Study on the new strategy and key techniques for accurate prevention and treatment of nonalcoholic steatohepatitis based on intestinal target bacteria

Lili Zhuo, MMa, Jiali Xu, MBb, Ningning You, MMc, Liyan Wang, MBd, Yu Song, MMd, Yan Luo, MDe, Junping Shi, MDf.

Abstract
Background: Nonalcoholic fatty liver disease (NAFLD) has emerged as a major health problem worldwide; according to statistics, 10% to 25% of patients with NAFLD can progress to nonalcoholic steatohepatitis (NASH). A link between the composition and metabolites of intestinal microbiota and the development of NAFLD is becoming clearer. It is believed that microbiota factors are driving forces of hepatic steatosis and inflammation. The formulated food that contains prebiotics and dietary fiber may improve NAFLD by altering the intestinal flora and its metabolites.

Methods: The study plan to recruit adult patients (18–75 years, n = 120) with NAFLD, range of alanine aminotransferase is 1.5 to 5 times upper limit of normal (ULN) or liver biopsy is confirmed as NASH. Participants will be randomly allocated into 2 groups: formulized food (n = 80) and a placebo group (n = 40) for 24 weeks. Both groups will receive lifestyle and nutritional advice. The primary endpoint is a decrease in MRS-PDFF by more than 30% from baseline at 24 weeks. The secondary endpoints include the change of anthropometric, liver function, glycolipid metabolism, and systemic inflammation at 4, 12, and 24 weeks. In addition, we consider the changes in intestinal microbiota as an exploration to assess the abundance and diversity at 24 weeks. Weeks 24 to 36 are the follow-up period of drug withdrawal.

Discussion: This clinical trial will provide evidence of efficacy and safety of formulized food as a potential new therapeutic agent for NAFLD patients.

Trial Registration: The trial is registered in the China Clinical Trial Center (ChiCTR1800016178).

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, CK18 = cytokeratin 18, Cr = creatinine, DPP-4 = dipeptidyl peptidase-4, eGFR = estimated glomerular filtration rate, ECG = Electrocardiogram, FIN = fasting insulin, FPG = fasting plasma glucose, GG = γ-glutamyl transferase, GLP-1 = glucagon-like peptide-1, HbA1c = glycosylated hemoglobin A1c, HDG = human chorionic gonadotropin, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis model assessment insulin resistance, HROQL = health-related quality of life, IL-6 = interleukin-6, LDL-C = low-density lipoprotein-cholesterol, NAFLD = nonalcoholic fatty liver disease, NASH = nonalcoholic steatohepatitis, OCA = obeticholic acid, PDDF = proton density fat fraction, SGLT2 = sodium-dependent glucose transporters 2, TC = total cholesterol, TG = triglycerides, TSH = thyroid stimulating hormone, UDCA = ursodeoxycholic acid, ULN = upper limit of normal, WHR = waist to hip ratio.

Keywords: clinical trial, gut microbiota, nonalcoholic fatty liver disease, soluble dietary fiber
1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a chronic progressive liver disease caused by accumulation of intrahepatic fat. With the significant increase in the incidence of obesity and type 2 diabetes, NAFLD has surpassed chronic hepatitis B to become the most common chronic liver disease in China. According to statistics, 10% to 25% of patients with NAFLD can progress to nonalcoholic steatohepatitis (NASH), of which about 20% progress to cirrhosis, and liver cancer has been increasing rapidly in recent years.[1]

The current treatment of NAFLD mainly includes lifestyle intervention: refers to weight control through reasonable diet and exercise. There is evidence that the improvement of liver histology is related to weight loss. When weight loss is 10%, liver histology (steatosis, inflammation, and fibrosis) can be relieved, but due to poor compliance, only 9.9% of patients lose weight within 1 year more than 10%.[2,3] In addition, whether exercise has independent benefits for NAFLD is still inconclusive. Drug therapy: Some drugs such as insulin sensitizers and antioxidants can improve the effect, but they are not approved for the treatment of NAFLD. These drugs have their limitations in treatment. For example, pioglitazone can improve liver steatosis, insulin resistance, and liver enzyme levels, but weight gain of about 3 to 5 kg is the most common adverse reaction, and patients with obvious heart failure also should not be taken, and it is also related to bladder cancer and bone loss after menopause.[4] Human glucagon-like peptide-1 (GLP-1) analogs, such as liraglutide, can suppress appetite, delay gastric emptying, and reduce weight. However, GLP-1 receptors may be downregulated after long-term activation, and the drug may be stopped. Debeounce may occur afterwards. In addition, PIVENS studies have shown that antioxidant vitamin E can significantly improve liver inflammation in nondiabetic-NASH patients, but it is not effective for liver fibrosis and is related to the risk of prostate cancer and hemorrhagic stroke,[5,6] so safety needs further evaluation. Surgical treatment: Bariatric surgery can significantly improve inflammation and ballooning, but it is an invasive operation and is not recommended. Therefore, the actual treatment of NAFLD has not been resolved.

