In the development of NAFLD plays an important role the intestinal microflora. Our aim was to characterize role microbiota in children. Distinctive gut microbiota composition was observed in children, characterized and short-chain fatty acid producing bacteria. For the treatment of NAFLD it is possible by therapeutic manipulations with prebiotics and probiotics to modulate the gut microbiota and maintain the integrity of the intestinal barrier are potential agents.

Keywords: pediatric NAFLD, dysbiosis, short-chain fatty acids (SCFAs), gut microbiome (GM), Butyrate

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is now recognized as one of the most common disorders in the proper functioning of the liver, both in the pediatric and adult population. It is estimated that it affects up to 10% of the entire pediatric population, including 26–41% of children with obesity (Anderson et al., 2015; Schwimmer et al., 2006). In the adult population, this percentage ranges from 25 to 40% (Estes et al., 2018). Younossi and others (Younossi et al., 2016) in a systematic review estimated that the global prevalence of NAFLD reached 25%, which resulted in a significant increase in the interest of scientists, who are trying to find out the causes leading to its development. The global increase in NAFLD prevalence among children constitutes a poor prognosis for the future (Obesity Collaborators The GBD 2015). NAFLD by itself is defined as a metabolic dysfunction-associated liver disease caused by excessive accumulation of fat in hepatocytes, where it is possible to raise secondary factors leading to accumulation of fat in liver cells, such as alcohol consumption, hereditary disorders, and drug intake (Chalasani et al., 2012). Within this disease, we can distinguish a number of liver conditions that vary in severity degree, from steatosis to non-alcoholic steatohepatitis (NASH) with or without fibrosis, to cirrhosis (Buzzetti et al., 2015). In the long term, NASH might progress to hepatocellular carcinoma (HCC), a condition that increases liver-related morbidity and mortality. NAFLD is also related to metabolic syndrome (MS), including insulin resistance, dyslipidemia and visceral obesity (Boyraz et al., 2013). The pathological mechanism leading to the onset and development of NAFLD is not fully known. According to the multiplet hit hypothesis we do know, that there are many factors leading to NAFLD. Among them, we can set apart genetic and epigenetic factors, lipotoxicity, insulin resistance, dietary determinants proinflammatory factors, and gut microbiome, which might be crucial due to the production of large amounts of molecular mediators that affect liver cells (Clemente et al., 2012).

GUT MICROBIOME AND IMMUNOLOGICAL RESPONSE

The gut microbiota affects host physiology at least in part, through the release of metabolites and complex bacterial structural components such as lipopolysaccharides (LPS), peptidoglycans and flagellins. Metabolites derived from the gut microbiota include short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs), aromatic acid metabolites, vitamins, amino acids and other metabolites. Recent studies have shown that gut microbiome (GM) has a significant impact on the development of NAFLD. The gut microbiota includes microorganisms belonging to different kingdoms, such as bacteria, viruses, archaea and fungi. As a whole, they form a complex ecosystem that lives in symbiosis with its host. Human GM is dominated by four bacteria phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria, where Firmicutes and Bacteroidetes are most common (Tremaroli et al., 2012). Bacteria are involved in many metabolic processes in the gut. They participate in energy harvest, regulation level of bile-acid, fermentation of polysaccharides, and lastly choline metabolism (Dumas et al., 2006). Intestinal bacteria can also produce substantial amounts of endogenous ethanol, which is transported via the portal vein to the liver, where it might trigger inflammation. Disturbances in the composition of the intestinal microbiota are associated with chronic metabolic syndromes such as obesity, NAFLD and type 2 diabetes mellitus (T2DM) (Turnbaugh et al., 2009; Loomba et al., 2013; Donaldson et al., 2016). Diet is one of the main factors that modulate the composition of gut microbiota – it has an influence on species richness. When the number of species decreases, then it comes to a condition known as dysbiosis, which has been associated with the pathogenesis of inflammatory diseases (Jennison et al., 2021). Gut microbiota is responsible for maintaining of the intestinal epithelial barrier, thus preventing the entry of bacteria and their metabolites into the blood (Fig. 1). It is also known that bacterial metabolites play
Figure 1. Gut microbiota imbalance contributes to the development of NAFLD, may lead to the nutrition imbalance and change the gut microbiota composition and its metabolites, such as SCFAs, and bile acids.

