

Update 2013: the Role of Probiotic in Non-alcoholic Fatty Liver Disease, an Evidence Based Approach

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ABSTRACT

During the last two decades, non-alcoholic fatty liver disease (NAFLD) has been a topic in many discussions. The major risk factors for NAFLD is metabolic syndrome, which include obesity, insulin resistance and hypertension. Beside insulin resistance, oxidative stress has been linked with the disease.

There is accumulating evidence that intestinal bacterial overgrowth plays an important role in NAFLD pathogenesis. Intestinal bacteria influence the progression of NAFLD through endogenous ethanol production and cytokine that would eventually induce hepatic oxidative stress. Probiotic intervene pathogenic intestinal flora so it is a potential treatment for NAFLD.

Many animal studies documented the beneficial effect of probiotic in NAFLD. Probiotic reduce hepatic inflammation, reduce hepatic steatosis and improve insulin resistance. There is still limited human studies upon this topic. However, preliminary result showed potential role of probiotic in NAFLD treatment. Probiotic is safe, cheap and widely available therefore it is a promising new approach for NAFLD therapy. Upcoming study would hopefully provide firm foundation regarding the use of probiotic for NAFLD on human.

Keywords: NAFLD, probiotic, metabolic syndrome

ABSTRAK

Dalam dua dekade terakhir, perlemakan hati non-alkoholik (PHNA) merupakan topik hangat yang terus diperbincangkan. Faktor risiko dari PHNA meliputi sindrom metabolik, yang meliputi obesitas, resistensi insulin dan hipertensi. Disamping resistensi insulin, stres oksidatif juga diduga berhubungan dengan penyakit ini.

Beberapa penelitian menunjukkan adanya hubungan antara pertumbuhan bakteri usus yang berlebihan dengan kejadian PHNA. Bakteri usus mempengaruhi perkembangan PHNA melalui produksi etanol endogen dan sitokin yang pada akhirnya menyebabkan stres oksidatif pada hati. Probiotik menghambat flora usus patogen sehingga dianggap sebagai terapi potensial untuk PHNA.

Beberapa penelitian pada objek hewan mendokumentasikan efek yang menguntungkan dari probiotik pada PHNA. Probiotik mengurangi inflamasi hati, mengurangi steatosis hati dan memperbaiki resistensi insulin. Penelitian pada manusia mengenai topik ini masih sangat terbatas. Namun beberapa hasil penelitian pendahuluan menunjukkan peran potensial dari probiotik untuk PHNA. Probiotik adalah terapi yang aman, murah dan tersedia secara luas, karena itu merupakan pendekatan yang menjanjikan untuk pengobatan PHNA. Penelitian di masa yang akan datang diharapkan dapat menjadi landasan bukti yang kuat untuk memberikan rekomendasi penggunaan probiotik untuk PHNA pada manusia.

Kata kunci: PHNA, probiotik, sindrom metabolik

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as fat accumulation in the liver exceeding 5% to 10% by weight, but is estimated practically as the percentage of fat-laden hepatocytes observed by light microscopy.¹ The histologic finding of NAFLD encompasses a various degree of inflammation and fibrosis. It is similar to that of alcoholic liver disease but in the absence of significant alcohol intake. If the liver damage continues, it may progress to liver cirrhosis or even hepatocellular carcinoma.^{2,3,4}

Certain metabolic risk factors have been strongly linked to NAFLD. The association between obesity, diabetes mellitus (insulin resistance) and dislipidemia with NAFLD has been well documented.⁵ NAFLD affects 10% to 24% of the general population and the number increases up to 74% in obese population.⁶ Besides insulin resistance and oxidative stress, intestinal flora is thought to cause NAFLD. Although the exact mechanism remains obscure, small intestinal bacterial overgrowth (SIBO) is postulated to induce inflammation through the production of cytokine.⁷

Therapy for NAFLD has been a field of active research. There were multiple agents that were proved to be beneficial when given to NAFLD patients. These agents targeted certain process in the pathogenesis of NAFLD. Insulin sensitizer (metformin, thiazolidinediones), vitamin E, betaine, ursodeoxycholic acid are some agents that have been studied. Besides improving the liver transaminase enzymes profile, some of these agents even have the capability of improving the stage of fibrosis. However there is still no proven effective therapy for NAFLD to date.^{3,8,9,10}

As mentioned briefly in previous paragraph, pathogenic intestinal flora is one potential culprit of NAFLD progression. Probiotics seems a promising therapy for NAFLD treatment through the enhancement of commensal intestinal flora. Probiotics' beneficial effect has been widely accepted for the management of diarrheal disease. However, evidence on the role of probiotics in NAFLD is still limited.¹¹ This review would provide evidence based information regarding the role of probiotic in NAFLD.

