Intestinal hormones, gut microbiota and nonalcoholic fatty liver disease (NAFLD)

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Keywords: NASH, fibrosis, GLP-1, leptin, probiotics

Funding: None

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of the metabolic syndrome and has a complex pathophysiology with multiple pathways of development and progression implicated. Intestinal hormones regulate multiple biological functions and may play a role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) by affecting food intake, body weight and insulin resistance. Bacterial products can affect the secretion of these hormones and thus have an effect on metabolism. Gut microbiota are normally involved in the intestinal energy harvest and their role has been increasingly been implicated in the pathogenesis of obesity and NAFLD. The intestinal hormone pathways as well as in the intestinal microbiota populations are potential therapeutic targets in the management of NAFLD. We review the evidence on the associations of the intestinal hormones and gut microbiota in the development, progression and treatment of NAFLD.
Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as the accumulation of fat in the liver of patients who do not consume excessive alcohol (1). It is the most common hepatic disease and depending on the population and the diagnostic methods that have been used has a prevalence of up to 35% (2). NAFLD is the hepatic manifestation of the metabolic syndrome and is usually associated with central obesity, dyslipidemia, insulin resistance and diabetes (3, 4). In terms of pathology, NAFLD includes two separate entities: non alcoholic fatty liver (NAFL) which is the accumulation of fat defined by the presence of steatosis in >5% of hepatocytes ('fatty liver’ or steatosis) with no inflammation or fibrosis and non alcoholic steatohepatitis (NASH). The latter requires the joint presence of steatosis, ballooning and lobular inflammation, carries a worse prognosis and might be associated with fibrosis, cirrhosis and hepatocellular carcinoma (5).

The pathogenesis of NAFLD is still unclear but appears to be multifactorial (1). Dietary factors including high calorie diet and high fructose intake as well as genetic factors have been implicated (5). In addition to these, the role of gut microbiota has been increasingly implicated over the last years as a possible factor contributing to NAFLD. The human gut contains an extensive number of microorganisms, known as the microbiota (6). The gut microbiota are important for several physiological functions including carbohydrate digestion, contribution of nutrients, vitamin biosynthesis, bile acid degradation and regulation of intestinal hormones (7). It consists of approximately $10^{11}$–$10^{12}$ bacteria that
reside in the colon, and $10^5$–$10^9$ bacteria in the jejunum and ileum (8). In healthy adults, Bacteroidetes (mainly gram negative bacteria like Bacteroides fragilis) and Firmicutes (mainly gram positive clostridia), are the predominant phyla of the large intestine (9). However, there is a significant variability and each individual has a unique composition of microbes (8).

The so-called liver–gut axis is the result of the tight anatomical connection between the liver and the gut. The liver receives 75% of its blood supply from the portal circulation; the blood flow originating from the intestines passes entirely through the liver where the necessary metabolic and immunologic processes take place before the blood finally flows to the systemic circulation (10). Therefore, the liver is exposed to the metabolic and inflammatory products of the intestinal bacteria that are transported there through the portal circulation. Multiple studies both in humans and animals have investigated the complex symbiotic relationship between the gut microbiota and the host. Available data indicate that there may be a possible causative role of microbiota in the development of obesity and NAFLD. Several mechanisms have been proposed and investigated.

Intestinal hormones are produced by the entero-endocrine cells, in response to nutritional and hormonal signals and regulate multiple biological functions including food intake, gastric emptying, gut motility, gut barrier formation, and glucose metabolism (11). Bacterial products can also affect the secretion of these hormones and thus have an effect on metabolism. These hormones, may play a role in the NAFLD pathogenesis by affecting food intake,
body weight and insulin resistance. Therefore, the metabolic pathway of the intestinal hormones has been the target not only for the treatment of diabetes but also for the treatment of NAFLD (11, 12).

In this review we describe the role of intestinal hormones that are implicated in the pathogenesis of NAFLD and the therapeutic interventions in the intestinal hormone pathway that have been found to be useful in the treatment of this expression of the metabolic syndrome. We also review the physiological aspects of the microbiota - human host symbiosis, the role of microbiota in energy harvest, intestinal hormone regulation and pathogenesis and treatment of NAFLD.

**Search strategy and selection criteria**

We searched Medline using the following search terms: “Intestinal hormones AND NAFLD” that indentified 66 results, “gut microbiota AND obesity” that indentified 1369 results and “gut microbiota AND NAFLD” that revealed 179 results. We largely included publications from the past 5 years, but we did not exclude highly relevant older publications. We also selected further relevant publications from the reference lists of articles identified by this search strategy.