Microbiota disorders promote the intestinal endocrine L cells to secrete GLP-1 to act on the vagus nerve to activate the gut–brain–liver axis in his research found out that gut microbiota is involved in adipsic tissue growth, due to modulation of energy absorption and stimulation of increased triglycerides synthesis in the liver. Bacteria overgrowth may cause intestinal permeability to increase the amount of endotoxins in the systemic circulation and that will allow for the development of endotoxemia with inflammation in the liver. Some other commensal metabolites may also influence the metabolism of the host and that impact can lead to the development and progression of NAFLD (Aron-Wisnewsky et al., 2020).

The human intestine contains at minimum 100 trillion microbes, which belong to at least 1000 different species with 150 times more genes than the human genome has (MetaHIT Consortium et al., 2010; Chassaing et al., 2014). The most common phyla in the human gut are Firmicutes and Bacteroidetes, which are followed by frequently identified Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria (Kamada et al., 2014). There are many factors that influence the composition of GM such as gender, age, diet, hormonal changes, drug intake, travel and pregnancy (Rodríguez et al., 2011). Symbiotic or al., 2015; Uchiyama et al., 2019). It is important to keep in mind internal factors that affect the composition of the intestinal microbiota, such as the state of the intestinal mucosa, which affects the permeability of the intestinal barrier and the state of the host immune system (Adams et al., 2008). These factors can lead to unfavorable conditions for some bacteria, leading to lower microbial diversity – dysbiosis. Maintaining homeostasis of GM is crucial for keeping the human body (especially the liver) in good condition. It is linked with beneficial metabolites produced by commensal bacteria. Dysbiosis may occur due to an imbalance in the gut, which will manifest itself through excessive proliferation and overproduction of harmful substances that affect the liver, due to the fact that the portal vein transports about 70% of the total amount of blood to the liver. This is the main reason why it is exposed to endotoxins or peptidoglycans first – they are transported with blood to the liver, where they will be neutralized. The continuous influx of microbial-derived products may cause disturbances in liver functioning and that might end up with inflammation in liver cells. That generates a response from receptors located on certain types of liver cells such as Kupffer cells, sinusoidal cells, biliary epithelial cells and hepatocytes (Chen et al., 2019). Gut microbiota produce many metabolites and part of them have a negative impact on the liver causing inflammation. Among them, we can highlight bile acids, short-chain fatty acids (SCFAs), choline and ethanol (Chiang et al., 2020). All of them are involved in the development of NAFLD. In this study, not all microbiome metabolites were included due to the character and purpose of this study.

