

## UPDATE ON THE EFFECT OF PROBIOTICS AND PREBIOTICS FOR THE TREATMENT OF NON-ALCOHOLIC FATTY LIVER DISEASE: A SYSTEMATIC REVIEW

Sarah El-Nakeep

### ABSTRACT

**Introduction:** Non-alcoholic fatty liver disease (NAFLD) is the accumulation of fat vacuoles in liver cells. It can progress to non-alcoholic steatohepatitis (NASH), which could eventually lead to liver cirrhosis and hepatocellular carcinoma. Until now, there is no effective treatment for these conditions, except managing through modifications in lifestyle and diet (including controlling weight). Several studies have tried to establish the effects of various treatments, but unfortunately the results have been disappointing so far. Prebiotics and probiotics have been shown to improve hepatic steatosis and steatohepatitis through their modulatory effect on the immune system.

**Objective:** The objective of this review is to assess the effectiveness of probiotics and prebiotics in the treatment of NAFLD and NASH.

**Materials & Methods:** A systematic search of relevant literature was conducted on PubMed, the Cochrane Library, Science Direct and MEDLINE databases (for the period 1990 to 2014) using the keywords mentioned below.

**Results:** These include several observations – various prebiotic/probiotic combinations used in different randomized control trials (RCTs) and non-RCTs indicating signs of histological and radiological improvement, a decrease in ALT and AST, and an improvement in lipid profile and insulin resistance – that could justify the use of this therapy on NAFLD/NASH patients.

**Conclusion:** Research in this domain is constantly being updated due to the continuous accumulation of vast data. However, there is need for specifying the type, dose and duration of the probiotic/prebiotic used, and evaluation of its clear impact on the general condition of the patient. Therefore, large-scale RCTs are needed to provide evidence for the generalization of the treatment.

**Keywords:** Non-alcoholic fatty liver disease (NAFLD), fatty liver, non-alcoholic steatohepatitis (NASH), prebiotics and probiotics

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### INTRODUCTION

NAFLD is a disorder that covers an array of diseases from steatosis to steatohepatitis and late cirrhosis. It is characterized by the accumulation of vacuoles of triglycerides in liver cells<sup>1,2</sup>.

The prevalence of fatty liver disease has increased due to the rise in average BMI,

especially in Western countries, where fast food is consumed widely. However, NAFLD and NASH are not chiefly associated with increase in weight in Asian countries; here, these are prevalent in patients with lean body weight. Prevalence is also low in patients of African descent, even with high BMI<sup>3,4</sup>.

Diet and lifestyle modifications are the only proven ways to manage NASH, as weight loss has been found to improve liver histology and cardiometabolic profile<sup>5</sup>. Several treatments have been tried in clinical trials (for example, orlistat, metformin, thiazolidinediones, ursodeoxycholic acid, statins, bariatric surgery,

**Correspondence:** Dr. Sarah El-Nakeep (MD), Internal Medicine Department, Ain Shams University, Cairo, Egypt. Email: sarahnakeep@yahoo.com

vitamin E, probucol, N-acetylcysteine, and low-dose carnitine), but concrete evidence about these being effective is yet to come<sup>1,6</sup>.

Trials on probiotics and prebiotics in humans have yielded some promising results recently, paving way for further research.

## MATERIALS & METHODS

A systematic search of literature was conducted using the following databases for the period 1990 to 2014:

1. PubMed
2. The Cochrane Library (CENTRAL)
3. ClinicalTrials.gov
4. Science Direct
5. MEDLINE

### Proposed Pathophysiology

The NASH hepatocellular injury can be explained by the *double hit hypothesis*; the accumulation of fat is followed by cellular inflammation due to oxidative stress<sup>7, 8</sup>. The mitochondria serve as targets and sources of free radicals, causing swelling, formation of intramitochondrial crystals in NASH patients and increase in reactive oxygen species (ROS)<sup>9</sup>. Ballooning occurs due to the damage to intracellular organelles. This contributes to systemic effects leading to metabolic syndrome<sup>10</sup>.

