

Systematic Review

Fecal Microbiota in Non-Alcoholic Fatty Liver Disease and Non-Alcoholic Steatohepatitis: A Systematic Review

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Abstract

Background: With the increasing prevalence of obesity, non-alcoholic fatty liver disease (NAFLD), has become a frequent cause of chronic liver disease, often leading to cirrhosis. In recent decades, gut microbiota have been evaluated as an effective factor in NAFLD pathogenesis, causing steatohepatitis by involving the host immune system. The aim of this study is to evaluate gut microbiota dysbiosis in NAFLD/NASH patients in comparison to healthy controls.

Methods: We conducted a systematic search of published studies that have examined the composition of gut microbiota in relation to NAFLD. PubMed, Scopus and ISI Web of Science were searched. After the exclusion of irrelevant studies, 15 eligible studies were included and summarized.

Results: Overall, some studies reported the composition of microbiota at the phyla level, while others reported them at smaller subgroups; the results of studies were contradictory in some cases.

Conclusion: Overall, study findings indicate a relationship between microbial composition and NAFLD. Study methods and sequencing techniques influenced these results.

Keywords: Fecal microbiota, Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis

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Introduction

Non-alcoholic fatty liver disease (NAFLD), a common disease worldwide, ranges in severity from simple steatosis to non-alcoholic steatohepatitis (NASH). This disease can advance and cause serious complications such as cirrhosis of the liver. About 20%–30% of individuals with NAFLD go on to develop NASH and subsequently liver cirrhosis, with over 80% of the liver becoming nonfunctional. NAFLD is caused by genome-environment interactions, with diet, hormonal imbalance and epigenetics having major involvement.^{1,2}

Different hypotheses regarding NAFLD development have been proposed over the years, forming the idea that NAFLD is a multi-factorial disease.^{3,4} Of course, the most important factor associated with NAFLD/NASH development is obesity. However, one special topic that has gained much attention over the past few decades is the role of gut microbiota in liver diseases pathogenesis.

Microbes are present in many body surfaces and compartments. Contrary to beliefs about them not being present in sterile environments, some evidence is emerging

about microbiota in blood.⁵ It is estimated that the human body contains over 10 000 microbial species,⁶ the most diverse of which can be found in the gut.⁷

Microbes are highly involved in the development of many diseases, especially through dysbiosis, or their imbalance. One liver disease known to be affected by microbiota dysbiosis is NAFLD.^{8–10} Furthermore, recent studies on animals and humans suggest a link between metabolic disorders such as metabolic syndrome, diabetes, obesity etc. and gut microbiota imbalance.¹¹ Several animal studies have also demonstrated an association between microbial dysbiosis and development of NAFLD; microbiota transplantation experiments in mice suggest that certain microbiota are capable of inducing obesity and NAFLD independent of other environmental factors.¹²

Previous studies have shown that the composition of gut microbiota varies in lean and obese individuals. In overweight/obese individuals, following the same diet as leans, *Firmicutes* and *Bacteroidetes* can be found in increased and decreased levels, respectively. Various studies evaluating microbiota in NAFLD patients have shown

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that *Bacteroidetes* decrease in this patient population, while *Prevotella* and *Porphyromonas* levels increase compared to healthy controls.^{13,14}

The reason why gut microbiota can affect liver function lies in the close anatomical and physiological relationship between the liver and the gastrointestinal (GI) tract, also known as the gut-liver axis. The liver and the GI tract interact through the portal circulation, where all metabolites and immunological factors retrieved from the gut enter the liver for detoxification.^{15,16}

Through this process, microbiota enter the liver as well. Under normal conditions, a small amount of microbiota enters the liver, all of which is removed by Kupffer cells. However, any disruption in the mucosal lining of the intestine, or at times of high portal vein pressure, large amounts of microbiota enter the liver. While Kupffer cells and hepatic stellate cells are activated, inflammatory cytokines are also released, prolonged exposure to which causes liver damage. Any factor disrupting gut mucosal lining, such as antibiotic use, special diets, etc. can cause this cascade of events, leading to liver damage.¹⁷

In a systematic review aiming to evaluate the relationship between microbiota dysbiosis and NAFLD, a wide range of findings were reported, while evaluating possible pathways in the gut-liver axis.¹² However, since the study of microbiota is a rapidly evolving field with many other articles having been published since the last review, the current systematic review was generated to evaluate gut microbiota dysbiosis in NAFLD/NASH patients in comparison to healthy controls.