THE PATHOGENESIS OF NAFLD

The specific etiology of NAFLD remains an unresolved question. However "multiple hits" has been the leading theory in the pathogenesis of NAFLD. According to this theory, the initial insults (hits), in this case insulin resistance leads to hepatic steatosis.

The first hit made liver cells vulnerable to the second insults, which is oxidative stress. The latter eventually kills hepatocytes, promotes the accumulation of inflammatory cells and further worsen the condition which contribute to the occurrence of non-alcoholic steatohepatitis (NASH). After years of chronic hepatic inflammation, some NASH patients develop further fibrogenic response and could progress to cirrhosis.^{3,7}

The First Hit: Insulin Resistance

A combination of genetic and environment factors contribute to insulin resistance. Insulin resistance plays a central role in the pathogenesis of NAFLD. Insulin resistance causes multiple lipid metabolism disorders, which include the accumulation of triglycerides in hepatocytes as a result of more fatty acids being synthesized, more free fatty acids being delivered to the liver, less fatty acids being degraded, and less triglycerides being released from the liver.⁷

Excessive amount of free fatty acids may be deleterious for the liver through a variety of mechanism, including de novo synthesis of ceramides, which may cause apoptosis, further contributes to insulin resistance by interfering with intracellular phosphorylation process and lipid peroxidation. Chronic hyperinsulinemia also promotes hepatic lipogenesis through upregulation of lipogenic transcription factors and activate profibrotic cytokines such as connective tissue growth factor, which finally contribute to liver fibrosis.^{5,7}

The Second Hit: Oxidative Stress

Mitochondrial dysfunction appears to play a critical role in the second hit. There are many etiologies of oxidative stress, some of which are oxidation of fatty acids and iron load. Fatty acids could induce the progression from simple steatosis to steatohepatitis through oxidative stress production. TNF- α also contributes to the second hit in NASH pathogenesis through its effect on mitochondrial radical formation which would finally promote cell death.^{12,13} Intestinal flora also plays an important role in cytokine production, as will be further explained.¹⁴

Hepatic oxidative stress would activate IKB Kinase β (IKK β). IKK β activation would result in the phosphorylation of insulin receptor substrate (IRS)-1, thereby causing disruption of insulin-related intracellular signaling and would ultimately result in insulin resistance. As can be seen, although there are two distinct "hits" in the pathogenesis of NAFLD, there are some overlaps along the process (Figure 1).⁷