### Effect of Gut Microbiota on Fatty Liver Disease

The common embryological origin of the liver and intestine justifies the search for a link between intestinal microbiota and NAFLD. Studies confirmed the link between gut permeability caused by small intestinal bacterial overgrowth (SIBO) and NAFLD; also, SIBO can affect the severity of steatosis<sup>11, 12</sup>. At least nine phyla are present in the gut, with *Bacteroides* and *Firmicutes* constituting the bulk in mice and humans (refer to Table 1). Although the gut microbiota tend to stimulate toll-like receptors (TLRs), their low presence in a normal liver prevents inflammation of hepatocytes. On the contrary, in NAFLD, the presence of gut microbiota is high, inducing inflammatory

response that causes the destruction of hepatocytes<sup>7</sup>. High fat diet increases the permeability of the intestine to lipopolysaccharides (LPS), which increase portal endotoxemia, resulting in direct injury to the liver. Also, LPS induce inflammatory response by increasing TLR-2, tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6)<sup>3, 7, 13, 14</sup>.

**Table 1.** Classification of gut microbiota based on gram staining<sup>7</sup>

Gram-Positive Bacteria	Gram-Negative Bacteria	Unclassified
<i>Actinobacteria</i>	<i>Bacteroides</i>	<i>Deferribacteres</i>
<i>Firmicutes</i>	<i>Cyanobacteria</i>	<i>Tenericutes</i>
TM7	<i>Verrucomicrobia</i>	

Short-chain fatty acids (SCFA), produced from fermentation by anaerobic gut microbiota, play an important role in ensuring the proper functioning of epithelial barriers by stimulating mucus production and secretion, reducing intestinal permeability, and improving epithelial protection and survival<sup>15–18</sup>. The SCFA also mediate the secretion of hormones in the glucagon group – peptide GLP-1, peptide YY (PYY) and leptin – causing reduction in appetite<sup>19, 20</sup>. In addition, they prevent inflammation as they inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and TNF- $\alpha$  activation; decrease the release of IL-6 and IL-12; and increase IL-10 and Fiaf response<sup>21–25</sup>.

### Effect of Probiotics/Prebiotics on Fatty Liver Disease

It was found that probiotic treatment in mice with high fat diet (HFD)-induced diabetes reduces mucosal dysbiosis, bacterial translocation and improves glucose metabolism<sup>26</sup>. Overfeeding may lead to decreased intake of dietary fibers and omega 3 polyunsaturated free fatty acid (*n*-3 PUFA), resulting in inadequate nutrition and chronic diseases<sup>27</sup>.

Many animal studies have proposed the theory of the underlying effect of probiotics in NASH induction by the *gut-liver axis*, where the gut microbiota, through their products endotoxin and bacterial DNA, activate the immune system. Prebiotics and probiotics act

by modifying this immune stimulation process<sup>28, 29</sup>.

On the other hand, studies have shown that the composition of the gut bacteria could be altered by diet. However, the identity of the strains did not change, where the carbohydrates decreased *Prevotella*, while protein and fat decreased *Bacteroides*<sup>30</sup>. The *Bifidobacterium* species is responsible for fermenting carbohydrates (for example, glycans, galactans and fructans). These fermentable carbohydrates are in fact prebiotics promoting the *Bifidobacteria*<sup>27, 31</sup>. Increasing the level of beneficial bacteria will induce an anti-inflammatory response and decrease the *Firmicutes/Bacteroidetes* ratio.

Second, prebiotics induce satiety through fermentation of oligofructose by bacteria, resulting in the production of SCFA, and by altering the gene expression of peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) and G-coupled receptors protein, which induces lipolysis, inhibits lipogenesis, and improves insulin resistance. Also, prebiotics will decrease the ghrelin level and increase response to hormones such as leptin, which will decrease food intake<sup>32–35</sup>.

### **Clinical Studies Conducted on Humans to Evaluate the Effect of Probiotics and/or Prebiotics on NAFLD**

This search helped identify nine studies: six double blinded RCTs, two case control studies (where one of the arms contained NAFLD or NASH patients) and one open labeled study that was stopped prematurely after being conducted on only four patients<sup>36–38</sup>.