Methods

Eligibility Criteria and Information Sources

In order to conduct the present systematic review, we followed the Preferred Reporting Items for Systemic Reviews and Meta-Analysis statement (PRISMA) guidelines. The following items were established as the inclusion criteria:

- Study design: cross-sectional, case-control and cohort studies.
- Participants: Case: individuals diagnosed with NAFLD/NASH using different methods of detection, including imaging techniques (fibroscan, MRI, abdominal ultrasound or CT scan) or biopsy. Controls: population-based or hospital-based controls without NAFLD/NASH or related liver diseases.
- Main outcome reported: major phylum, class, order, family, genus and species of fecal microbiota.
- Publishing time cutoff: November 17, 2018.

Studies with the following features were excluded:

- Studies including animal subjects.
- Any interventional trials.
- Studies without a healthy control group.
- Gray literature, including conference papers.

In a comprehensive search, the PubMed, SCOPUS and

ISI Web of Science databases were searched. Additionally, the references of all selected articles were manually reviewed. The syntax used in the PubMed database is presented as follows. This syntax was adjusted for the other two databases.

((Metagenome*[tiab] OR microbiota *[tiab] OR (stool[tiab] AND microbi*[tiab]) OR (fecal[tiab] AND flora[tiab]) OR (intestinal[tiab] AND flora[tiab]) OR (intestinal[tiab] AND bacteria[tiab]) OR (gut[tiab] AND flora[tiab]) OR (gut[tiab] AND bacteria[tiab]) OR microbiot*[tiab] OR (feces[tiab] AND microflora[tiab]) OR (colon[tiab] AND flora[tiab]) OR dysbiosis[tiab] OR dysbioses[tiab] OR disbiosis[tiab] OR disbioses[tiab] OR (bacterial[tiab] AND overgrowth[tiab]) OR (bacterial[tiab] AND translocation[tiab]) OR dys-symbiosis[tiab] OR "Dys symbiosis"[tiab] OR dys-symbioses[tiab] OR dysbacteriosis[tiab] OR dysbacterioses[tiab] OR disbacteriosis[tiab] OR disbacterioses[tiab] OR (bacterial[tiab] AND overgrowth[tiab]) OR probiotic*[tiab] OR prebiotic* OR symbiotic OR synbiotic) AND (nafl[tiab] OR nash[tiab] OR (non-alcoholic[tiab] AND steatohepatitis[tiab]) OR ("non alcoholic"[tiab] AND steatohepatitis[tiab]) OR (nonalcoholic[tiab] AND steatohepatitis[tiab]) OR (nonalcoholic[tiab] AND fatty[tiab] AND liver[tiab]) OR ("non alcoholic"[tiab] AND fatty[tiab] AND liver[tiab]) OR (non-alcoholic[tiab] AND fatty[tiab] AND liver[tiab]) OR (nonalcoholic[tiab] AND liver[tiab]) OR (non-alcoholic[tiab] AND liver[tiab]) OR ("non alcoholic"[tiab] AND liver[tiab]) OR (liver[tiab] AND steatosis[tiab]) OR (hepatic[tiab] AND steatosis[tiab]) OR (fatty[tiab] AND liver[tiab]) OR (fatty[tiab] AND livers[tiab]) OR steatohepatitis[tiab] OR (gut[tiab] AND liver[tiab] AND axis[tiab]) OR (hepatocyte[tiab] AND steatosis[tiab]) OR (hepatocytes[tiab] AND steatosis[tiab]) OR (liver[tiab] AND lipid[tiab]) OR (liver AND lipids) OR (visceral[tiab] AND steatosis[tiab]) OR (Steatoses[tiab] AND Visceral[tiab]) OR (liver AND fatty AND degeneration) OR (nonalcoholic[tiab] AND steatohepatitides[tiab]) OR (Steatoses AND Liver)) AND 1913/01/01:2018[dp])

Study Selection and Data Extraction

After removing duplicate results, the abstracts of all articles were screened for eligibility by two independent reviewers (ZM and NMG). Afterwards, the full-texts of relevant articles were retrieved and thoroughly reviewed by both reviewers, separately. In case of non-reported data, the authors of original studies were contacted via their email address. Inconsistencies between the reviewers were directed to a third (senior) author (SE). Author ZM extracted all relevant data, including study design, number of participants, the method of NAFLD diagnosis and basic patient characteristics (age and gender). Extracted outcomes comprised gut-related factors, including

microbiota composition, poly-unsaturated fatty acids (PUFAs), volatile organic compounds (VOCs) and fecal bile acids.