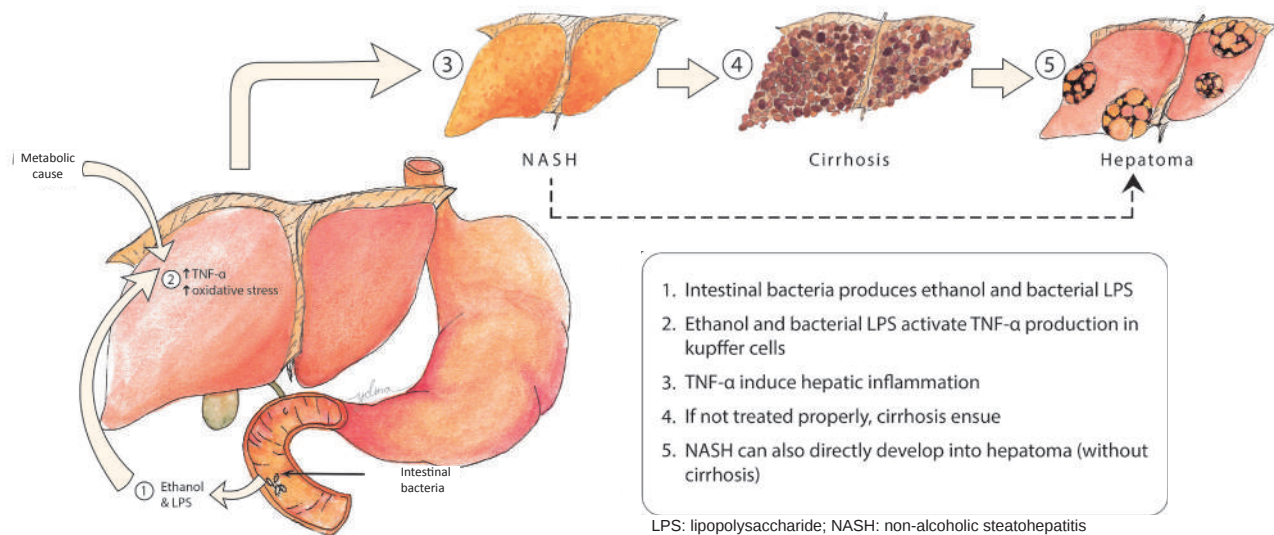


Figure 1. The pathogenesis of non-alcoholic fatty liver disease⁷

THE ROLE OF INTESTINAL BACTERIA OVERGROWTH IN NAFLD

As a matter of fact, the association between gut flora and liver disease has long been established. This association is not limited only to NAFLD. The practise of using lactulose for the treatment of hepatic encephalopathy suggests the involvement of gut flora in the management of liver disease. The association between gut flora with hepatic disease is popularly known as gut-liver axis. The understanding of the gut-liver axis will provide new opportunities for the management or even prevention of NAFLD.^{11,15}

There are emerging evidence about the association between intestinal bacterial and fatty liver. The relationship between intestinal flora and alcoholic fatty liver disease has long been elaborated. When ethanol-fed rats are given neomycin (to partially decontaminate the gut) or polymixin (to bind lipopolysaccharide/LPS) or lactobacillus (to modify intestinal flora), they are protected from alcohol-induced liver damage. This protective effect is the result of reduced hepatic exposure to intestinal products, such as LPS, that induce the release of TNF- α from hepatic macrophage. As previously explained, TNF- α is the cytokine that promote cell death in the liver.¹⁴

It is logical to assume that similar effect of intestinal flora and NAFLD also exist, since TNF- α is also the key mediator in this disease. Currently there are two mechanisms by which intestinal flora may increase hepatic oxidative stress. The first one is increased endogenous ethanol production, and the second is direct activation of inflammatory

cytokines in luminal epithelial cells, non-parenchymal liver cells (macrophages), or both via release of lipopolysaccharide (LPS). Both ethanol and bacterial LPS can activate TNF- α production in Kupffer cells and thus induce hepatic inflammation, oxidative stress and fibrosis.^{14,16}

The role of endogenous ethanol production in NAFLD was initially studied in experiments with mice. The administration of neomycin significantly reduces endogenous ethanol production. This finding demonstrate a direct link between intestinal bacteria with ethanol production, and suggest that treatment of bacterial overgrowth might reduce the harmful effect of endogenous ethanol in NAFLD.¹⁴

While human experiment on this particular topic is still limited, one study noteworthy to know is a study by Wigg et al.¹⁷ According to this study, patients with NASH have a higher prevalence of small intestinal bacterial overgrowth. Small intestinal bacterial overgrowth was present in 50% of patients with NASH and 22% of control subjects ($p = 0.048$). Mean TNF- α levels in NASH patients and control subjects were 14.2 and 7.5 pg/mL, respectively ($p = 0.001$). The level of endotoxin and intestinal permeability were similar in the two groups. This finding further supported the role of bacterial overgrowth in NAFLD, however failed to demonstrate a statistically significant correlation between TNF- α and endotoxin levels. Nevertheless, since it is a small scale study, the result need to be interpreted with cautious and future study with larger sample would hopefully provide valuable information concerning this topic.¹⁷

