive probiotic treatment or usual care for 6 months. Randomization was carried out through the use of computer-generated list of random numbers in blocks of 4. Treatment assignment was kept in sealed envelopes until the time of randomization. All study medications were dispensed by a research nurse. Compliance with the study medication was calculated from the unused sachets of study medications at each visit. The study protocol was approved by the ethics committee of The Chinese University of Hong Kong. All subjects gave informed written consent. While the subjects in both groups did not receive monetary compensation, they received free clinical care and medical tests. The study was registered at ClinicalTrials.gov (NCT00870012).

Subjects

Patients aged 18 to 70 years had histology-proven NASH, defined as hepatic steatosis of more than 5% and the presence of either hepatocyte ballooning or intralobular hepatocyte necrosis on a liver biopsy performed within 6 months before inclusion. Histological scoring was also performed according to the NASH Clinical Research Network system.²² These patients had elevated alanine aminotransferase (ALT) level of over 30 IU/L in men and 19 IU/L in women. We excluded patients with positive hepatitis B surface antigen, antibody against hepatitis C virus, or anti-nuclear antibody titer above 1/160; significant alcohol consumption (over 20 g per day in men or 10 g per day in women); ALT above 10 times the upper limit of normal; liver decompensation or

malignancy; or consumption of systemic corticosteroids or methotrexate in the last 6 months.

Study treatment and follow-up

At baseline, all NASH patients received lifestyle advice from an investigator. They were instructed to lose weight, reduce fat intake, and exercise at least 3 times per week. Patients randomized to the probiotic group also received one sachet of Lepicol probiotic and prebiotic formula (Healthy Bowels Company Ltd, Birmingham, UK) twice a day for 6 months. The formula contained 5 probiotic cultures including *Lactobacillus plantarum* (ATCC 14917), *Lactobacillus delbrueckii* ssp. *bulgaricus* (ATCC 11842), *Lactobacillus acidophilus* (ATCC 4356), *Lactobacillus rhamnosus* (ATCC 7469) and *Bifidobacterium bifidum* (ATCC 29521). Each 10 g sachet contained 200 million probiotic cultures. Other ingredients of the formula included 3 g of fructo-oligosaccharides (prebiotics), cellulose, magnesium stearate, silica and milk.

A research nurse contacted each patient by phone at week 4 to identify new symptoms. Thereafter, the patients were followed up at months 3 and 6. At each visit, new symptoms and concomitant drugs were recorded. Body weight, body height, waist circumference and blood pressure were measured. Waist circumference was measured at a level midway between the lower rib margin and iliac crest with the tape all around the body in the horizontal position. Body mass index (BMI) was calculated as body weight (kg) divided by body height (m) squared. Blood was taken for liver biochemistry, glucose and lipids after fasting for at least 8 h. All biochemical tests were performed using the cobas® 8000 modular analyzer (Roche Diagnostics, West Sussex, England).

Proton-magnetic resonance spectroscopy

Intrahepatic triglyceride content (IHTG) was measured at baseline and month 6 by proton-magnetic resonance spectroscopy using the whole body 3 Tesla scanner (Philips Healthcare, Best, the Netherlands). Whole-body 3.0T scanner with a single voxel point-resolved spectroscopy sequence and an echo time of 40 ms and repetition time of 5,000 ms was used. A survey scan was first performed in the abdominal region to help positioning a volume measuring 20 (AP) x 15 (RL) x 40 (FH) mm within the liver. The scanner's built-in body coil was used for both signal transmission and reception. No-water-

suppressed spectra was acquired using 32 signal averages and the data were exported for offline spectral analysis. Water (4.65 ppm) and lipid (1.3 ppm) peak amplitudes were measured to determine vertebral marrow fat content, which was defined as the relative fat signal amplitude in terms of a percentage of the total signal amplitude (water and fat) and calculated according to the following equation:

$$\text{Fat content} = [\text{Ifat}/(\text{Ifat} + \text{Iwater})] \times 100.$$

Where Ifat and Iwater are the peak amplitudes of fat and water, respectively. Radiologists performing spectral analysis were blinded to the treatment assignment.