**Intestinal hormones and NAFLD**
Multiple biological functions are physiologically regulated by gut hormones that are produced by the entero-endocrine cells, which consist about 1% of the intestinal cells (11). Among these, glucagon-like peptide 1 (GLP-1) has attracted the greater interest. GLP-1 is an incretin (i.e. an hormone that is released from the gut into the bloodstream in response to food ingestion) which is produced from the entero-endocrine cells in the distal small intestine and colon (13). The fasting plasma levels of GLP-1 increase approximately 2-3 folds reaching the peak levels about 20-30 minutes after a meal (13). The secretion of GLP-1 is mainly induced by nutritional elements like carbohydrates, lipids and proteins. Interestingly, it can also be induced by gut bacterial products: non digestible carbohydrates that reach the colon are metabolized by bacteria to short chain fatty acids (SCFA) like butyrate, propionate, and acetate which serve as an energy source to colonic epithelium (14). These bacteria-derived SCFA can also interact with the host and modify the levels of gut hormones that are produced by entero-endocrine cells and thus regulate energy homeostasis (11, 14). SCFA can activate selected G-protein-coupled receptors (GPCRs) on these cells and thus promote secretion of gut hormones like GLP-1 (13).

GLP-1 is a significant hormone that has attracted great interest since it maintains glucose-dependent insulin secretion, promotes augmentation of b-cell mass in the pancreas and improves oral glucose tolerance and insulin sensitivity (11, 14). In addition, GLP-1 inhibits gastric emptying and GI motility, mainly via vagal nerve mediated mechanisms and also targets the brain by improving satiety and thus decreasing food intake (15, 16). Results from a randomized
controlled trial that showed ultrasonographic improvement in patients with NAFLD taking a probiotic called VSL#3, showed that this benefit was mediated by GLP-1 increase (17).

GLP-1 secretion may be also affected by hormonal factors since enterochromafine cells express receptors for hormones like insulin and leptin (13). Leptin is mainly produced by the adipose tissue and is involved in the pathogenesis of NASH by contributing to the development of insulin resistance and subsequently to steatosis (3).

DPP-4 is an enzyme that degrades GLP-1 as well as other intestinal hormones like the gastric inhibitory polypeptide (GIP). The latter was the first isolated incretin which also induces insulin secretion (13). Commonly used anti-diabetic drugs like sitagliptin belong to the category of DPP-4 inhibitors that maintain their antihyperglycemic action mainly by preventing the degradation of GLP-1. DPP-4 also metabolizes peptide tyrosine tyrosine (PYY). PYY is secreted postprandially mostly by the same intestinal L cells which also express GLP1. This hormone delays gastric emptying and has an anorectic effect (18). The potential role of DDP-4 inhibitors in NAFLD has not been extensively tested. In a mouse model, sitagliptin seemed to prevent the development of hepatic steatosis in animals fed with diet rich in sucrose and fatty acids (19). Some small trials in humans have shown some benefit in liver biochemistry and steatosis but there are no studies available that include histological data after DDP-4 inhibition therapy (20). A recent randomized, double-blind, placebo-controlled trial that included 50 NAFLD patients with pre-diabetes or early
diabetes did not show any benefit of sitagliptin over placebo in reducing liver fat or improving liver biochemistry. This study assessed fat in liver with MRI-derived proton density-fat fraction and MR spectroscopy (21).

Another category of antidiabetic drugs are the GLP-1R agonists which are resistant to DPP-4 inactivation. Liraglutide is probably the best studied drug for NAFLD that acts through the GLP-1 pathway. It is a long-acting GLP-1 analogue that has been licensed for glycaemic control in overweight patients with type 2 diabetes (12) and for the treatment of obesity. A large meta-analysis of patients with type 2 diabetes and elevated liver enzymes treated with liraglutide showed an improvement in liver biochemistry (22), whereas a pilot study demonstrated that treatment with liraglutide had a good safety profile and significantly improved liver function and histological features in NASH patients (23). An important recent multicentre, double-blinded, randomized, placebo-controlled trial assessed the safety and efficacy of liraglutide, in patients with NASH. Liraglutide given subcutaneously was found to be safe, well tolerated, and led to histological resolution of NASH, in 9 out of 26 patients in the drug group, compared to 2 out of 26 in the placebo group (12). On the contrary, a recently published placebo-controlled randomised trial that included 52 patients and assessed the effects of a 12-week course of liraglutide or sitagliptin on spectroscopy-measured hepatic steatosis in patients with type 2 diabetes, did not show a significant effect on hepatic steatosis (24).