SHORT-CHAIN FATTY ACIDS (SCFAs) ON NAFLD

Another important group of bacterial metabolites are short-chain fatty acids (SCFAs). SCFAs are mainly produced as a result of dietary fiber fermentation in the colon. Then they might be absorbed in the intestine (Liu et al., 2021; Rau et al., 2018). The most common SCFAs are acetate, propionate and butyrate, which are absorbed via the portal vein during lipid digestion. SCFAs are binding to G-protein coupled receptor (GPCR), which occurs in many organs, such as the liver, skeletal muscles, adipose tissue, and enteroendocrine L cells, exerting influence on their metabolic and immunological functions (Li et al., 2022). The short-chain fatty acids, mostly acetate and propionate can stimulate gluconeogenesis and lipogenesis, leading to fat accumulation in liver cells (Bastos et al., 2022). Butyrate is responsible for the suppression of insulin resistance and obesity while high fatty diet (HFD) intake in mice model (Gao et al., 2009). Butyrate also has anti-inflammatory potential – it can reduce inflammation in the intestines and reduce inflammatory response due
to activation of regulatory T cells, which will inhibit T cells and Th17 cells (Arpaia et al., 2013). Moreover, it reduces levels of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNFα) or monocyte chemotactic protein-1, while increasing levels of prostaglandin E2, which has an anti-inflammatory effect (Smith et al., 2013; Usami et al., 2008; Mouzaki et al., 2019). Butyrate is the most potent anti-inflammatory mediator and can reduce local inflammation in the gut and prevent the progression of inflammatory responses into the systemic circulation. Butyrate can also promote tight junction function and intestinal integrity. Another beneficial effect of butyrate is to provide colonicocytes with an energy source to maintain intestinal health. Dysbiosis leads to a decrease in butyrate levels which may cause increased gut permeability, and translocation of bacteria or lipopolysaccharides (LPS) into the systemic circulation, which can lead to the development of NALFD (butyrate promotes the regeneration of enterocytes). T-cells are responsible for releasing Glucagon-like peptides, that stimulate fatty acids β-oxidation in hepatocytes. They also increase insulin sensitivity in liver cells, thus they are important to NAFLD development (SvegliatiBaroni et al., 2011). The gut microbiota regulates bile acid homeostasis through biotransformations such as deconjugation, dehydroxylation, oxidation and desulfation. In terms of deconjugation, Bacteroides, Lactobacillus, Bifidobacterium, Clostridium and Listeria produce bile salt hydroxylases (BSH) that deconjugate the taurine and glycine groups in the primary bile acids produced in the liver (Ticho et al., 2019). In experimental animals with dysbiosis or treated with antibiotics, taurine deconjugation by intestinal bacteria was blocked, shifting the balance toward almost exclusively taurine-conjugated bile acids, resulting in increased taurine-conjugated bile acids in the liver, heart and kidneys (Arab et al., 2017). For dehydroxylation, Clostridium and Eubacterium of the Firmicutes tribe produce 7α-dehydroxylase of bile acid, which converts primary bile acids (cholic acid and chenodeoxycholic acid) into secondary bile acids (deoxycholic acid, lithocholic acid and ursodeoxycholic acid) (Lund et al., 2018). 7α-dehydroxylation occurs after deconjugation and is the most physiologically relevant conversion of bile acids in humans. Regarding oxidation, bacteria from genera such as Bacteroides, Clostridium, Eubacterium, Escherichia, Eubacterium lentum, Peptostreptococcus and Ruminococcus produce hydroxysteroid bile acid dehydrogenases (HSDH), which convert toxic bile acids into ursodeoxycholic acid, which is less toxic to human cells and more water-soluble (Ni et al., 2022). Finally, several gut bacteria, such as Clostridium sp. Strain S2, produce sulfatases that are able to enhance the desulphation of bile acids (Jackson et al., 2022). Desulfation of bile acids by intestinal bacteria facilitates the reabsorption of bile acids (Song et al., 2019) and is essential for the homeostasis of the bile acid pool (Moszak et al., 2021). In the liver are synthesized bile acids by the rate-limiting enzyme cytochrome P450 7A1 (CYP7A1) and secreted into the intestine. Intestinal epithelial cells (IECs) are reabsorbed bile acids by the distal ileum via apical sodium-dependent bile acid transporter (ASBT) where they activate farnesoid X receptor (FXR), inducing the expression and secretion of fibroblast growth factor 15/19 (FGF15/19) to inhibit hepatic bile acid synthesis. Luminal and basolateral bile acids activate Takeda G protein-coupled receptor 5 (TGR5) in enteroidendrine L-cells, resulting in the release of the incretin glucagon-like peptide-1 (GLP-1), promoting glucose tolerance (Chiang et al., 2020; Zhou et al., 2014). Overproduction of bile acids can promote diarrhea by several distinct mechanisms. In the colon, bile acids disrupt barrier integrity allowing bile acids to reach the basolateral membrane of epithelial cells and induce chloride secretion, though the receptors underlying this phenomenon are unknown. Colonic FXR and TGR5 activation inhibits the secretion of chloride and other electrolytes, which may be a compensatory mechanism. Colonic bile acids activate TGR5 on enterochromaffin cells (ECs), promoting 5-hydroxytryptamine (5-HT) release and motility. Bile acids activate neuronal TGR5, which either stimulates or inhibits motility, depending on the type of neuron and region of the GI tract. Overproduction of bile acids induces colonic secretion and enhances motility, producing a diarrheal phenotype (Wang et al., 2021; Krishnan et al., 2018).