Current studies have shown that the occurrence and development of NAFLD are closely related to the composition and metabolites of the intestinal flora.[7] In obese patients, the diversity and abundance of intestinal microbes have undergone significant changes, prone to overgrowth of small intestinal bacteria, increased intestinal permeability, and increased absorption of endotoxins,[8,9] thereby activating the NF-kB pathway and its related inflammatory pathways increase the possibility of NAFLD formation.[10,11]

It has been projected that dietary factors play a more important role in shaping the composition of the gut microbiota.[12] At present, it is mainly regulated by probiotics, prebiotics, and synbiotics. Prebiotics are defined as “a selective fermentation component that causes a specific change in the composition and/or activity of the gastrointestinal microbiota to confer on a host’s health benefit.”[13] In pig microbiome analysis, high-fiber/low-fat diet is associated with higher concentration of Bifidobacteria, Lactobacilli, and Faecalibacterium prausnitzii, which have a protective role in intestinal inflammation.[14] This phenomenon also exists in a human experimental research that healthy subjects with improved glucose metabolism following fiber supplementation have a higher Prevotella/Bacteroides ratio than those who do not respond to increased fiber.[15]

Fermentation of fiber by gut microbiota yields SCFAs that not only provide energy for the host but also play an immune regulatory role. Recent studies have shown that SCFAs exerts anti-inflammatory and immune effects through the effects on Treg cell expansion/generation via SCFAs-GPCR or their HDAC-inhibiting ability.[16] Prebiotics can also improve intestinal barrier function and alleviate inflammation and insulin resistance associated with obesity by increasing gut hormone release, such as glucagon-like peptides 1 and 2 (GLP-1 and GLP-2), and by regulating endogenous cannabinoid systems.[17,18] In addition, Bomhof et al[19] through liver biopsy follow-up found that the overall NAS score decreased after taking prebiotics.

These results suggest that improving the gut microbiota by taking dietary supplements may be a potential treatment for patients with NAFLD. The most commonly used prebiotics in practice are oligofructose, galacto-oligosaccharide, lactulose and nondigestible carbohydrate inulin, cellulose, resistant starch, hemicellulose, gums, and pectin, while formulated food is rich in prebiotics, mainly oligofructose, isomalto-oligosaccharide, and dextrin. Therefore, we designed a randomized, double-blind, placebo-controlled trial to evaluate the role of formulated food in improving NAFLD.

2. Methods

2.1. Trial design

The aim of the trial is to evaluate the efficacy and safety of formulated food 2 packs per day vs placebo for 24 weeks in NAFLD. During this time, subjects need to visit the hospital regularly and comply with an individualized diet and lifestyle provided by a professional dietitian, at the same time increasing exercise, the frequency is maintained 4 to 5 times a week, 40 minutes each time. Dietary intake at these stages will be recorded by an app for easy management assessment. The study plans to recruit 120 adult NAFLD patients from the Affiliated Hospital of Hangzhou Normal University, randomly assigned.

2.2. Ethical considerations and registration

The study protocol is in line with the Helsinki Declaration and related laws. We conducted an ethical review in accordance with this declaration and regulations, and the trial was approved by the Ethics Committee of the Hangzhou Normal University Affiliated Hospital. The trial was also registered with the China Clinical Trial Center with registration number ChiCTR1800016178. All subjects will sign a written informed consent form and retain a copy before receiving treatment, which have been approved by the qualified Ethics Committee. During and after the study, we will follow the requirements of the Ethics Committee.

