Review

Gut dysbiosis: does the gut microbiome affect the development of hepatic steatosis and how can this knowledge influence future treatment?

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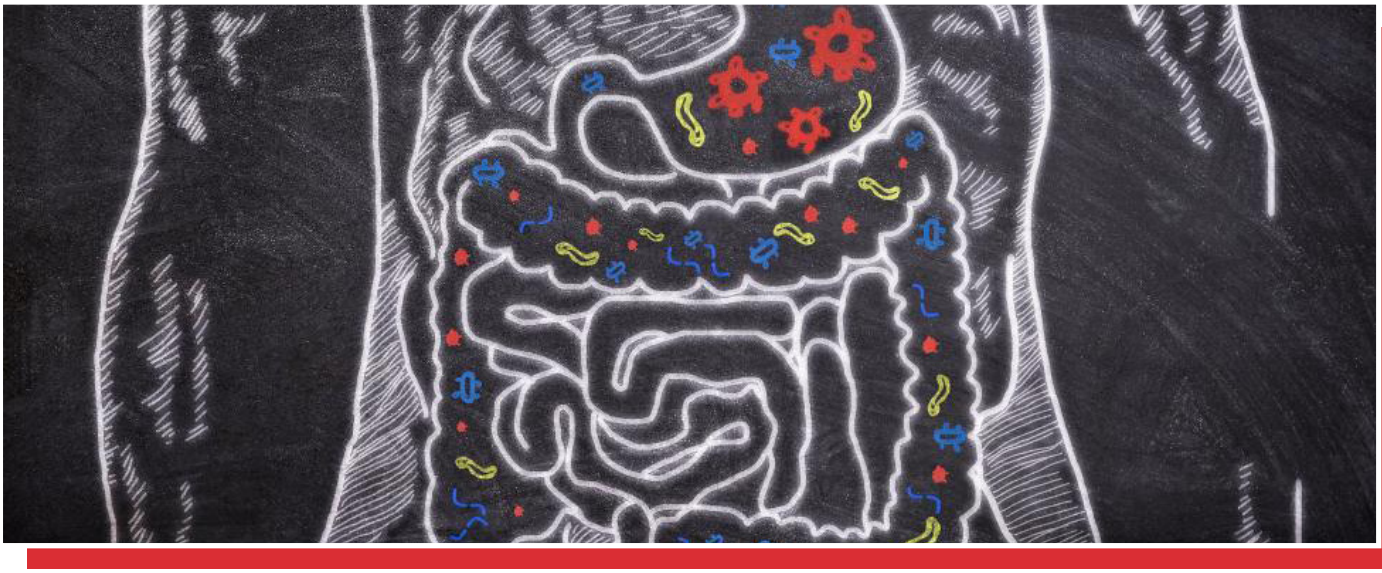

Abstract

There is confounding evidence to support the theory that a mutualism exists between the human body and the rich, diverse microbial community that resides within the human gut, in particular, between the microbial community and the liver. This paper will consider the influence of this 'gut-liver axis' in relation to the development of non-alcoholic fatty liver disease (NAFLD). Sources were gathered using PubMed, NICE evidence and BMJ databases, using the key search terms "(gut microbiome OR microflora) AND (non-alcoholic fatty liver disease OR NAFLD)" and "gut microbiome AND hepatic steatosis". When a gut microbiome is poorly managed, its interface with the liver is thought to have the potential to induce pathology, altering hepatic lipid metabolism and generating an excessive immune response. There are multiple mechanisms proposed in the literature regarding hepatic steatosis which will be discussed, such as the translocation of bacterial and microbial products generating inappropriate lipogenesis within the liver, and the relationship between increased body-mass index (BMI) and an overgrowth of gram-negative bacteria. This review will outline the potential utility of probiotic and prebiotic treatment to manage the composition of the microbial community and reduce states of active disease. Application of these findings in future human trials could increase our knowledge of the causes of liver steatosis and vastly improve the prevention schemes and treatment of NAFLD available in the NHS. Fundamentally, this article will consider how active management of the gut microbiome beyond diet and lifestyle changes can be altered to improve the prognosis for patients suffering from NAFLD.

Abbreviations

BMI - Body-mass index
LPS - Lipopolysaccharides
NAFLD - Non-alcoholic fatty liver disease
NLRP3- Nod-like receptor family pyrin domain containing 3
PAMP - Pathogen-associated molecular pattern
rRNA - Ribosomal RNA
SCFA - Short-chain fatty acid
TLR - Toll-like receptor
TNF-α - Tumour necrosis factor α

Introduction

The systemic interaction of the gut microbiome with the rest of the body is currently controversial and the ways in which gut microflora may be factorial in the progression of liver disease is a topic of recent debate.[1] The proposed mechanisms for how gut dysbiosis influences the development of non-alcoholic fatty liver disease (NAFLD) is in their early stages of clinical application; however, awareness of the active management of the gut microbiome through diet and lifestyle changes, alongside the role of pre and pro-biotics, is important for clinicians to consider when evaluating the causes of liver steatosis and when aiming to maximise the prognosis for patients suffering from NAFLD.[2]

The aim of this article was to determine how the contents of the gut microbiome can impact upon the pathophysiology of NAFLD.