Risk of Bias (Quality) Assessment

Methodological quality assessment was performed by two independent reviewers using a modified STROBE checklist.¹⁸ Disagreements were resolved by a third independent reviewer.

Results

This systematic review yielded 15 original human studies evaluating the relationship between fecal microbiota and NAFLD/NASH (Figure 1). All studies met the quality assessment requirements based on the modified STROBE checklist, and therefore, none were excluded from this review. No human studies were retrieved before 2013. Study characteristics are provided in Table 1.^{14,19-32} Biodiversity (alpha diversity and beta diversity), a characteristic often evaluated in studies regarding microbial dysbiosis, was reported in eight studies, three of which found alpha diversity to be significantly decreased in individuals with NAFLD.^{25,30,32} The results from all the studies found are summarized below.

Overall, different molecular methods were used by

researchers such as group-specific real-time polymerase chain reaction (RT-PCR), denaturing gradient gel electrophoresis (DGGE), and sequencing of the 16S rRNA genes. With the exception of one study, all microbiota analyses were performed in adults. Fifty-three percent of studies were performed in North America, and the rest in Asia.

Microbiota Differences in Studies

The first study found was published by Zhu in 2013, in which 16S rRNA pyrosequencing was used in three groups of obese (with or without NASH), healthy, and NASH-diagnosed children. Evaluating the microbiota composition showed a significant difference at the phylum, family and genus levels between the obese and healthy groups. The observed difference between the obese and NASH groups was smaller and significant differences were only seen among taxa prevalent at amounts greater than 1% in each group: Proteobacteria phylum, *Enterobacteriaceae* family and *Escherichia* genus. Firmicutes and Bacteroidetes were the two dominant phyla in all three groups. The amount of these phyla in the NASH and obese groups were similar but there was a significant increase in the amounts of Bacteroidetes and a decrease in Firmicutes in those two groups in comparison

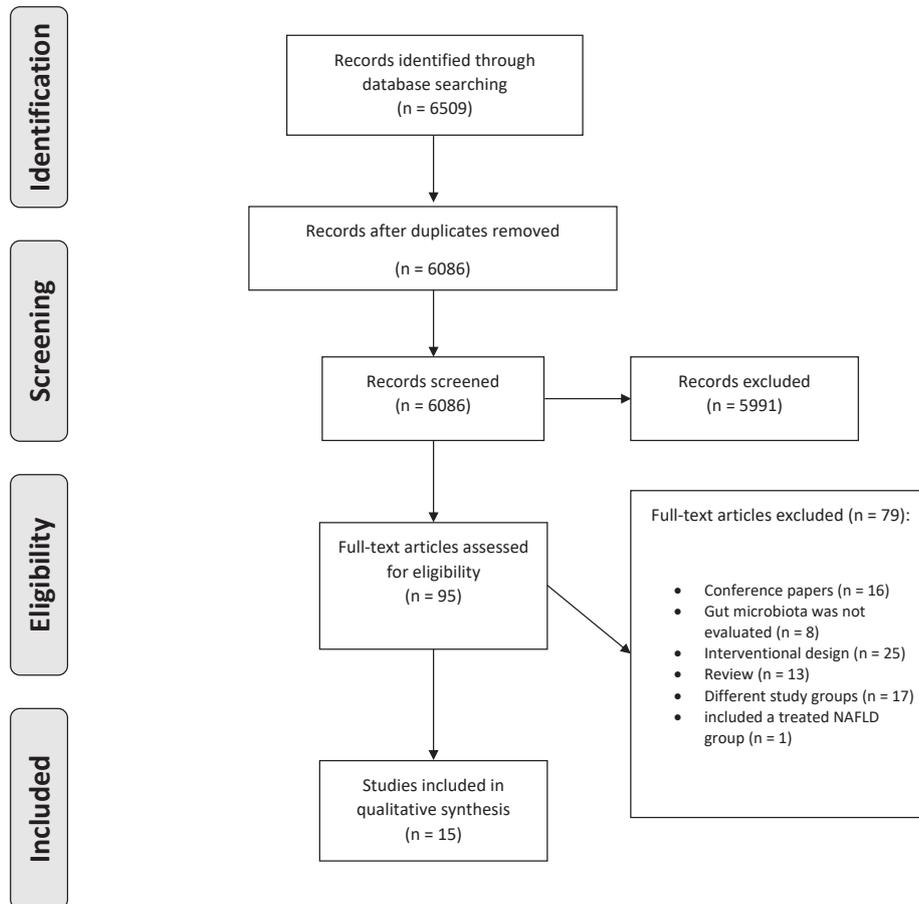


Figure 1. Flow Diagram of the Literature Search.