2.3. Eligibility

In the NAFLD patients enrolled in this study, we require ALT >1.5ULN or liver biopsy to confirm NASH with a 25 kg/m² < BMI < 35 kg/m². In addition, we excluded patients who had been taking drugs, which can influence ALT in the past 3 months. Detailed enrollment and exclusion criteria are summarized in Tables 1 and 2.

2.4. Study flow and schedule

The flow chart of the study is shown in Figure 1, and the study schedule is summarized in Table 3. For study visits at week 0,
Table 1

| Criteria type | Description of inclusion criteria |
|---------------|-----------------------------------|
| Sex           | Men and women                     |
| Age           | 18–75 yr                          |
| Alcohol consumption | No history of significant alcohol consumption (<70 g/wk for women and < 140 g/wk for men) |
| Body mass index | BMI > 35 kg/m²                    |
| Evidence of NAFLD | ALT > 1.5 ULN at the start of this study, or liver biopsy to confirm NAFLD |
| Blood pressure | Hypertension patients will be required to take a stable antihypertensive drug(s) to keep blood pressure stable (<140/90 mm Hg) 2 mo before randomization, patients may continue to take antihypertensive drug(s) during clinical trials |
| Blood lipid   | If a participant is using a statin or fibrate, he/she will be required to be on a fixed dose to keep lipids stable within 3 mo before enrollment |
| Other drug use | The following medications were not received within 3 mo before enrollment (metformin, thiazolidinediones, hypoglycemic agents, DPP-4 inhibitors, GLP-1, SGLT2 inhibitors, polypeptide phosphatidylcholine, glycyrrhizin preparation, bicyclol, reduced glutathione, S-adenosylmethionine, silymarin, OCA/UDCA, betaine, fish oil, phosphodiesterase inhibitor, gemfibrozil) |
| Informed consent | Understand the study and sign an informed consent form |

Table 2

| Criteria type | Description of exclusion criteria |
|---------------|-----------------------------------|
| Alcohol consumption | Alcohol and/or drugs abuse and dependence over the past 5 yr |
| Liver comorbidities | Patients with cirrhosis |
| Patients with any other acute or chronic active liver diseases such as viral hepatitis, hereditary hemochromatosis, hepatolenticular degeneration, alpha-antitrypsin deficiency, alcoholic liver disease, drug-induced liver diseases, etc |
| Other comorbidities | Known heart failure of New York Heart Association class 2, 3, or 4 |
| Known acceptance of cardiac pacemaker implantation |
| Uncontrolled hypertension |
| With uncontrolled hypothyroidism (serum level of thyroid-stimulating hormone is 2 times higher than the upper limit of normal value) |
| Renal insufficiency, serum creatinine > upper limit of normal |
| Evidence of positive human immunodeficiency virus antigen or antibody (HIV-Ag/Ab) |
| Other | Pregnant or lactating women |
| Female early pregnancy screening test positive, or unwilling to contraception during clinical trials |
| Cannot accept MRI examiners |
| Other investigators believe that clinical trials cannot be completed |
| Those who cannot sign informed consent |

Manager will assign a drug or placebo to each patient during the follow-up period. All test drugs are packaged identically. Until the end of the trial, all subjects and investigators were blinded to the specific allocation of the drug. Adverse events (AEs) will be closely monitored throughout the course of the study and all AEs will be recorded in the case report form. If a serious AE occurs and the study drug is suspected to be a potential cause, the drug manager will only open the assignment information to the doctor in attendance.

2.7. Supply of formulated food and placebo

Only the person responsible for registering the patient knows the distribution of the drug and ensures that the subject is double-blind with the investigator. Formulated food and placebo are indistinguishable in appearance. They are manufactured and supplied by Jintong Special Medical Food Co., Ltd. (Guangzhou, China) and are not commercially available. We generated 120 nonrepeating random numbers through SPSS, and randomly assigned them to the formulated food group and the placebo group. Each number corresponding to a serial number. Subjects get unique serial number according to the time of enrollment that
determines which group he or she is. Double-blind will remain throughout the study and all study data will be kept confidential until all subjects complete the 36-week study.