Study endpoints

The primary endpoint was the reduction in IHTG from baseline to month 6 as measured by proton-magnetic resonance spectroscopy. Secondary endpoints included changes in ALT, aspartate aminotransferase (AST), BMI, fasting glucose, lipids and liver stiffness measurement by transient elastography. Liver stiffness measurement was performed using the Fibroscan machine (Echosens, Paris, France). The procedure was performed according to the manufacturer's instructions as described previously.^{23,24} Liver stiffness measurements were considered reliable only if 10 successful acquisitions were obtained, the success rate was above 60%, and the interquartile range (IQR)-to-median ratio was below 0.3.

Statistical analysis

Continuous variables were expressed in mean \pm standard deviation, or median (IQR) if they were not in normal distribution. Continuous variables were compared between the two treatment arms using unpaired t test or Mann-Whitney U test. Categorical variables were compared using χ^2 test or Fisher exact test as appropriate. The change in IHTG within group was tested using paired t test. Statistical significance was taken as a two-sided P value of < 0.05 .

This was a pilot study. We considered a reduction of IHTG by 30% to be clinically significant. Assuming that the reduction of IHTG was 30% in the probiotic group and 10% in the usual care group with a standard deviation of 15%, a sample size of 9 patients per arm would achieve 80% power to detect the difference at a 5% significance level. Allowing a 10% dropout rate, a total of 20 patients were required.

RESULTS

From March to September 2009, 20 patients were enrolled and underwent randomization (Figure 1). Ten patients were randomized to the probiotic group and ten patients were assigned to the usual care group. The two groups were well matched, although there was a trend that patients in the usual care group were less obese (Table 1). None of the patients were lost to follow-up. All patients in the probiotic group took at least 80% of the study medication.

Changes in liver fat

At month 6, IHTG by proton-magnetic resonance spectroscopy decreased from $22.6 \pm 8.2\%$ to $14.9 \pm 7.0\%$ in the probiotic group (mean reduction 7.7%; 95% confidence interval 0.7%, 14.7%; $P = 0.034$). In contrast, IHTG remained static in the usual care group at $16.9 \pm 6.1\%$ at baseline and $16.0 \pm 6.6\%$ at month 6 (mean reduction 1.0%; 95% confidence interval -2.5%, 4.5%; $P = 0.55$). The probiotic group tended to have greater reduction in IHTG (Table 2). Overall, 8 subjects in the probiotic group and 4 subjects in the usual care group had reduced IHTG at month 6 ($P = 0.17$) (Figure 2). Six subjects in the probiotic group had IHTG reduced by more than 30% from baseline, compared to 2 subjects in the usual care group ($P = 0.17$).

Biochemical and metabolic response

Similarly, the probiotics group had greater reduction in AST level than the usual care group (Table 2). On the other hand, there was no significant effect of probiotic treatment on the body mass index, waist circumference, glucose and lipid levels, and liver stiffness.

Adverse events

Adverse events occurred in 4 patients in each treatment group (Table 3). All reported adverse events were minor and did not differ between treatment groups.

DISCUSSION

In this proof-of-concept study in human patients with histology-proven NASH, probiotics treatment for 6 months resulted in reduction in liver fat and AST level.