Table 1. Characteristics of Human Studies Found in the Systematic Review Regarding Microbial Dysbiosis and NAFLD/NASH

Name and Year of Publication	Location	Type of Study	Study Participants	NAFLD/NASH Diagnosis Method	Microbiota Analysis Method	Differences at Phylum Levels	Differences at Class, Order, Family, Genus, Species levels
Zhu L, 2013 ¹⁴	USA	Case-Control	22 NASH, 25 Obese, 16 Controls	Biopsy	16S rRNA Pyrosequencing on a 454-FLX-Titanium Genome Sequencer	<p>NASH: Bacteroides†, Firmicutes↓ Actinobacteria↓</p> <p>Obese: Bacteroides†, Firmicutes↓</p> <p>Control: Proteobacteria↑</p>	<p>NASH: Prevotellaceae†, Rikenellaceae↓, Lachnospiraceae↓, Ruminococcaceae↓, Clostridium↓, Blautia↓, Faecalibacterium↓, Enterobacteriaceae†</p> <p>Obese: Prevotellaceae†, Rikenellaceae↓, Lachnospiraceae↓ Ruminococcaceae↓, Clostridium↓</p> <p>Control: Bifidobacteriaceae↓, Bifidobacterium↓</p>
Wong VW, 2013 ¹⁹	China	Case-Control	16 NASH patients, 22 controls	Biopsy used only for NASH patients	16S rRNA pyrosequencing	<p>Control: Firmicutes†</p>	<p>NASH: Aeromonadales†, Succinivibrionaceae†, Porphyromonadaceae†, Parabacteroides† Allisonella†, Clostridia↓, Faecalibacterium↓ Anaerosporbacter↓</p> <p>C. coccoides†</p>
Mouzaki M, 2013 ²⁰	Canada	Cross-sectional	11 simple steatosis, 22 NASH, 17 controls	Biopsy	qPCR	<p>NASH: Bacteroidetes↓</p>	
Raman M, 2013 ²¹	Canada	Case-Control	30 Obese NAFLD patients, 30 Controls	Sonography	Multitag pyrosequencing	<p>NAFLD: Firmicutes†</p>	<p>NAFLD: Lactobacillus†, Lachnospiraceae† Dorea†, Robinsoniella†, Roseburia† Under-represented: Ruminococcaceae, Oscillibacter</p>
Yuan J, 2014 ²²	USA	Case-Control	19 NASH, 14 Obese, 18 Control	Biopsy	16S rRNA by pyrosequencing on a 454-FLX-Titanium Genome Sequencer	<p>NASH and Obese: Bacteroidetes†, Proteobacteria↑ Firmicutes↓, Actinobacteria↓</p> <p>NAFLD: Gammaproteobacteria†, Epsilonproteobacteria† Bacteroidetes†</p>	—
Michail S, 2015 ²³	USA	Case-Control	13 NAFLD, 11 Obese no evidence of NAFLD, 26 Controls	Sonography/Biopsy	16S rRNA gene microarray	<p>Control: Lentisphaerae†</p>	<p>NAFLD: Prevotella† Obese children without NAFLD: Clostridia↓, Alphaproteobacteria†</p>
Jiang W, 2015 ²⁴	China	Cross-sectional	53 NAFLD, 32 Controls	Sonography OR Biopsy	16S rRNA Illumina next-generation sequencing technologies		<p>NAFLD: Clostridium†, Anaerobacter†, Streptococcus†, Lactobacillus†, Escherichia†</p> <p>Control: Ruminococcaceae† (Oscillibacter† and Flavonifractor†), Odoribacter† Porphyromonadaceae†, Alistipes†</p>