The secretion of a similar peptide called glucagon-like peptide 2 (GLP-2) by the entero-endocrine cells can be also induced by bacteria derived SCFA.
GLP-2 has been found to maintain the intestinal barrier by inducing intestinal epithelial cell proliferation and increasing the production of intestinal tight junction proteins (11). Prebiotic treated mice exhibited a decreased hepatic expression of inflammatory and oxidative stress markers. This decrease was associated with a lower intestinal permeability and improved tight-junction integrity compared to controls, which occurred in parallel with increased endogenous (GLP-2) production. Importantly, when the mice were given a GLP-2 antagonist, most of the prebiotic effects were abolished (25).

Ghrelin is a gut hormone produced mainly by the stomach and the small intestine which has the opposite functions in basically all endocrine and metabolic target organs compared to GLP-1, as well as the opposite secretion patterns in response to food intake (16). Ghrelin is the only well-established peripherally produced orexigenic or hunger hormone and exerts its effect mainly through receptors in the central nervous system and possibly through afferent vagal mechanisms. It increases adiposity and decreases insulin secretion while stimulating glucagon secretion (16). In a study that included 75 morbidly obese patients with biopsy-proven NAFLD (41 of which had NASH), it was shown that patients with NASH had a two-fold higher concentration of des-acyl ghrelin than non-NASH patients (26). In addition, ghrelin concentrations in NASH patients with fibrosis stage ≥2 were almost double the concentration of NASH patients with fibrosis stage <2 indicating that the products of the ghrelin pathway may be important for the pathogenesis of NASH and fibrosis (26). The potential role of intestinal hormones in the pathogenesis of NASH is illustrated in Figure 1.
The role of gut microbiota in energy harvest and obesity

The human intestinal microbiota has a symbiotic relationship with its host and contributes nutrients and energy by metabolizing dietary components in the large intestine. Non-digestible carbohydrates of plant origin that reach the colon are metabolized by bacteria to SCFA like butyrate, propionate, and acetate which serve as an important energy source to colonic epithelium (14, 27). Microbiota-derived butyrate enters the portal circulation and is transferred to the liver. There it enters the citric acid cycle via the production of acetyl-CoA and can thus enhance glycogen synthesis, decrease glucose oxidation and increase hepatic glycogen storage (14, 28). As mentioned above, SCFA can induce insulin secretion and satiety through the GLP-1 pathway. Therefore, the role of SCFAs is somehow complex, since on the one hand they enhance energy harvest and contribute to excess lipogenesis in the liver, but on the other hand they concurrently increase insulin secretion and sensitivity, and enhance satiety (29). This depends on the particular SCFA; butyrate and propionate are considered predominantly anti-obesogenic. Butyrate is a major energy source for colonocytes but on the other hand improves insulin sensitivity, increases leptin expression, possess anti-inflammatory potential, increases intestinal barrier function and protects against diet-induced obesity (30). Propionate inhibits cholesterol synthesis, thereby antagonizing the cholesterol increasing action of acetate, and also inhibits the expression of resistin in adipocytes. Moreover, both
these SCFAs have been found to cause weight regulation through their stimulatory effect on anorexigenic gut hormones and to increase the synthesis of leptin. On the other hand, acetate shows more obesogenic potential, as it acts as a substrate for synthesis of cholesterol and contributes in the synthesis of lipids in the liver (30).

Turnbaugh et al showed that the microbiome from genetically obese mice has an increased capacity to harvest energy from the diet since they have significantly less energy remaining in their feces when compared to their lean littermates (31). Even more interesting was the finding that this trait was transmissible: colonization of germ-free mice with an ‘obese microbiome’ resulted in a significantly higher increase in total body fat than colonization with a ‘lean microbiome’ (31). Similarly, a recent study by Panasevich et al showed that the type of populations and the metabolic capacity of the microbiota in low-aerobically fit rats may contribute to their susceptibility to acute high fat diet (HFD) induced hepatic steatosis (32). Low-aerobically fit rats had a greater propensity to gain weight and develop steatosis in response to an acute HFD compared with high-aerobically fit rats. It was suggested that the physiologic changes observed in the low-aerobically fit rats fed with an acute HFD appeared to be associated with decreases in SCFA-producing microbiota (32).