Methodology

The search term "(gut microbiome OR microflora) AND (non-alcoholic fatty liver disease OR NAFLD)" was searched to produce the following results: BMJ 175 results, NICE 16 results, PubMed 195 results. The term "gut microbiome AND hepatic steatosis" was also used and produced: BMJ 253 results, NICE 7 results, PubMed 364 results. Of the two search terms, the former produced fewer, more relevant papers and was used preferentially over the latter search term. To elaborate, the search term "(gut microbiome OR microflora) AND (non-alcoholic fatty liver disease OR NAFLD)" produced the same list of appropriate, relevant papers found also when using the search term "gut microbiome AND hepatic steatosis", as well as additional, more recent research. Of the papers identified by the chosen primary search term, relevant papers were selected if they focussed primarily upon NAFLD and the gut microbiome, were written in English and were published within the last 15 years. In total, 25 papers were selected.

Establishing the role of the gut-liver axis in NAFLD

Within western society, NAFLD is becoming an increasing concern.[1] A global epidemiological meta-analysis studying NAFLD estimated it to be prevalent in 25.24% of the world's adult population, a figure thought to be a consequence of the increasing rates of obesity and type 2 diabetes.[2]

It is well-established that NAFLD is characterised by the elevation of pro-inflammatory markers, such as cytokines, and disturbed hepatic lipogenesis.[3] Yet, mechanisms have been proposed for the causation of liver disease as a result of gut dysbiosis and dysregulation of the intestinal microbiota.[4,5] The liver acts as an intersection between portal venous blood from the gut and peripheral organs, interacting with the microbiota-derived compounds that may pass through the gut barrier and intestinal lumen following digestion.[5] The translocation of these bacterial and microbial products has been suggested to bring about changes in metabolism in the liver and immune responses of the liver, which can lead to disease.[5]

Altered lipid metabolism and NAFLD

Many studies have questioned the relevance of genetic variation within the microbiome in relation to the pathogenesis of obesity.[7] Statistical homogeneity can be seen in results from studies using mouse models, where there is a noteworthy increase in the Firmicutes:Bacteroidetes phyla ratio in the gut microbiome of obese mice compared to lean mice.[7]

A 2008 study looking at the metagenomics of gut flora in mouse models suggested that Firmicutes dominance within obese-associated bacterial cultures results in a larger capacity for fermenting polysaccharides, a compound metabolised by the microbiome into monosaccharides and short-chain fatty acids (SCFAs).[8] These SCFAs are eventually converted to complex lipids within the liver and are likely to contribute to substantial fat accumulation and steatosis.[2,7,8] Transplantation of these microbial samples to the gut of healthy mice has proven to induce an obese phenotype, suggesting that

> *the genetic richness of the microbiome is active in regulating metabolism and the metabolic pathogenesis seen in obesity and NAFLD.*[7,9]

However, the application of this obesity model to the human gut is not so straightforward. One study examined the microbial composition of faecal matter from obese patients to approximate the contents of their gut microbiome. It was demonstrated that bacterial ratios in human microbiota were different to that of mouse models; disparities were reported with regard to the representation of Firmicute and Bacteroidetes following ribosomal RNA (rRNA)-based analysis. These findings demonstrate that the biotic changes seen in mice are not always a true reflection of the human microbiome.[9]

Further research has acknowledged that the distribution of gram-negative and gram-positive bacteria may be dependent on body mass index (BMI), with models with a higher BMI showing gut microbial colonisation predominated by gram-negative species.[10,11] An overgrowth of gram-negative species within the gut microbiota has been shown to increase the formation of hepatotoxic substances, such as lipopolysaccharides (LPS), and eventually lead to the deposition of metabolised triglycerides and other SCFA derivatives within the liver, and disturbed lipogenesis.[12,13]

Inflammatory changes and NAFLD

LPS are considered to hold pro-inflammatory properties when over-produced by the gram-negative bacteria that dominate obesity-associated microbiomes.[14,15] The recognition of LPS by Toll-like receptor 4 (TLR-4), most commonly found in liver cells and resident Kupffer cells, has been shown to be accelerated by mediator inflammatory cytokines, such as tumour necrosis factor α (TNF-α).[16,17] Subsequently, upregulation of the LPS/TLR4 cascade may be influential in the development of progressive inflammation, which is established as a hallmark of NAFLD.[16]