2.8. Efficacy and safety evaluation

2.8.1. Primary endpoints. The study endpoints are summarized in Table 4. The primary endpoint is a decrease in MRS-PDFF by more than 30% from baseline at 24 weeks. MRS-PDFF and MRI-PDFF are closely related to liver fat content assessed by liver biopsy, with no superiority between them. Preliminary results from a phase II clinical trial confirmed the feasibility of MRI-PDFF in longitudinal observation of clinical trials and highlighted the evidence that liver stiffness by MRI-PDFF is a biomarker of steatosis. In addition, data confirm that changes in MRI-PDFF are associated with hepatic steatosis and overall NAS score response. At the same time, MRI achieves 100% accuracy by a fat fraction threshold of 5.36% to distinguish normal and abnormal fat fractions in liver steatosis testing as long as all known confounding factors are resolved. Therefore, MRS-PDFF and MRI-PDFF potentially replace liver biopsy to assess liver fat content.\[26–28\]

2.8.2. Secondary endpoints. Anthropometrics: This mainly includes body mass index (BMI) and waist-to-hip ratio (WHR). At each visit, body weight and waist and hip circumferences will be measured using a standardized stadiometer and a calibrated electronic scale.

Serum markers: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), fasting insulin, glucose, markers of inflammation (IL-6, CK18), lipid profile (total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride (TG) will be assayed. Insulin resistance (HOMA-IR) was calculated using measurements for fasting glucose and fasting insulin. Hepatocyte apoptosis is a major mechanism of NAFLD progression, and cytokeratin-(CK-)18 is a marker derived from hepatocyte apoptosis produced by caspase3, which is significantly higher in patients with NAFLD than those without NAFLD.\[29,30\] Importantly, in 2 large randomized controlled trials of children and adults, serum CK18 levels were significantly reduced with improved liver histology after treatment.\[31\] These findings suggest that serum CK18 fragments may be an attractive biomarker for monitoring response to different therapeutic agents.\[32\]

Quality of Life: We will use SF-36 Life Scale to assess subjects.

2.8.3. Safety endpoints. The safety of formulated food will be assessed on the basis of AE, clinical laboratory tests, physical

![Figure 1. Study design.](image-url)
examination, and vital signs. Clinical laboratory tests include liver, kidney, urine routine, glucose metabolism, lipid metabolism, electrocardiogram (ECG), and the like.

2.8.4. Exploratory end point. Stool samples: Changes of intestinal flora abundance and diversity at 24 weeks.

The data will be entered by 2 persons and managed by the hospital’s electronic data collection system. They will be kept confidential and only relevant personnel can view them. The data regulator will check them. They are independent of the sponsor.

### Table 4
### Study endpoints.

| Study time point, wk | Treatment period | Follow-up period |
|---------------------|-----------------|-----------------|
|                     | 0               | 4               | 12              | 24              | 36              |
| Study objectives    |                 |                 |                 |                 |                 |
| Primary objectives  |                 |                 |                 |                 |                 |
| Change in liver fat content (MRS/MRI-PDFF) | × | × | × | × | × |
| Change in anthropological indicators (BMI and WHR) | × | × | × | × | × |
| Change in liver function (ALT, AST, GGT) | × | × | × | × | × |
| Change in diabetic factors (FG, FINS, HOMA-IR, HbA1c) | × | × | × | × | × |
| Change in lipids (TG, TC, HDL-C, LDL-C) | × | × | × | × | × |
| Change in IL-6 and CK18 | × | × | × | × | × |
| Change in HRQOL (SF-36) | × | × | × | × | × |
| Safety objectives   |                 |                 |                 |                 |                 |
| Renal function (BUN, Cr, eGFR) | × | × | × | × | × |
| Blood routine       | × | × | × | × | × |
| Urine routine       | × | × | × | × | × |
| Fecal routine       | × | × | × | × | × |
| Coagulation function | × | × | × | × | × |
| TSH                 | × | × | × | × | × |
| Exploratory endpoint|                 |                 |                 |                 |                 |
| Change in intestinal flora | × | × | × | × | × |

All objectives will be compared between formulated food and placebo.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BMI = body mass index, CK18 = cytokeratin 18, Cr = creatinine, eGFR = estimated glomerular filtration rate, ECG = Electrocardiogram, FINS = fasting insulin, FPG = fasting plasma glucose, GGT = γ-glutamyl transferase, HbA1c = glycated hemoglobin A1c, HCG = human chorionic gonadotropin, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis model assessment insulin resistance, HRQOL = health-related quality of life, IL-6 = interleukin-6, LDL-C = low-density lipoprotein-cholesterol, PDFF = proton density fat fraction, TC = total cholesterol, TG = triglycerides, TSH = thyroid-stimulating hormone, WHR = waist to hip ratio.
and have no conflict of interest. The final data can be contacted with the sponsor after the trial is over.