A recent study by Chevalier et al suggested that intestinal energy harvest was increased during acute cold and that this increase contributed to maintaining stable body temperature. In parallel to this, exposure to cold resulted in marked changes in the composition of gut microbiota. Importantly, this shift in the
bacterial composition was associated with an increase in energy harvest thus highlighting the role of gut microbiota in energy harvest and host homeostasis (33). Additionally, mice transplanted with ‘cold microbiota’ showed increased sensitivity to insulin, suggesting that ‘cold microbiota’ alone is sufficient to transfer part of the increased insulin sensitivity phenotype (33).

Gut microbiota have been implicated in the development of obesity and thus contribute in the development of NAFLD. Available data from studies performed in mice support this hypothesis. Ley et al compared the microbiota of lean and obese mice and regardless of kinship, obese animals had a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes (34). Backhead et al found that exposure of adult germ-free mice to a normal microbiota originating from the distal intestine of conventionally raised animals resulted in a significant increase in body fat content and also in insulin resistance. Interestingly these results became apparent within days and occurred despite reduced food intake (6). Duca et al demonstrated that obese prone (OP) and obese resistant (OR) mice phenotypes were associated with distinct and differing gut microbial communities only during high fat. Strikingly, phenotype and behavioral differences between OP and OR rats were reliably transferred to animals as long as they were on a high fat diet. OP as well as mice inoculated with OP microbiota had a significantly greater 24-h food intake and adiposity index than the others during HF feeding but not chow feeding. In addition, circulating leptin and insulin levels were significantly increased in OP recipient animals as were triglyceride and glycemia levels, features all associated with
metabolic syndrome (35). Finally, on HF feeding but not on chow feeding, OP and the OP recipient animals both exhibited altered tight junction protein levels indicating an impaired mucosal barrier (35).

Apart from increased energy harvest, a link between gut microbiota and obesity can be found in the impact that microbiota might have on appetite control. A recently published study by Breton et al suggested that E. Coli derived proteins may have a direct short-term effect on satiety by acting locally in the intestine. The release of gut hormones like GLP-1 and PYY could mediate this effect (36). In addition, the same study showed that bacterial derived proteins may also have a long term impact on the central control of appetite by activating central anorexic circuitries (36).

Studies in humans have shown differences between obese and lean people with regard to the two dominant groups of bacteria that reside in their gut. The relative proportion of Bacteroidetes is decreased in obese people compared to lean people. This proportion, however, reverses with weight loss on low-calorie diet indicating that manipulation of gut microbial communities could be a possible approach in the treatment of obesity (37). Nevertheless, there are several studies that found contradictory results with regard to the ratio of abundance of Firmicutes to Bacteroidetes (10). Scwiertz et al found that the ratio of Firmicutes to Bacteroidetes changed in favor of the Bacteroidetes in overweight and obese subjects (38). Hence, available data are still inconclusive and the question as to whether obesity alters the microbiome, or if the microbiome alters the risk for obesity remains and requires further long term research (10).
An inpatient study that included 21 individuals showed that an altered nutrient load induced rapid changes in the bacterial composition of the human gut microbiota. During this study the amount of calories that were ingested and expelled in stool were measured. It was found that the alteration of the nutrient load induced a change in gut microbiota; it resulted in an increase in the abundance of Firmicutes and a corresponding decrease in Bacteroidetes which was associated with an increased energy harvest of approximately 150 Kcal (39).

In an interesting study by Kalliomaki et al it was suggested that differences in the intestinal microbiota may precede the development of obesity. The abundance of Bifidobacteria in the first year of life was higher in children who had a normal weight at the age of seven compared to children who were overweight (40).