Moreover, the involvement of nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasomes, sensor molecules involved in recognising bacterial pathogen-associated molecular patterns (PAMPs) within the innate immune system, has been shown to promote insulin resistance.[18,19] Following gut dysbiosis, the liver will receive PAMPs in high concentrations and be considerably vulnerable to their accumulation.[19] NLRP3 is said to activate the cleavage of pro-inflammatory cytokines, pro-IL-1β and pro-IL-18, during the inflammatory drive associated with NAFLD.[19] Pro-inflammatory cytokines, such as the aforementioned, have been recognised as signalling molecules within the insulin signalling pathways of hepatocytes.[20] Thus, the inappropriate cleavage of such proteins may be a potential driver in deranged insulin signalling within the liver and increased insulin resistance.[19,20]

Improving treatment: the involvement of probiotics

Probiotics are live microorganisms administered to confer a beneficial, mutualistic relationship with the host. Prebiotics are fermented products given to induce beneficial changes in the host's gut microbiota and aid healthy gut metabolism. For many years, probiotic and prebiotic treatment has been recognised as a successful therapy in the management of diarrhoeal illness due to their anti-inflammatory properties within the gastrointestinal system.[21] These products are thought to act by reforming an already poorly functioning microbiota and, thus, their role is larger in treating active disease than it is in preventing gut dysfunction.[20,21]

Faecal samples, although not direct quantitative measures of the intestinal microflora, are thought to be accurate surrogate measures of the intestinal content.[21] A study of human faecal samples following treatment suggested that probiotics act as effective competitors against unfavourable bacteria within the gut.[21] The introduction of live gram-positive bacteria via probiotics, in particular Bifidobacteria and Lactobacillus, results in these bacteria dominating the gut microbiome, preventing the over-growth of damaging gram-negative species by way of competition for space and nutrients. In doing so, they reduce the inflammatory consequences of endotoxaemia and prevent stimulation of lipogenesis within the liver.[22]

A meta-analysis of the effects of probiotics upon liver health has also been largely positive, highlighting that probiotics also improve the histological picture of epithelial junctions and liver parenchyma by

reducing gut-epithelial permeability, and reducing the translocation of hepatotoxic products, such as LPS and PAMPS, to the portal circulation.[23,24]

Prebiotics are not themselves digestible by the human host but are intended to support the metabolism of beneficial gut microbiota, allowing them to outgrow unwanted and potentially harmful bacterial microbes.

> *The fibrous content of prebiotics has been shown to minimise the effect of pro-inflammatory cytokines, such as TNF-α, and are considered supportive for those patients who have struggled to maintain a 'gut-friendly' diet.*[25]

For those who have had a consistently large intake of cholesterol and high calorie food over many years, the addition of prebiotics alongside weight loss and dietary changes is thought to have the potential to expedite positive changes to their microbiome.[25]

Furthermore, the use of prebiotics as a form of co-therapy has been suggested as vital in influencing the efficacy of probiotic treatment.[26] Prebiotics, such as fructo-oligosaccharides, can be fermented by both Bifidobacteria and Lactobacillus, aiding their growth and metabolic activity and demonstrating success in reducing hepatic inflammation and improving glucose tolerance.[22,26]

Currently, diet and lifestyle modifications are elemental in the treatment regime of NAFLD, with adequate exercise and restrictions on the consumption of fructose, carbohydrates and saturated fats being advised to patients with weight-related, metabolic conditions.[20] However, the application of probiotics and prebiotics are becoming increasingly more popular in combating hepatic steatosis. The use of these co-therapies is considered favourable to both patients and clinicians due to their availability, accessibility by addition to the diet in food and liquid form, and safety in pregnancy.[27]

Strengths, limitations, and recommendations for future research

It is worth noting that many of the research studies discussed in this paper are mouse models or molecular studies. Whilst these provide a promising, sensible explanation of the role of the gut-liver axis in NAFLD, further research is needed to allow the application of these theories to the human microbiome. Although some high-quality studies and meta-analyses clearly demonstrate the ability of probiotics and prebiotics to reduce hepatic inflammation and improve insulin resistance in humans on a molecular level, the implications of this treatment in improving patient prognosis and recovery is yet to be elucidated.[2,21,22] The use of these treatments in the clinical setting for some gastrointestinal diseases suggests that their therapeutic ability within the human body, and potentially specifically in the liver, should not go overlooked. However, in order to accelerate the incorporation of prebiotic and probiotic treatment into the established management for NAFLD, long-term cohort studies and clinical trials evaluating their efficacy and cost-effectiveness are necessary.

To conclude

The role of gut dysbiosis in amplifying fat accumulation in the liver and development of NAFLD is highly likely to be linked to the imbalance of bacterial colonies within the gut flora and the production of pro-inflammatory metabolites. Research is currently in its infancy, with much of the evidence coming from the use of mouse studies; the mouse microbiome may or may not be comparable to the human microbiome. However, the use of probiotic and prebiotic treatment to actively manage the composition of gut microflora is a promising, novel avenue for treatment that clinicians should be aware of.

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