Table 1. Continued

Wang B, 2016 ²⁵	China	Cross-sectional	43 non-obese NAFLD, 83 controls	Sonography	DGGE in all subjects and further examined by 454 pyrosequencing in the sub-cohort (10 NAFLD, 15 HCs).	NAFLD: Bacteroidetes↓, Firmicutes↓ G- bacteria↑, G+ bacteria↓	NAFLD: Bacteroidia↑, Clostridia↓, Lachnospiraceae↓ Ruminococcaceae↓, Lactobacillaceae↓ Peptostreptococcaceae↓, Coprococcus↓ Pseudobutyrvibrio↓, Moryella↓ 12Roseburia↓, Anaerospirrobacter↓ Anaerotruncus↓, Ruminococcus↓
Mouzaki M, 2016 ²⁶	Canada	Cross-sectional	12 NAFLD, 16 NASH, 25 Controls	Biopsy	qPCR	NASH: Bacteroidetes↓, Clostridium leptum↓	—
Özgül C, 2017 ²⁷	Turkey	Case-Control	46 NASH, 38 Controls	Biopsy	16S rRNA by qPCR	NASH: Mucimiphila↓, Fragilis↓, Enterobacteriaceae↑ Control: Bifidobacterium↑	—
Yan J, 2017 ²⁸	China	Cross-sectional	100 NAFLD patients with type 2 diabetes, 50 NAFLD patients with normal glucose metabolism, 60 17 normal metabolism without NAFLD	Biopsy, Sonography	Real Time PCR	NAFLD patients with type 2 diabetes and normal control group: Eubacteriu↑, Lactobacillus↓, Bacteroides↓ Tairatamicron↓ NAFLD patients with type 2 diabetes group and normal control group: Eubacteriu↑, Lactobacillus↑, Bacteroides↓, Tairatamicron↓, Bifidobacterium↓ NAFLD patients with normal glucose metabolism group and normal control group: Bifidobacterium↓	—
Sobhonsidsuk A, 2018 ²⁹	Thailand	Case-Control	16 NASH, 8 controls	Biopsy	16S rRNA MiSeqinstruments (Illumina)	NASH: Bacteroidetes↑, Firmicutes↓, Actinobacteria↓	NASH: Ruminococcus↓, Phascolarctobacterium↑
Shen F, 2017 ³⁰	China	Case-Control	25 NAFLD, 22 controls	Biopsy, for normal subject: Fibroscan	454 GS-FLX platform	NAFLD: Proteobacteria↑, Fusobacteria↑ Control: Bacteroidetes↑	NAFLD: Lachnospiraceae↑, Enterobacteriaceae↑ Clostridium_XVIII↑, Streptococcus↑ Erysipelotrichaceae↑, Strepto-coccaceae↑ Control: Prevotella↑, Ruminococcaceae↑, Faecalibacterium↑
Vemkar M, 2018 ³¹	India	Cross-sectional	11 NASH patients and 9 controls	Sonography/Biopsy	16S rRNA by Ion torrent personal Genome Machine (PGM) next generation sequencer platform	NASH: Firmicutes ↑, Bacteroidetes ↓	NASH: Streptococcus↑, Clostridium ramosum↑, Lactobacillus↑ Control: Ruminococcus ↑,
Da Silva HE, 2018 ³²	Canada	Cross-sectional	39 NAFLD (15 Simple Steatosis& 24 NASH), 28 controls	Biopsy, for controls Biopsy, MRI, CT scan	16S rRNA MiSeqinstruments (Illumina)	NAFLD: Bacteroidetes↓, Bacteroidetes↓	NAFLD: Lactobacillus↑, Lactobacillaceae↑ NASH: Ruminococcus↓, F. prausnitzii↓, Coprococcus↓

to healthy individuals. Actinobacteria and Proteobacteria were detected in amounts greater than 1% in at least one of the three study groups and were significantly different among them. Actinobacteria were found in significantly smaller amounts in the NASH group in comparison to the healthy group. There was a gradual increase in Proteobacteria levels from the healthy group to the obese and then the NASH group.

