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Gut microbiota and non-alcoholic fatty liver disease: new insights

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a severe liver disease that is increasing in prevalence with the worldwide epidemic of obesity and its related insulin-resistance state. A 'two-hit' mechanism has been proposed; however, the complete physiopathogenesis remains incompletely understood. Evidence for the role of the gut microbiota in energy storage and the subsequent development of obesity and some of its related diseases is now well established. More recently, a new role of gut microbiota has emerged in NAFLD. The gut microbiota is involved in gut permeability, low-grade inflammation and immune balance, it modulates dietary choline metabolism, regulates bile acid metabolism and produces endogenous ethanol. All of these factors are molecular mechanisms by which the microbiota can induce NAFLD or its progression toward overt non-alcoholic steatohepatitis.

Keywords: hepatic steatosis, microbiota, non alcoholic fatty liver disease, obesity, steatohepatitis
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Introduction

The epidemic of obesity [1,2] has led to the dramatic increase of its related metabolic diseases, namely insulin resistance, type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) [3]. The mechanisms involved in weight gain and the development of obesity are numerous and complex, and research continues to uncover new factors. In the past few years, a potential role of the gut microbiota has emerged in weight regulation [4–8]. The gut microbiota is now considered as a major metabolic internal organ, composed of $>10^{14}$ microorganisms and containing a second genome (named the metagenome), which is up to 100–400 times that of humans [9]. Culture-independent, large-scale tools [10] and associated projects such as the Human Microbiome Project [11] or the MetaHit consortium [9] have enabled major breakthroughs in the understanding of gut microbiota composition and functions in different pathological conditions. Data suggest an important impact of the gut microbiota on health [12] and in the pathogenesis of certain inflammatory and metabolic [13] diseases such as type 2 diabetes [14] and obesity. Recent literature also points to a potential role in the development of NAFLD.

Non-alcoholic fatty liver disease is a very prevalent and severe disease that can lead to cirrhosis, liver carcinoma [15] and death related to liver morbidity. In addition, data show that NAFLD correlates with increased cardiovascular risk assessment scores and most of the clinical surrogates of cardiovascular diseases. A few smaller studies have suggested that NAFLD induces not only increased risk of patent cardiovascular disease, independently of usual risk factors [16,17] and of other components of metabolic syndrome, but also increased risk of related mortality [18,19]. However, no treatment has yet proven effective to improve non-alcoholic steatohepatitis

(NASH) [20,21]. Finding novel mechanisms for the pathogenesis of NASH, in particular involving the microbiota, could emphasize new research areas to develop new therapeutic targets.

This review summarizes what is currently known of microbiota composition in obesity and the physiopathogenesis of NAFLD in that context. Recent literature suggests a role of the gut microbiota in NAFLD but mainly in animal models. We therefore aim to present the putative molecular mechanisms linking fatty liver and gut microflora.

NAFLD and Obesity

Non-alcoholic fatty liver disease is a frequent disease, occurring in 16–30% of the general population, depending on the assessment method [22,23]. Its prevalence rises [24] in parallel with the worldwide epidemic of obesity and metabolic diseases [25], reaching 50–90% of obese individuals, among whom more than one-third present with overt NASH [26]. The diagnosis of NAFLD is made on histological findings, first described by Kleiner and Brunt and their colleagues [27,28]. It includes a wide spectrum of lesions, starting with steatosis, characterized by the accumulation of triglycerides within the hepatocytes [29], in the absence of other liver disease or significant alcohol consumption [21]. Some patients will also develop hepatocyte injuries, such as ballooning and inflammatory infiltrates, both responsible for NASH, with or without concomitant collagen deposition (fibrosis) that potentially leads to end-stage cirrhosis [30]. Recently, a new algorithm also based upon histological determination has been proposed and validated to better define early stages of NASH, most particularly in morbidly obese patients [31]. Although liver biopsy is the gold standard to confirm the diagnosis, it is an invasive and costly procedure, potentially responsible for secondary effects [32,33]. Furthermore, considering the ever-increasing prevalence of both diabetes [34] and obesity [35], and the subsequent number of patients at risk for liver alterations, it cannot be considered a practical, efficient and large-scale tool to identify those at risk of NASH or advanced fibrosis. In that context, a clinical scoring system including type 2 diabetes, hypertension, sleep apnea and ethnicity has been suggested to propose liver biopsy for those at high risk of NASH [36]. Other imaging techniques include fast magnetic resonance imaging [37], computed tomography, ultrasound [38] and emerging proton magnetic resonance spectroscopy, which is a reliable non-invasive tool to quantify hepatic triglyceride content [39]. The last of these correlates closely with histopathological grade [40] and allows larger sampling than liver biopsy (8–27 cm^3). Recently, non-invasive tests, initially used in viral hepatitis B [41] and C [42], have been developed, that are currently validated to screen NAFLD in at-risk populations. For example, Fibromax®, which is an algorithm including an association of gender, age, weight, height and numerous serum biomarkers, has shown good reliability in the prediction of liver abnormalities including steatosis [43], NASH [44] and fibrosis, in both overweight patients and in those at different stages of obesity [45–47].

Regarding the pathogenesis of NAFLD, for a long time, a 'two-hit' mechanism was proposed to explain the progression in liver alteration stages, where the first hit consists in hepatocyte lipid accumulation mainly due to obesity and insulin resistance [48]. Then a second hit, occurring in some patients only, plays a role in the shift from steatosis to NASH [49,50]. Several factors have been incriminated in the pathophysiology of NAFLD such as oxidative stress, systemic inflammatory mediators and chronic intermittent hypoxia [51–53]. Furthermore, adipose tissue inflammatory tone [54] also seems to play an important role, because patients with more severe stages of NASH display increased macrophage accumulation in visceral adipose tissue [55]. Moreover, their deep subcutaneous adipose tissue not only displays increased macrophage infiltration but also increased inflammatory gene expression compared with superficial adipose tissue [56]. These associations between macrophage accumulation in adipose tissue and NASH stages were found independently of the diabetes status in morbid obesity.

More recently, an alternative theory favours the determinant effect of free fatty acid lipotoxicity in liver injury, which leads to NASH and occurs in parallel with triglyceride droplet accumulation (i.e. steatosis) [57]. Hepatic steatosis is known to develop in the context of an imbalance of triglycerides afflux in the liver, which is dramatically increased during obesity and even more so when associated with insulin resistance. Indeed in that context, there is a concomitant increased supply (coming from diet, *de novo* lipogenesis [58] and adipose tissue) and decreased degradation (via impaired β-oxidation) [57]. A special focus should be placed on fructose consumption, which is dramatically increased in the western diet [59]. Indeed, its metabolism induces both *de novo* hepatic lipogenesis and reactive oxygen species (ROS) production and therefore participates in the two 'hits' of NAFLD physiopathology [60]. Yet to date, the complete pathogenesis of NAFLD is still unknown and many more factors remain still to be uncovered, in particular the precise role of gut microbiota.

Microbiota Composition in Obesity

Studies in mice indicated a relation between gut microbiota and weight regulation. Indeed, germ-free mice displayed reduced adiposity despite increased food consumption when