MICROBIAL SYNTHESIS OF ETHANOL

Some bacteria have the ability to produce ethanol in the intestine as a result of polysaccharide fermentation. Normally, the amount of produced ethanol is small, but if dysbiosis occurs its amount may be significantly bigger than that in healthy controls (de Medeiros et al., 2015). The health effect of a high concentration of ethanol is an increase in the permeability of the intestinal barrier, which may result in the development of NAFLD and liver steatosis (Chen et al., 2020). Recent studies have shown that increased ethanol levels in the human body are associated with a high abundance of genera Escherichia, Bacteroides, Bifidobacterium, Clostridium and Klebsiella pneumoniae (Engstler et al., 2016; Mir et al., 2016). Zhu et al., in their study have observed that obese NASH patients have higher levels of endogenous ethanol than non-obese NASH patients or healthy controls. In turn, Yuan and others (Yuan J et al., 2019) proved that the presence of Klebsiella pneumoniae K14 was common for patients with NAFLD after transfer, isolates of bacteria cause NAFLD-like changes in mice. Also in children with NAFLD who were on the same diet as healthy children noticed that ethanol levels were significantly higher. Gut microbiota have enzymes which are involved in the metabolism of ethanol, e.g. aldehyde dehydrogenase. That enzyme converts ethanol into acetaldehyde and acetate. Acetaldehyde takes part in the translocation of microbiota metabolites due to the weakening tight junctions of the intestinal barrier (Schwimme et al., 2020).