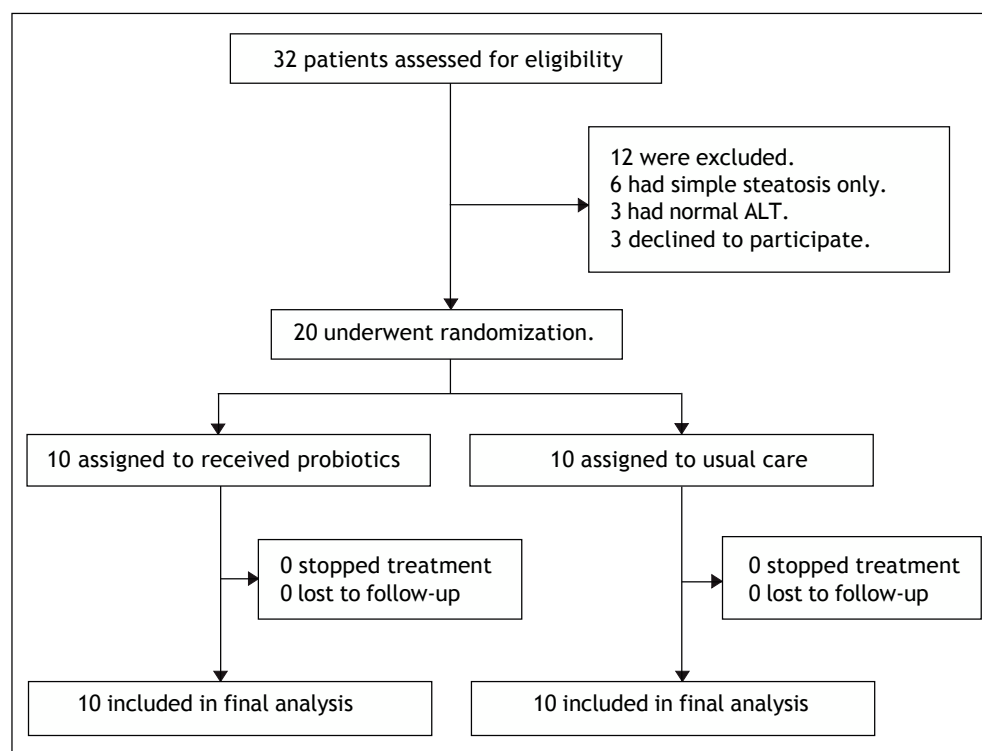


Figure 1. Screening, randomization and follow-up of study subjects.

Table 1. Baseline characteristics of subjects with and without NASH.