Different combinations of bacteria were used in these studies.

VSL#3 – A mixture of eight probiotic strains: *Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii subsp. Bulgaricus*; it is the most studied probiotic in NAFLD<sup>39</sup>

LAB – A mixture of *Lactobacillus acidophilus*, *Bifidus*, *Rhamnosus*, *Plantarum*, *Salivarius*, *Bulgaricus*, *Lactis*, *Casei*, *Breve*, fructo-oligosaccharides (FOS) as prebiotic vitamins B6, B2, B12, D3, and C+ folic acid, zinc oxide, ferrous gluconate and potassium iodide, Bio-Flora cps, and Dermo Duemila Italia<sup>28</sup>

Lepicol probiotic formula – Contains *Lactobacillus plantarum*, *Lactobacillus deslbrueckii*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum*

Other new mixtures are still under trial (for example, BioFemale by SOLGAR containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus*, *Lactobacillus casei* and *Streptococcus thermophilus*).

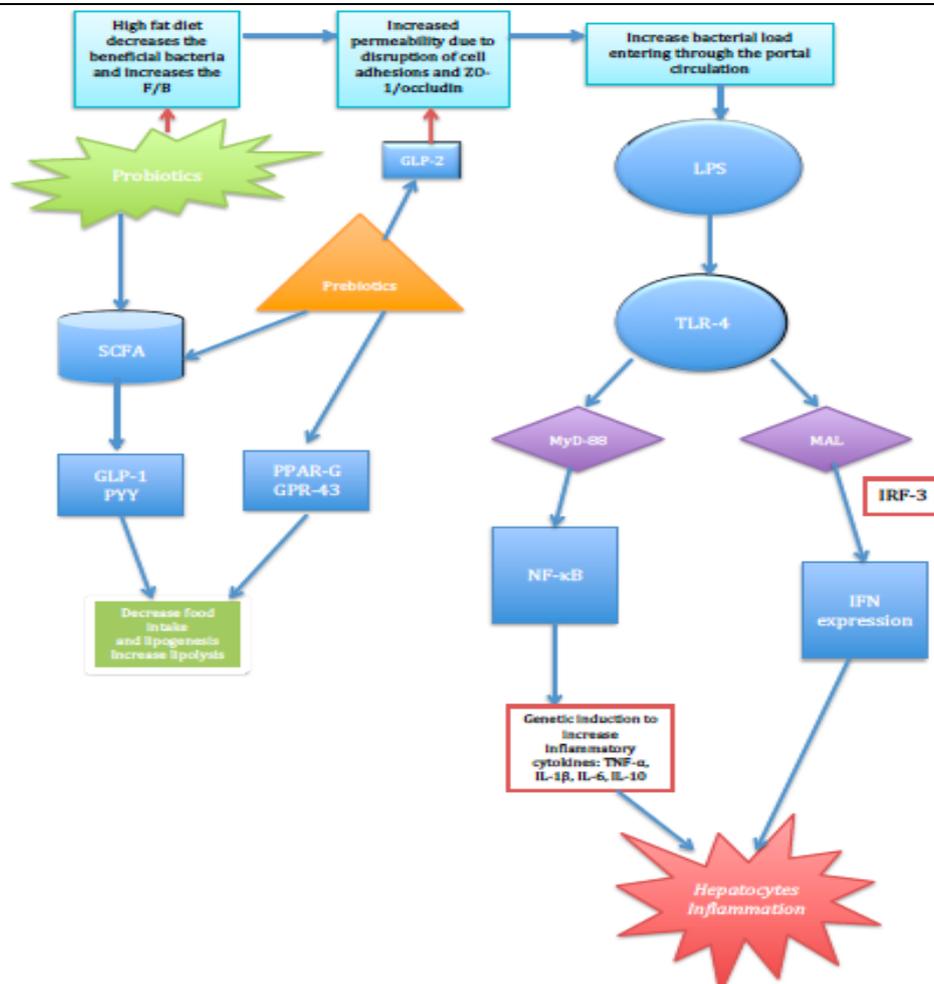
The only prebiotic used in these studies is FOS, either alone or in combination with probiotics.