PROBIOTICS IN NAFLD

As intestinal bacteria plays a significant role in NAFLD, one obvious way to control the development of NAFLD is by manipulation of the intestinal bacteria. Currently, the manipulation of intestinal bacteria can be done by the administration of antibiotics and probiotics. Antibiotics seems a logical choice since this agent can decontaminate intestinal bacteria. The efficacy of antibiotics has been proven in a mice model. The administration of metronidazole and tetracycline reduced hepatic injury as shown by the decreased hepatic aminotransferase. However, long term use of antibiotics may cause bacterial resistance. Not to mention the side effects when these antibiotics are taken in long term.^{14,18}

Competitive inhibition of pathogens by probiotics alters their inflammatory effects in intestinal bacterial overgrowth. The beneficial effects of probiotic administration in NAFLD have been documented in multiple animal studies. According to Ma et al, the administration of probiotic improved insulin resistance and steatosis in mice with diet-induced steatosis.¹⁹ Xu et al performed a study on a rat model of NAFLD and they also documented the beneficial effect of probiotic in reducing hepatic fat accumulation.²⁰

Li et al also concluded that the administration of probiotic named VSL#3 (mixture of bifidobacteria,

lactobacilli and streptococcus thermophilus) significantly reduced liver inflammation and slightly reduced hepatic steatosis. VSL#3 also decreased the DNA binding activity of nuclear factor κ B (NF- κ B), the target of IKK β , another TNF-regulated enzyme that causes insulin resistance.²¹ Esposito et al, also found that VSL#3 reduced the level of TNF- α in NAFLD rats.²² All these animal studies agrees that probiotics are beneficial for NAFLD. Probiotics reduced hepatic inflammation, reduced hepatic steatosis and improved insulin resistance.

There are multiple pathways by which probiotics alter the progression of NAFLD. Probiotic may eradicate directly the pathogenic bacteria in the intestine. Probiotic also reduces bacteria ethanol production and reduces the inflammatory effect of intestinal bacteria through alteration in cytokine signaling (Figure 2).^{14,23}

The beneficial effect of probiotic in other digestive disease like diarrheal and inflammatory disease has been studied in many human trials.¹¹ However from recent literature searching, there is still limited clinical trial evaluating probiotic treatment involving human subject with NAFLD. A result from one pilot study seems promising though. Aller et al documented that a tablet containing 500 million of lactobacillus bulgaricus and Streptococcus thermophilus improved

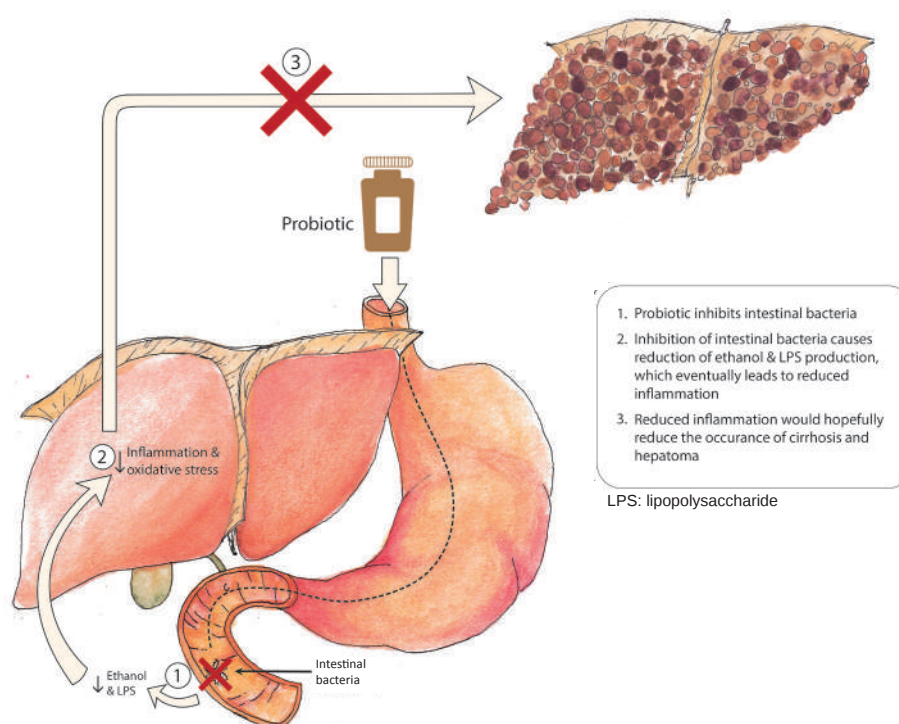


Figure 2. Probiotics role in the treatment of non-alcoholic fatty liver disease through its effect on endogenous ethanol production and LPS¹⁴