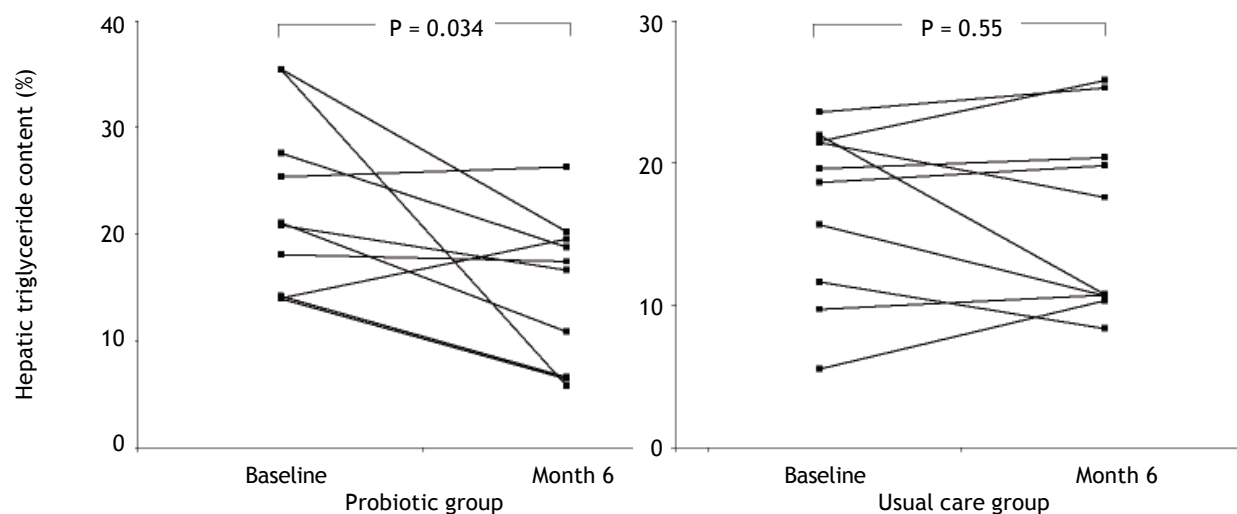
| Characteristics | Probiotic group (n = 10) | Usual care group (n = 10) |
|--|--------------------------|---------------------------|
| Age (years). | 42 ± 9 | 55 ± 9 |
| Male:Female. | 8:2 | 5:5 |
| BMI (kg/m ²). | 30.2 ± 5.0 | 28.7 ± 5.7 |
| Waist circumference (cm). | 101 ± 10 | 97 ± 14 |
| ALT (IU/L). | 96 ± 75 | 72 ± 30 |
| AST (IU/L). | 50 ± 25 | 38 ± 15 |
| Fasting glucose (mmol/L). | 6.1 ± 1.4 | 6.2 ± 1.0 |
| Total cholesterol (mmol/L). | 4.8 ± 0.8 | 5.0 ± 1.0 |
| HDL-cholesterol (mmol/L). | 1.2 ± 0.2 | 1.3 ± 0.4 |
| LDL-cholesterol (mmol/L). | 2.6 ± 0.6 | 2.8 ± 0.9 |
| Triglycerides (mmol/L). | 2.7 (1.4, 3.1) | 1.8 (1.3, 2.6) |
| Diabetes (n). | 3 | 4 |
| Hypertension (n). | 5 | 7 |
| Sulfonylurea (n). | 2 | 1 |
| Metformin (n). | 3 | 4 |
| Thiazolidinedione (n). | 0 | 0 |
| Insulin (n). | 1 | 0 |
| Steatosis grade (1/2/3). | 0/5/5 | 1/5/4 |
| Lobular inflammation (0/1/2/3). | 3/6/1/0 | 2/8/0/0 |
| Ballooning (0/1/2). | 0/10/0 | 0/9/1 |
| Fibrosis stage (0/1/2/3/4). | 3/5/1/1/0 | 3/4/0/1/2 |
| NAFLD activity score. | 4 (4, 5) | 5 (4, 5) |
| Intrahepatic triglyceride content (%). | 22.6 ± 8.2 | 16.9 ± 6.1 |
| Liver stiffness (kPa). | 8.0 (6.2, 12.0) | 8.8 (5.2, 11.5) |

Continuous variables were expressed as mean ± standard deviation or median (interquartile range). ALT: alanine aminotransferase. BMI: body mass index. HDL: high density lipoprotein. LDL: low density lipoprotein. NASH: nonalcoholic steatohepatitis.

Table 2. Changes in hepatic and metabolic parameters between baseline and month 6.

| Parameters | Probiotic group | Usual care group | P* |
|-------------------------------|------------------|-------------------|-------|
| Hepatic triglyceride content. | | | |
| Absolute percentage. | -7.7 ± 9.8 | -0.9 ± 4.9 | 0.065 |
| Percentage of baseline value. | -29 ± 35 | -7.7 ± 9.8 | 0.083 |
| BMI (kg/m ²). | -1.0 ± 2.3 | -0.5 ± 1.1 | 0.57 |
| Waist circumference (cm). | -2 ± 6 | 1 ± 4 | 0.34 |
| ALT (IU/L). | | | |
| Absolute change. | -26 ± 91 | 2 ± 41 | 0.39 |
| Percentage of baseline value. | -13 ± 56 | 15 ± 59 | 0.28 |
| AST (IU/L). | | | |
| Absolute change. | -13 ± 31 | 23 ± 32 | 0.021 |
| Percentage of baseline value. | -16 ± 39 | 37 ± 40 | 0.008 |
| Fasting glucose (mmol/L). | 0.8 ± 2.9 | 0.2 ± 0.7 | 0.51 |
| Total cholesterol (mmol/L). | 0 ± 0.4 | 0.2 ± 0.5 | 0.39 |
| HDL-cholesterol (mmol/L). | 0 ± 0.1 | 0.1 ± 0.1 | 0.14 |
| LDL-cholesterol (mmol/L). | 0.1 ± 0.6 | 0.3 ± 0.5 | 0.50 |
| Triglycerides (mmol/L). | -0.1 (-0.8, 0.3) | -0.3 (-0.5, -0.1) | 0.68 |
| Liver stiffness (kPa). | -0.7 (-2.0, 0) | 0 (-2.5, 0.9) | 0.56 |