The most common parameter to determine the efficacy of any drug used for fatty liver disease is its effect on liver enzymes. While this test is noninvasive and feasible, it does not provide solid evidence on the degree of effectiveness of the drug used, as it could be altered by various causes such as other drugs the patient is already on or change in BMI.

No adverse effects were noticed in any of the regimens used, and the drugs were safe.

**Figure 1.** Effect of probiotics and prebiotics on NAFLD (original figure)

GLP-1: Glucagon-like peptide; SCFA: Short chain fatty acids; PYY: peptide YY; GPR-43: G protein coupled receptor 43; NF-KB: nuclear factor kappa-light-chain-enhancer of activated B cells; IFN: Interferon; IRF-3: Interferon regulatory factor 3; MAL: MyD88-adaptation-like; TLR-4: Toll-like receptor 4; LPS: Lipopolysaccharide

**Table 2.** Studies on the effect of probiotics/prebiotics in NAFLD in humans

Type of probiotic/prebiotic	Effect assumed	References
LAB associated to prebiotics (FOS) and vitamins (B6, B2, B12, D3, C and folic acid)	Decreased serum ALT, MDA, 4-HNE and TNF- $\alpha$	Loguerico <i>et al.</i> , 2002 <sup>39</sup>
Oligofructose	Significantly decreased serum AST, moderately decreased ALT, and insulin level; no difference in ultrasound picture; a non-significant decrease in triglyceridaemia	Daubioul <i>et al.</i> , 2005 <sup>43</sup>
VSL#3	In NAFLD and AC groups, improved plasma levels of lipid peroxidation markers: MDA, 4-HNE; in AC patients, improved cytokines (TNF- $\alpha$ , IL-6 and IL-10); S-NO plasma levels improved in all groups	Loguerico <i>et al.</i> , 2005 <sup>37</sup>
VSL#3	A significant increase in liver fat; no significant differences in glycosylated hemoglobin TNF- $\alpha$ , IL-6, interferon $\gamma$ , and height, weight, body mass index and medication use	Solga <i>et al.</i> , 2008 <sup>38</sup>
<i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i>	Decreased serum ALT, AST, Gamma GT	Aller <i>et al.</i> , 2011 <sup>46</sup>
<i>Lactobacillus GG</i>	Significantly decreased serum ALT and antipeptidoglycan-polysaccharide antibodies; no significant changes in BMI z score, visceral fat, TNF- $\alpha$ and ultrasound bright liver parameters	Vajro <i>et al.</i> , 2011 <sup>47</sup>
<i>Bifidobacterium longum</i> + FOS	Significant decrease in AST, LDL cholesterol, CRP, TNF- $\alpha$ , HOMA-IR, serum endotoxin, steatosis and NASH activity index	Malaguarnera <i>et al.</i> , 2012 <sup>42</sup>
Lepicol probiotic and prebiotic formula	Intrahepatic triglyceride content (IHTG) and AST levels decreased; no changes in body mass index, waist circumference, glucose and lipid levels	Wong <i>et al.</i> , 2013 <sup>45</sup>
VSL#3	No between-group differences (groups included: none, light, moderate or severe fatty liver) detected in triglycerides, HOMA and ALT, while BMI decreased and GLP-1 and aGLP1 increased in VSL#3 group	Alisi <i>et al.</i> , 2014 <sup>39</sup>