Table 1. Existing studies on the role of probiotic in NAFLD^{19,20-25}

Study	Population	Intervention	Duration of treatment	Outcome
Ma X et al ¹⁹	Mice with high fat diet	VSL#3 probiotics	4 weeks	Oral probiotic treatment significantly improved the high fat diet-induced hepatic NKT cell depletion, insulin resistance and hepatic steatosis
Xu RY et al ²⁰	Rat with high fat diet	<i>Bifidobacterium longum</i> and <i>Lactobacillus acidophilus</i>	12 weeks	Oral supplementation with probiotics attenuates hepatic fat accumulation. <i>Bifidobacterium longum</i> is superior in terms of attenuating liver fat accumulation as compared to <i>Lactobacillus acidophilus</i>
Li et al ²¹	Mice with high fat diet	VSL#3 probiotics	4 weeks	Treatment with VSL#3 improved liver histology, reduced hepatic total fatty acid content, and decreased serum ALT levels.
Esposito E et al ²²	Rat with high fat diet	VSL#3 probiotics	4 weeks	VSL#3 administration could limit oxidative and inflammatory liver damage in rats with high fat diet. In rats treated with VSL#3, liver TNF α levels, MMP-2 and MMP-9 activities, and expression of iNOS and COX-2 were significantly lower than in the group without intervention
Aller R et al ²⁴	Human with NAFLD	<i>Lactobacillus bulgarius</i> and <i>Streptococcus thermophilus</i>	3 months	Probiotic administration improved liver aminotransferase level in patients with NAFLD
Loguercio et al ²⁵	Human with NAFLD	VSL#3	4 months	Probiotic administration improved oxidative stress and lipid peroxidation, as shown by the improvement of two parameters such as: malondialdehyde and 4-hydroxynonenal

VSL#3 probiotics: a mixture of viable, lyophilized *bifidobacteria*, *lactobacilli* and *Streptococcus thermophilus*; NKT cell: natural killer and T cell receptors cell; ALT: alanine aminotransferase; TNF- α : tumor necrosis factor- α ; MMP-2: matrix metalloproteinase-2; MMP-9: matrix metalloproteinase-9; iNOS: inducible nitric oxide synthase; COX-2: cyclooxygenase-2; NAFLD: non alcoholic fatty liver disease

liver aminotransferase levels in patients with NAFLD.²⁴ Table 1 summarizes existing studies on animal and human subject concerning the role of probiotic in NAFLD.

Along with the progress of our knowledge about NAFLD, new treatments targeted at certain level of the disease would emerge. Lifestyle modification remains the first line treatment for NAFLD. In recent years, some potential NAFLD treatments have been the topic of discussion and study, one of which is probiotic. Not only probiotic is inexpensive, it is also safe and widely available, so the administration of this agent for NAFLD holds a promising future. Based on the efficacy and safety profile of probiotics, it can be an option for NAFLD treatment. However, limited data on human use made it unwise to give a supportive recommendation from an evidence based perspective, but neither is it opposed.

Existing human trials are small scale trials with limited period of follow up, therefore larger clinical trial with longer follow up is needed.^{24,25} There are some questions need to be addressed in future research. First, the strain of probiotic bacteria that would be best for NAFLD treatment. Second, the amount of probiotic needed to produce significant effect among NAFLD patients.

CONCLUSION

As our understanding about the pathogenesis of NAFLD advances, new insight regarding potential treatment for this disease would ultimately emerge. There are accumulating evidences that probiotic is a new approach in the treatment of NAFLD. The characteristic of probiotic is suitable for NAFLD treatment. All existing studies provide favorable result for the use of probiotic in NAFLD treatment.

However up to this time, there is still limited data on the use of probiotic for NAFLD on human. So, evidence-based recommendation cannot be decided yet. This review hopefully provides new information and triggers upcoming study that would eventually become solid evidence for wide implementation in clinical practice.

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