Continuous variables were expressed as mean ± standard deviation or median (interquartile range). *Comparison between groups was performed using unpaired t test or Mann-Whitney U test. ALT: alanine aminotransferase. AST: aspartate aminotransferase. BMI: body mass index. HDL: high density lipoprotein. LDL: low density lipoprotein.

**Figure 2.** Intrahepatic triglyceride content at baseline and month 6.**Table 3.** Adverse events.

| Event | Probiotic group (n = 10) | Usual care group (n = 10) |
|----------------------|--------------------------|---------------------------|
| Dyspepsia | 2 | 1 |
| Musculoskeletal pain | 2 | 1 |
| Cough | 0 | 1 |
| Skin rash | 0 | 1 |

The effect of probiotics on NASH has been tested in a number of animal studies. In general, probiotics treatment leads to improvement in hepatic steatosis and necroinflammation.^{13-15,25,26} On the other hand, the effect of probiotics in human NASH patients has not been adequately tested. A non-randomized study on the use of probiotic VSL#3 for 3 months in patients with different liver diseases (22 NAFLD, 20 alcoholic cirrhosis and 36 chronic hepatitis C) showed improved plasma levels of malondialdehyde, 4-hydroxynonenal and S-nitrosothiols.²⁷ Recently, a randomized controlled trial of 28 patients with biopsy-proven NAFLD showed that a mixture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for 3 months led to greater reduction in ALT, AST and gamma-glutamyl transferase levels.²⁸ In our study, probiotics treatment with four strains of *Lactobacillus* and *Bifidobacterium* resulted in significant reduction in IHTG and AST level in patients with biopsy-proven NASH. IHTG by proton-magnetic resonance spectroscopy is currently one of the most accurate non-invasive tests for liver fat.^{1,29} These encouraging results provide foundation for further evaluation of probiotics treatment in larger studies using histological endpoints.

At present, the mechanism of action of probiotics in the treatment of NASH is incompletely understood. In murine models of obesity and fatty liver, probiotics reduce the expression of proinflammatory cytokines and modulate the activity of natural killer T cells in the liver.^{13-15,25} Interestingly, several strains of probiotics also ameliorate gut inflammation in the dextran sulfate sodium colitis model.^{30,31} Along the same line, the bacterial product lipopolysaccharide is increased in the portal blood of animals with colitis.³² In return, this promotes hepatic necroinflammation and fibrosis. Furthermore, NASH patients have increased small bowel bacterial overgrowth and higher blood level of endotoxin.¹¹ In animal models, inflammasome-mediated dysbiosis also regulates progression of NAFLD through influx of toll-like receptors 4 and 9 in the portal circulation and overexpression of tumor necrosis factor- α .³³ These data suggest the close relationship between gut bacteria and the pathogenesis of NASH, and these mechanisms may be modulated by the use of probiotics.

Our study has a few limitations. First, the sample size was relatively small. However, the consistent improvements in IHTG and AST level support further investigation in this area. Second, the results cannot be directly extrapolated to other probiotic formulae. Future studies are required to

identify the roles and actions of individual bacterial strains.

In conclusion, probiotics treatment may reduce liver fat and AST level in NASH patients. The therapeutic potential of probiotics in NASH should be tested in larger studies.

ABBREVIATIONS

- **ALT:** alanine aminotransferase.
- **AST:** aspartate aminotransferase.
- **BMI:** body mass index.
- **IHTG:** intrahepatic triglyceride content.
- **IQR:** interquartile range.
- **NAFLD:** nonalcoholic fatty liver disease.
- **NASH:** nonalcoholic steatohepatitis.

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COMPETING INTERESTS

None declared.

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