2.10. Sample size estimation

The sample size was calculated using Medcalc version 18.2.1 software. On the basis of the results of the pre-test, we expected the formulated food to improve the ratio by about 50% and about 13% in the placebo group, with an α level of 2-sided type I error of 0.05, and with a β level of type II error of 0.02. The placebo group was calculated to be 35 patients. Because the formulated food group vs the placebo group is 2:1, the total number is about 120 taking into account the 10% shedding rate.

2.11. Statistical analyses

This study will use intent-to-treat patient population. Values with a bias distribution will be log transformed before analysis. Baseline characteristics, categorical variables will be tested by χ² test, and continuous variables will be compared by t test. Repeated measures analysis of variance (ANOVA) will be used for variables measured over time to test treatment efficacy, time, and interaction between them. To compare differences between baseline and 24 weeks, paired t tests will be used for primary and secondary outcomes (liver function biomarkers, diabetic biomarkers, liver fat content, diabetic biomarkers, lipid biomarkers, BMI, WHR). Secondary analysis will be performed in all secondary outcomes (liver function biomarkers, diabetic biomarkers) and interaction between them. To compare differences between baseline and 24 weeks, paired t tests will be used for primary and secondary outcomes (liver function biomarkers, diabetic biomarkers, liver fat content, diabetic biomarkers, lipid biomarkers, BMI, WHR). Secondary analysis will be performed in all secondary outcomes (liver function biomarkers, diabetic biomarkers, liver fat content, diabetic biomarkers, lipid biomarkers, BMI, WHR).

3. Discussion

The dietary supplements have received a lot of attention in recent years. Although some studies have shown that they can improve metabolism, they have not been formally used in clinical practice. The purpose of the double-blind, randomized, placebo-controlled clinical trial is to investigate the efficacy and safety of formulated food in patients with NAFLD. A follow-up plan was made to sequence the stool samples to explore the relationship between intestinal flora, NAFLD, and formulated food. We acknowledge some of the limitations of this research. Inclusion and exclusion criteria are proposed to maximize the safety of enrolled patients, while also reducing the likelihood of extending the results to patients with more severe conditions or those with a higher degree of comorbidity. If formulated food can achieve the desired effect, it will provide a new treatment idea for NAFLD.

Acknowledgments

The authors thank those who collected data or performed the measurement: Qianru Zhu and Jin Chen.

Author contributions

Junping Shi is responsible for conceiving and designing the trial, making the final decision to terminate the trial, and approving the final manuscript. Lili Zhuo and Jiali Xu are drafting the manuscript and in charge of recruitment. Ningning You and Liyan Wang will participate in data collection and analysis. Yu Song and Yan Luo are responsible for supervising the study.

Investigation: Junping Shi.
Methodology: Ningning You.
Software: Liyan Wang.
Supervision: Yu Song, Yan Luo.
Writing – original draft: Lili Zhuo, Jiali Xu.
Writing – review & editing: Lili Zhuo, Jiali Xu.