**Table 3.** Registered trials in ClinicalTrials.gov

Title	The Effect of Probiotics on Non Alcoholic Fatty Liver Disease	Supplementation of Probiotics and Fruit Fibre to Patients With Fatty Liver	The Effect of Good Bacteria on Nonalcoholic Fatty Liver Disease in Diabetics	Treatment of Nonalcoholic Fatty Liver Disease With Probiotics and Prebiotics	The Effect of a Probiotic on Hepatic Steatosis
Sponsor	Rabin Medical Center	Region Skane Collaborator: The Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS)	National Center for Complementary and Alternative Medicine (NCCAM)	Chinese University of Hong Kong	VSL Pharmaceuticals
Registered date	2008	2011	2003	2009	2004
Status	Still not recruiting	Ongoing study	Terminated	Completed	Terminated
Responsible party	Weiss Hemda	Region Skane	Steve Solga	Henry LY Chan	Steve Solga
Drug	<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium bifidum</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus casei</i> , <i>Streptococcus thermophilus</i> .	Dietary Supplement: probiotics and fruit fiber	Probiotic -containing powder	Lepicol probiotic & prebiotic formula	VSL#3
Outcome measured	Lactulose breath test	Improvement in ALT, AST and MRI liver	Not specified	Reduction in hepatic triglyceride content from baseline to week 24	MRI Liver biopsy Blood work
Study phase	Not provided	Not provided	Phases I and II	Not provided	Phase I
Termination date	2015	2018	2006	2012	2010 (Published)

## DISCUSSION

In 2007, a systematic review was published in the Cochrane database for the treatment of NASH by probiotics; no RCTs had been conducted until then<sup>40</sup>. Later, a metaanalysis was done by Ma *et al.*<sup>41</sup> There were only four complete studies until 2013. Now, multiple studies are underway, information on which is available in the ClinicalTrials.gov database, with final results awaited.

Of the nine studies conducted, one used FOS alone, two used it in combination with probiotics, while the rest used probiotics alone<sup>36, 42, 43</sup>. Also, FOS was the only prebiotic used in the nine studies. FOS is an oligomer of beta-D fructose present in onion, asparagus, garlic, artichoke, chicory root, etc. It is fermented in the colon by the gut bacteria as it is not easy to digest and can enhance the growth of beneficial bacterial strains selectively.

Two of the three studies showed an apparent effect not only on aspartate aminotransferase (AST) but also on insulin resistance [detected by homeostatic model assessment insulin resistance (HOMA-IR) or insulin level]; however, the effect on plasma lipids was contradictory. The third study indicated a decrease in alanine aminotransferase (ALT),  $\gamma$ GT and TNF $\alpha$ ; however, the effect on plasma lipids was not assessed.

Two studies were done on patients with chronic liver disease (CLD) having different etiologies, although the arms containing NAFLD and NASH patients were relatively small (22 and 10, respectively)<sup>37, 43</sup>. Similar results of improvement in plasma level lipid peroxidation markers [malondialdehyde (MDA) and 4-hydroxynonenal (4HNE)] were observed; however, TNF $\alpha$ , ALT and  $\gamma$ GT improved only in one study<sup>43</sup> but not the other<sup>37</sup>. This might be

attributed to the usage of different combinations of bacteria (VSL#3 versus LAB) or the difference in the degree of damage to the liver in either condition (greater in NAFLD versus NASH).

Despite being the most popular probiotic used in NAFLD and NASH treatment, VSL#3 gave different outcomes in the three studies. This might be partially explained by the high variation in dosage administered. In a study done by Alisi *et al.*, the authors stated in the editorial comment that the discrepancies between their results and a former study done by Solga *et al.* were due to a tenfold increase in the dosage of the probiotic, which gave more significant results<sup>1, 38, 39, 44</sup>. Other factors also need to be considered, for instance, vast differences in the group of people selected (obese children, adults with chronic liver disease and patients with non-biopsy proven NAFLD)<sup>37–39</sup>. These factors contributed to the huge variations in outcomes measured that made it very difficult to interpret overall results [liver brightness by ultrasound, MDA for obese children, 4-HNE for adults with CLD and noninvasive proton magnetic resonance spectroscopy (<sup>1</sup>H MRS) for patients with non-biopsy proven NAFLD].

Two studies used <sup>1</sup>H MRS as the primary outcome with contradictory results<sup>38, 45</sup>. One indicated a decrease in liver fat, while the other showed an increase, prompting the authors to stop the trial after only four patients. This could be attributed to the usage of different combinations of bacteria (Lepicol probiotic formula versus VSL#3) or selection of patients with different degrees of damage to the liver (NASH confirmed by liver biopsy versus NAFLD where no biopsy was done). Again, improvement was observed in the NASH group, not NAFLD group. This raises the question of relating the effectiveness of the treatment to the degree of inflammation of the liver.