Serum ethanol levels were also evaluated in this study and were the same in the healthy and obese groups, but significantly increased in those with NASH. Given the role of alcohol in oxidative stress and hepatic inflammation, the results of this study showing an increase in alcohol-producing bacteria and subsequently serum ethanol levels in NASH patients shed light on the role these bacteria play in NASH pathogenesis.¹⁴

In another study by Michail et al published in 2015, the fecal microbiota composition of children with NAFLD was evaluated and compared to a healthy control group. This analysis was performed to evaluate phylogenetic, metabolomics, metagenomic and proteomic characteristics of fecal microbiota. Samples from children with NAFLD had significantly greater amounts of Gama Proteobacteria and *Prevotella*. Similar to the previous study, Michail et al also assessed ethanol levels, finding a significantly greater amount of serum ethanol in the NAFLD group, which is not surprising given that Gama Proteobacteria and *Prevotella* are composed of alcohol-producing bacteria.²³ The positive correlation between the increase in these two groups of bacteria and serum ethanol levels have been previously observed in animal studies.³³ Several other studies have also confirmed this finding that endogenous alcohols, produced during bacterial fermentation of carbohydrates, can cause fatty liver disease through oxidative stress.^{14,34}

Wong et al used 16S rRNA pyrosequencing to detect differences in the fecal microbiota composition of healthy individuals and those with NASH. Bacteroidetes were the most common phylum in both groups, followed by Firmicutes which were more significantly present in healthy individuals compared to NASH patients (30.3% and 22.3% respectively, $P = 0.029$).¹⁹

In another study by Mouzaki et al performed in 2013, fecal microbiota composition was analyzed using quantitative real-time PCR in the following three groups: healthy, NAFLD and NASH. Bacteroidetes, *Bifidobacteria*, *Clostridium leptum*, *Clostridium coccooides* and *Escherichia coli* were evaluated in this study. NASH patients had significantly less Bacteroidetes ($P = 0.006$) and more *Clostridium coccooides* ($P = 0.04$) in comparison to the other two groups. No other bacteria were different among the three groups. An evaluation of other factors in the study groups such as body mass index (BMI) and calorie, fat and carbohydrate intake showed no significant finding, except for an inverse relationship between caloric intake and

Bacteroidetes amounts in NAFLD patients ($P = 0.038$). The authors concluded that the role of Bacteroidetes in NASH pathogenesis is independent of BMI and dietary intake.²⁰

In a study by Raman et al using multitag pyrosequencing, differences in fecal microbiota composition were compared in healthy individuals and those with NAFLD. Individuals with NAFLD had higher amounts of *Lactobacillus* species and some Firmicutes (*Lachnospiraceae*, *Dorea*, *Robinsoniella* and *Roseburia*) in comparison to the healthy controls, which was a significant finding. Other Firmicutes (*Ruminococcaceae* and *Oscillibacter*) were found at significantly lower amounts. Fecal volatile organic compounds (VOC) were also assessed in this study using gas chromatography-mass spectrometry. Ester VOC, acting as metabolic and metagenomic factors, can lead to changes in fecal microbiota composition of obese NAFLD individuals and can be used as an influential factor in the etiology of obesity. Twelve fecal VOC, (such as ketones, furans, aldehydes) were significantly decreased and 18 VOC (such as aliphatic esters of ethanoic, propanoic, butanoic, pentanoic acids) were significantly increased in the NAFLD group.²¹

Yuan et al evaluated gram-negative bacteria in three groups including patients diagnosed with NASH, obese individuals with a normal liver, and healthy controls, using 16S rRNA pyrosequencing. Obese individuals and those with NASH had significantly greater amounts of Gram negative bacteria present in their fecal samples compared to the controls (54.5%, 55.7% and 29.7%, respectively, $P = 0.0019$). The authors believed that these changes were caused by an increase in Gram negative Bacteroidetes, Proteobacteria and a decrease in Gram positive Firmicutes and Actinobacteria. Serum endotoxins were also evaluated since Gram negative bacteria are their main source. Endotoxemia was significantly greater in obese and NASH individuals ($P = 0.029$). No correlation was observed between abundance of Gram negative bacteria and endotoxin levels. In the NASH group, there was no correlation between severity of disease and endotoxin levels.²²

Another study using 16S rRNA Illumina next-generation sequencing to evaluate fecal microbiota in healthy individuals and NAFLD patients also found Firmicutes and Bacteroidetes to be the most prevalently found phyla, but this finding was not significant between the two groups. In taxa present at amounts greater than 1% among different samples, *Alistipes* and *Prevotella* were more frequently observed in healthy individuals. *Escherichia*, *Anaerobacter*, *Lactobacillus* and *Streptococcus* were increased in individuals with NAFLD. Also, CD4+ and CD8+ lymphocyte levels decreased while TNF- α , IL6 and IFN- γ levels increased in the NAFLD group. Duodenum tight junctions were also assessed in this study using transition electron microscopes, revealing that duodenal surface was

more intact in healthy individuals while in those with NAFLD, irregularities were seen in microvilli and large junctions, supporting the idea that individuals with fatty liver have weakened intestinal barriers or a “leaky gut”. Based on these findings, it appears that lack of intestinal mucosal integrity, which allows for microbial-mediated inflammation and other immunological responses to take place in the gut, plays an important role in the pathogenesis of NAFLD.²⁴ Physiologic, chemical, immunologic and microbiologic barriers in the intestine play an important protective role when confronted with harmful substances and many human and animal studies have shown the relationship between these protective mechanisms and NAFLD pathogenesis and point it out as a possible way of treating NAFLD.³⁵⁻³⁷