References

[1] Rinella ME. Nonalcoholic fatty liver disease: a systematic review. JAMA 2015;313:2263–73.
[2] Musso G, Cassader M, Rosina F, et al. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia 2012;55:885–904.
[3] Vilas-Grégoire E, Martinez-Perez Y, Calzadilla-Berton L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology 2015;149:367–78. e365 quiz e314-365.
[4] Musso G, Gambino R, Cassader M, et al. A meta-analysis of randomized trials for the treatment of nonalcoholic fatty liver disease. Hepatology (Baltimore, Md) 2010;52:79–104.
[5] Lewis JD, Ferrara A, Peng T, et al. Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. Diabetes Care 2011;34:916–22.
[6] MacDonald MR, Petrie MC, Home PD, et al. Incidence and prevalence of unrecognized myocardial infarction in people with diabetes: a substudy of the Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes (RECORD) study. Diabetes Care 2011;34:1394–6.
[7] Auberle RE, Herrera V, Chen W, et al. Rosiglitazone and pioglitazone increase fracture risk in women and men with type 2 diabetes. Diabetes Obes Metab 2010;12:716–21.
[8] Levin D, Bell S, Sund R, et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. Diabetology 2015;58:493–304.
[9] Bjelakovic G, Nikolova D, Gluud LL, et al. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 2007;297:842–57.
[10] Gee PT. Unleashing the untold and misunderstood observations on vitamin E. Genes Nutr 2011;6:5–16.
[11] Schürks M, Glynn RJ, Rist PM, et al. Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials. BMJ (Clinical Research ed) 2010;341:c5702.
[12] Klein EA, Thompson IM, Tangen CM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2011;306:1549–56.
[13] Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. Gastroenterology 2014;146:1513–24.
[14] Altamirano-Barrera A, Urbe M, Chavez-Tapia NC, et al. The role of the gut microbiota in the pathophysiology and prevention of liver disease. J Nutr Biochem 2018;60:1–8.
[15] Wigg AJ, Roberts-Thomson IC, Dymock RB, et al. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut 2001;48:206–11.
[16] Soares JB, Pimentel-Nunes P, Roncon-Albuquerque R, et al. The role of lipopolysaccharide and toll-like receptor 4 signaling in chronic liver diseases. Hepatol Int 2010;4:659–21.
[17] Su GL. Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. Am J Physiol Gastrointest Liver Physiol 2002;283:G256–265.
[18] Carmody RN, Gerber GK, Lukevano JM, et al. Diet dominates host genotype in shaping the murine gut microbiota. Cell Host Microbe 2015;17:72–84.
[19] Robereford M, Gibson GR, Hoyle L, et al. Prebiotic effects: metabolic and health benefits. Br J Nutr 2010;104(suppl 2):S1–63.
[20] Miquel S, Martin R, Rossi O, et al. Faecalibacterium prausnitzii and liver diseases. Gastroenterology 2014;146:151–3.
[21] Kovatcheva-Datchary P, Nilsson A, Akrani R, et al. Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of prevotella. Cell Metab 2015;22:971–82.
[22] Koh A, De Vadder F, Kovatcheva-Datchary P, et al. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell 2016;165:1332–45.

[23] Zhu L, Baker SS, Gill C, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–9.

[24] Jandhyala SM, Talakdar R, Subramanyam C, et al. Role of the normal gut microbiota. World J Gastroenterol 2015;21:8787–803.

[25] Bomhof MR, Parnell JA, Ramay HR, et al. Histological improvement of non-alcoholic steatohepatitis with a prebiotic: a pilot clinical trial. Eur J Nutr 2018;58:1735–45.

[26] Meisamy S, Hines CD, Hamilton G, et al. Quantification of hepatic steatosis with T1-independent, T2-corrected MR imaging with spectral modeling of fat: blinded comparison with MR spectroscopy. Radiology 2011;258:767–75.

[27] Idilman IS, Keskin O, Celik A, et al. A comparison of liver fat content as determined by magnetic resonance imaging-proton density fat fraction and MRS versus liver histology in non-alcoholic fatty liver disease. Acta Radiol 2016;57:271–8.

[28] Jayakumar S, Middleton MS, Lawitz EJ, et al. Longitudinal correlations between MRE, MRI-PDFF, and liver histology in patients with non-alcoholic steatohepatitis: analysis of data from a phase II trial of selonsertib. J Hepatol 2019;70:133–41.

[29] Feldstein AE, Wieckowska A, Lopez AR, et al. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology (Baltimore, Md) 2009;50:1072–8.

[30] Wieckowska A, Zein NN, Yerian LM, et al. In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in nonalcoholic fatty liver disease. Hepatology (Baltimore, Md) 2006;44:27–33.

[31] Vuppalanchi R, Jain AK, Deppe R, et al. Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2014;12:2121–2.

[32] Sanyal AJ, Friedman SL, McCullough AJ, et al. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases-U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392–403.