Anthropometric parameters (including BMI, waist and hip circumference, and fat mass) were assessed in a number of studies<sup>39, 45</sup>.

<sup>46</sup>. Only one showed a decrease in BMI, with no change in the others.

Two studies were done on obese children, based on different parameters for the diagnosis of fatty liver, including high level of liver enzymes and liver brightness in ultrasound; only one study used liver biopsy to confirm NASH<sup>39, 47</sup>. Both showed contradictions in ALT results and change in liver brightness in ultrasound after treatment. On the other hand, in certain studies, these groups were assessed exclusively based on parameters, namely, anti peptidoglycan-polysaccharide antibodies PG-PS IgA, glucagon-like peptide GLP-1 and activated GLP-1, where improvement was observed with treatment.

Diet modification in the form of decreased calorie intake only, with or without probiotics, in the children studied by Alisi *et al.* indicated significant improvement in NAFLD<sup>39</sup>. Similar results were observed with lifestyle modification (in the form of diet and exercise together), with or without probiotics and prebiotics, in a study by Malaguarnera *et al.*<sup>42</sup>. Here, the group using probiotics and prebiotics showed a significant decrease in AST, LDL cholesterol, TNF $\alpha$ , CRP, HOMA-IR, serum endotoxin, steatosis, and NASH activity index. This confirms that probiotics/prebiotics play an important role in conjunction with lifestyle modifications.

The problem with the studies conducted is that there is no uniformity in outcomes measured due to the absence of set standards of measurement. Different laboratory and radiological methods were employed to evaluate inflammation (liver enzymes, liver biopsy, ultrasound, <sup>1</sup>H MRI, TNF- $\alpha$  and interleukins). The metabolic markers (HOMA test, insulin level, lipid profile and lactulose breath test) used were different. Even the starting stage of the conditions in patients, based on which they are selected for each study, was different, ranging from simple steatosis to steatohepatitis and fibrosis. The patients studied varied vastly (from obese children to adult patients with CLD, NAFLD and NASH), which made the generalization of results very difficult. Either

the number of patients in these studies was small, or the arm examining NASH or NAFLD was smaller than the other arms (for example, HCV or alcoholic patients).

This indicates the need for a uniform panel to assess the effects of probiotics/prebiotics on NAFLD. It should not only contain markers for fibrosis and inflammation but also metabolic markers; in addition, the procedure should be noninvasive to make it more accessible for studies and patients. Moreover, the results would be more comparable if the groups selected were not very different and had a large number of patients to facilitate generalization of data.

On the other hand, differences in lines of treatment (probiotics and/or prebiotics), bacterial types, combinations (VSL#3, LAB, Lепicol, etc.), concentrations per tablet or sachet, dosages, formulations (capsule, powder or tablets), medium of storage (air tight containers, refrigeration, with certain food, etc.) and duration of treatment can affect the outcome. Furthermore, other factors may interfere such as diet and lifestyle modifications, antibiotic intake and diseases causing congestion of the intestine (for example, heart failure and portal hypertension). Although the safety profile was high, none of the studies were conducted on immunodeficient patients.

This indicates the need for more experience in terms of aspects such as handling the treatment, knowing the best formulation, storage and dosage of the drug, and (therefore) signifies the scope for further research.

## CONCLUSION

Studies in this area are continuously being updated due to the constant accumulation of vast data amid increasing interest in healthy food, and need for further research on the mechanism underlying the effect of pro/prebiotics. The type of bacteria or prebiotic, the dose and the duration of treatment of the probiotic/prebiotic used need to be specified, and their clear impact on the general condition of the patient must be

evaluated. It is important to ensure uniformity in outcomes of the large number of trials. Therefore, further large size RCTs are needed to provide evidence for the generalization of treatment, taking into consideration special population groups (for example, diabetics, cirrhotic patients and immunocompromised persons).

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