OBESITY AND NON-ALCOHOLIC FATTY LIVER DISEASE

Obesity is associated with impaired intestinal barrier function and dysbiosis, resulting in metabolites and other bacterial products such as LPS and other molecular patterns associated with the microbiota, gaining access to the lamina propria in higher concentrations. Diet-induced increases in blood LPS concentrations are known as metabolic endotoxemia. Changes in the intestinal microbiota suggest that changes in diet and intestinal barrier function underlie elevated blood LPS concentrations. Alteration of intestinal TJ proteins, mainly zonula occludens-1 (ZO-1) and occludin, is a major molecular mechanism contributing to increased intestinal permeability (Lin et al., 2022). High-fat diets can lead to intestinal inflammation which in turn can result in TJ changes and increased intestinal permeability. Glucagon-like peptide 2 (GLP2), has been identified as a regulator of TJ protein expression and localization in obese mice. Although the expression pattern of metabolite receptors on
specific tissue-resident leukocytes, such as those in the lamina propria of the gastrointestinal tract, is still poorly understood, manipulation of immune cell metabolism by the gut microbiota may be a powerful approach. Gut-derived metabolites may be a powerful approach to direct the immune response. As is well known, obesity is a risk factor for the development of NAFLD, as well as metabolic syndrome (Rau et al., 2018). The prevalence of overweight and obesity has increased in children and adolescents worldwide over the past 30 years. Gut bacteria produce metabolites by modifying host metabolites, including secondary bile acids. In addition to the classic role of metabolites in bioenergetics and biosynthesis, metabolites can function as signaling molecules, much like hormones and neurotransmitters. Signaling metabolites generated from nutrients or by the intestinal microbiota primarily target the enterocorticoendoctrine, neuronal and immune cells in the lamina propria of the intestinal mucosa and liver, and through these tissues to the rest of the body. Metabolites from intermediary metabolism act as metabolic stressors in adipose tissue, liver and pancreas, being effective regulators of pro- and anti-inflammatory regulators of key immune cells, which function as important drivers of low-level inflammation associated with obesity. A large number of metabolite receptors are expressed on immune cells of both the innate and adaptive immune systems, and many different types of immune cells are found in the intestinal mucosa. In lean individuals, the immune cells of the lamina propria are dominated by anti-inflammatory subtypes of innate immune cells, including tolerogenic macrophages and dendritic cells, cosinophils, IL-22-producing innate lymphoid cells and resident regulatory T cells (Tregs) (Miura et al., 2014). Lean but not obese fat is enriched with a unique population of regulatory T cells that influence metabolic parameters. Diet and a sedentary lifestyle are determinants of obesity in children, but obesity in infants is influenced by the gut microbiota profile. As indicated by recently published studies Bacteroides and Lacto Bacillus spp. are the most common microbes involved in overweight in infants. However, to date, there are no certain species or genera identified as causing childhood obesity per se (Clemente et al., 2016). Early gut microbiota in infants can shape weight gain in childhood and controlling factors affecting early infant gut microbiota may prevent later overweight and obesity in children. Exposure to antibiotics changes the diversity of the gut microbiota. Infant exposure to antibiotics is strongly associated with childhood obesity. In children under <6 months of age, repeated antibiotic exposure puts boys at higher risk than girls of developing obesity.

CONCLUSION

In conclusion, bacterial metabolites mediate the communication between the commensal microbiota and the immune system, affecting the balance between pro- and anti-inflammatory mechanisms. Gut dysbiosis has been associated with NAFLD because the effects of an altered gut microbiota in abundance and diversity are mediated by many bacterial metabolites including bile acids, butyrate, choline, amino acids and ethanol. Modulation of the intestinal microbiota and supplementation of some bacterial metabolites may have a therapeutic benefit. The heterogeneous clinical features of NAFLD can be explained by consumption of various dietary factors, interpersonnel gut microbiome variability and genetics. In summary, the interplay of dietary factors, gut microbiota and intestinal barrier integrity plays an important role in the development of obesity and obesity-related NAFLD. Alteration of the gut microbiota is a result of diet, which can promote metabolic endotoxemia and inflammation contributing to the development of obesity and NAFLD. Understanding the role of the gut microbiome, and the mechanism of dietary factor influence gut microbiota, with the identification of components of the microbiome’s metabolic activity which significantly affect metabolism and possibly contribute to obesity and obesity-related NAFLD, might also be an important consideration for NAFLD treatment. Altogether, opportunities and challenges provided by microbiome research lead to future studies, which will hopefully elucidate the more specific role of gut microbiota in NAFLD and establish microbiota-targeted treatment approaches.

Highlights/Perceived strengths

• Intestinal microbiota plays an important role in the development of NAFLD.
• Impaired intestinal barrier integrity, metabolic endotoxemia and inflammation are closely associated with obesity and NAFLD.
• The gut microbiota and intestinal barrier function, promoting metabolic endotoxemia and inflammation can alter the absorption and metabolism of energy-reach foods and their ingredients (e.g., fat and fructose).
• Therapeutic manipulations with prebiotics and probiotics to modulate gut microbiota and maintain integrity of the intestinal barrier are potential efficient agents that should be further tested in NAFLD.

Declarations

Author Contributions. AWR, PC and PS drafted the manuscript. AWR and PS contributed to writing the manuscript and critical revision. All authors have read and approved the final manuscript.

Conflicts of Interest. All other authors report no conflict of interest.

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