Wang et al showed a 20% increase in Bacteroidetes ($P = 0.005$) and a 24% decrease in Firmicutes ($P = 0.002$) in the fecal microbiota composition of NAFLD patients compared to healthy individuals. Gram negative bacteria were also present at greater amounts in the NAFLD group ($P = 0.008$).²⁵ Many studies have focused on the mechanisms by which microbiota increase in NAFLD/NASH patients. The presence of Bacteroidetes in the gut is strongly correlated with an increase in other factors such as raffinose and choline and a decrease in short chain fatty acids, all of which play a role in NASH pathogenesis.³²

In another study, Mouzaki et al evaluated bile acids and their relationship with gut microbiota in NAFLD/NASH patients and healthy controls. The number of Bacteroidetes ($P = 0.028$) and *Clostridium leptum* ($P = 0.030$) was significantly decreased in those diagnosed with NASH compared to healthy controls, after adjusting for BMI and weight-adjusted caloric intake.²⁶

Ozkul et al, studying microbiota in NASH patients and healthy individuals, found a significant increase in *Enterobacteriaceae* ($P < 0.001$) and a significant reduction in *Akkermansia muciniphila* ($P = 0.003$) and *Bacteroides fragilis* levels ($P = 0.001$) in NASH patients. Individuals with fibrosis scores ≥ 2 also had significantly greater amounts of *Enterobacteriaceae* ($P < 0.001$) compared to those with fibrosis scores of 0-1. A positive correlation was observed between BMI and *Enterobacteriaceae* ($P = 0.021$). *L. ruminis* was the most abundant *Lactobacillus* in both groups (44.6% in patients vs. 50.0% in controls). Healthy microbiota such as *L. sakei* and *L. helveticus* were not observed in NASH patients while significantly increased serum endotoxin and high-sensitivity C-reactive protein levels were observed.²⁷

Yan et al investigated quantitative differences in *Eubacterium rectale*, *Bacteroides thetaiotaomicron*, *Lactobacillus* and *Bifidobacterium* in NAFLD patients with type II diabetes, NAFLD patients with normal glucose levels and healthy controls. The quantities of *Eubacterium rectale* and *Lactobacillus* were significantly greater in NAFLD patients with diabetes compared to those with

normal glucose metabolism, and healthy controls, while that of *Bacteroides thetaiotaomicron* was significantly smaller. Compared to the controls, *Bifidobacterium* had also decreased significantly in NAFLD patients with diabetes. The quantity of *Bifidobacterium* in NAFLD patients with normal glucose metabolism in comparison to controls had decreased significantly ($P = 0.00$). The results of this study show that glucose metabolism also affects microbiota composition in individuals with NAFLD.²⁸

In a study by Sobhonslidusk et al evaluating microbiota patterns and factors influencing them, patients with NASH had more abundant Bacteroidetes in comparison to healthy controls ($P = 0.002$). Firmicutes were also decreased in the NASH group, which could be because of lower *Ruminococcus* levels. As expected, the Bacteroidetes to Firmicutes ratio was also significantly increased in the NASH group in comparison to healthy controls ($P = 0.005$). Another phylum observed less significantly in NASH individuals was Actinobacteria.²⁹

Shen et al compared microbiota composition in a group of NAFLD patients and healthy controls. Their results showed that microbial diversity was less in NAFLD patients compared to healthy controls. They had more abundant Proteobacteria (13.5%) and *Fusobacteria* (2.76%). In addition, *Erysipelotrichaceae*, *Lachnospiraceae*, *Enterobacteriaceae*, *Streptococcaceae* and *Blautia* were enriched in the NAFLD group. *Prevotella* were seen more frequently in NAFLD patients ($P < 0.01$) while Bacteroidetes were more common in healthy individuals ($P = 0.01$).³⁰

The severity of NAFLD/NASH in relation to gut dysbiosis was evaluated by Boursier et al in individuals with NASH, Bacteroides and *Ruminococcus* were significantly increased while *Prevotella* levels were decreased. The results of this study showed the relationship between the amount of Bacteroidetes and NASH, independent of factors such as BMI, blood pressure, diabetes and metabolic syndrome. Comparing fibrosis scores and Bacteroidetes, *Prevotella* and *Ruminococcus* levels among NAFLD/NASH patients, there was a significant difference between scores 0 and 1, as well as 2 and greater. Individuals with fibrosis scores ≥ 2 showed greater amounts of Bacteroidetes and *Ruminococcus* and less *Prevotella* in comparison to those with lower fibrosis scores.¹¹

The study by Vernekar et al published in 2018 identified 6 phyla (Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Verrucomicrobia) in NASH and healthy individuals, the most dominant of which was Firmicutes at 59.46% and 45.69% in NASH and healthy individuals, respectively. Bacteroidetes, on the other hand, were less abundant in the NASH group (2.98%) compared to healthy controls (8.04%). None of the differences observed were statistically significant. Among the Firmicutes, individuals with NASH showed a significant increase in *Streptococcus* and *Clostridium*

ramosum ($P < 0.05$). *Ruminococcus*, however, was present at significantly greater amounts among healthy controls ($P < 0.05$).³¹

In a study performed by Da Sliva et al, the relationship between dysbiosis and NAFLD was assessed showing that both Firmicutes and Bacteroidetes were less frequently observed in the NAFLD group while genus *Lactobacillus* was more frequent in healthy individuals. While *Ruminococcus*, *Coprococcus* and *Faecalibacterium prausnitzii* were not significantly different among those with NAFLD and NASH, they were less frequent in these two groups compared to healthy controls. In NAFLD patients, the concentrations of propionate, isobutyric acid, serum 2-hydroxybutyrate and L-lactic acid were higher.³²

Dysbiosis and Obesity

NAFLD prevalence and severity is highly associated with the worldwide obesity epidemic.³⁸ In 2016, over 1.9 billion adults and 41 million children under the age of five were estimated to be overweight and obese and these figures are on the rise globally.³⁹ One common factor in the pathogenesis of both obesity and NAFLD is insulin resistance. Many studies have shown central obesity-associated insulin resistance to affect accumulation of lipids in the liver, causing steatosis.⁴⁰⁻⁴²

While lifestyle factors such as high calorie diets and physical inactivity influence obesity, in the past few decades, gut microbiota have also been shown to be directly associated with weight gain and obesity.⁴³ A recent systematic review published in 2018 investigating gut microbiota and obesity has reported 11 studies that have supported this relationship. Although the microbiome composition varied widely in different studies, the differences between obese individuals and controls were significant.⁴⁴

Gut microbiota are known to affect energy mobilization from ingested foods, by converting complex carbohydrates to short chain fatty acids, facilitating their absorption by the intestines. Certain variations in microbiome composition can negatively affect this process, by extracting more energy from food and allowing for more than usual lipid absorption, subsequently causing higher rates of *de novo* lipid production instead of lipid oxidation.^{45,46} Modifications in gut microbiome composition are proposed as a means of countering this process and preventing overweight and obesity, by inhibiting over-absorption of fatty acids, which can affect NAFLD/NASH development, as well.

Conclusion

In this article, differences in microbial composition in healthy individuals in comparison to those with NAFLD/NASH were reviewed. Dysbiosis was observed in all comparisons made between these two groups, indicating the role of microbiota in the pathogenesis of these conditions.

The most frequent changes were seen in the Firmicutes and Bacteroidetes phyla. The heterogeneity of the results could be partly explained by different methodologies used, as well as the fact that many factors including genetics, environmental exposures, body composition and diet also affect gut microbiota, which were not adjusted for in many of these studies. Due to the serious health consequences of NAFLD/NASH, we suggest greater attention to correcting gut microbial dysbiosis in individuals diagnosed with or prone to developing these conditions.

Authors' Contribution

ZM, SM, HP and AH designed and supervised the study. ZM, NMG, and SE conducted the independent literature search and review. ZM extracted data and prepared a first draft of the manuscript, and all authors (ZM, HP, NMG, SE, AH, PS and SM) finalized it.

Conflict of Interest Disclosures

The authors declare no conflict of interest related to this work.

Ethical Statement

Not applicable.

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