**Gut microbiota and NAFLD**

Data from animal studies provide evidence that gut microbiota could be a causative factor for the development of NAFLD and that the gut microbiota-mediated metabolic phenotype could be transmissible. A recent study by Le Roy et al showed that, germ-free mice that received intestinal bacteria from high blood glucose mice that were on a high-fat diet, were more likely to develop hepatic steatosis and insulin resistance compared to the subjects that were transplanted with bacteria from mice that although they were on a high fat diet as well, they had not developed high blood glucose levels (41, 42). Additionally, the
results presented by Hanao Mejia et al provided evidence that modulation of the intestinal microbiota through multiple inflammasome components is a critical determinant of NAFLD/NASH progression. In the gut, the combination of host related factors including inflammasome deficiency-associated dysbiosis resulted in abnormal accumulation of bacterial products in the portal circulation like toll-like receptors (TLR) agonists whose influx into the portal circulation was sufficient to drive progression of NAFLD/NASH (43). Importantly, co-housing of inflammasome-deficient mice with wild-type mice resulted to the transmission of a NASH phenotype through the transmission of the microbiome (43). A study by Zeng et al, also performed in mice, showed that high fat feeding promotes certain predominant hind gut bacteria like Lactobacillus gasseri and/or Lactobacillus taiwanensis in addition to the development of NASH (44).

NAFLD phenotypes have also been observed in humans; in a cohort of 61 pediatric patients with NAFLD/NASH and 54 healthy controls, there were specific microbiota signatures associated with NAFLD onset and progression to NASH (45). A recent study found that an increased abundance of the Bacteroidetes genus was independently associated with NASH, and in addition, an increased abundance of the Ruminococcus genus was independently associated with fibrosis (46). Mouzaki et al showed that the percentage of Bacteroidetes was significantly lower in patients with biopsy proven NASH compared to healthy controls and subjects with NAFL. Interestingly, the low abundance of Bacteroidetes in NASH was independent of BMI and energy intake from fat.
indicating a possible causative factor of the type of microbiota in the development of NASH (47).

A biochemical link has been suggested between bacteria derived volatile organic compounds and NASH. Recent findings from the study by Reid et al performed in mice showed that differences in portal venous bacteria derived volatile organic compounds levels were associated with diet-induced NASH (48). An observational study did not only find differences in the type of microbiota between NAFLD and healthy volunteers, but also in the volatile bacterial metabolites that were detected in the stools that are considered potentially toxic for the liver (49). Similar results came from the recent study by Chierico et al in which 26 organic compounds including alcohols, acids, aldehydes, ketones, amines, and esters that result from microbial actions were upregulated in the feces of pediatric patients with NAFLD compared to controls (45). The same study apart from significantly lower levels of Oscillospira, found significantly higher levels of 1-pentanol and 2-butanone, (both volatile organic compounds) in NAFLD patients compared to controls indicating that high levels of 2-butanone and low relative abundance of Oscillospira could be a potential fecal biomarker profile for liver steatosis (45).

Gut microbiota might also contribute to the development of NAFLD via the production of ethanol. Intestinal microbiota produces a number of potentially hepatotoxic substances including ethanol that are transported to the liver by the portal system. Acetaldehyde and acetate are two major metabolites of ethanol. Acetaldehyde and its metabolites may lead to the formation of reactive oxygen
species that are implicated with liver injury, whereas the latter is a substrate for fatty acid synthesis (50) Nair et al observed higher breath ethanol concentrations in obese women than in leaner ones (15). A study by Zhu et al performed in pediatric population showed evidence of higher ethanol blood levels and higher abundance of alcohol-producing bacteria in the gut of subjects with NASH compared to healthy controls, thereby supporting a possible role for alcohol-producing microbiota in the pathogenesis of NASH (51). This hypothesis could explain the similarities with regard to histological and biochemical findings that are present between alcoholic and nonalcoholic liver disease (1).

Choline is a component of cell membranes that is found in foods such as red meat and eggs but can be also endogenously synthesized. In the liver, choline is used for the synthesis of VLDL. Therefore, choline deficiency resulting from decreased intake, could prevent synthesis and excretion of VLDL, leading to hepatic triglyceride accumulation and hepatic steatosis (52). It has been suggested that gut bacteria affect the bioavailability of dietary choline to the host and can therefore influence the organism’s need for choline (53). Spencer et al showed that manipulations in dietary choline affected the type of gut microbiota as well as the amount of liver fat and indicated that specific members of the microbial community could predict susceptibility to choline deficiency induced fatty liver disease (53). The composition of gut microbiota before the induction of a low-choline diet intervention correlated with the development of NAFL, thus suggesting that the combination of choline dietary deficiency with a specific gut microbiota subtype could contribute to the development of NAFLD (53).
Butyrate, which is the basic bacteria derived SCFA, markedly increases epithelial cell proliferation and differentiation, and thus improves colonic barrier function in the normal gut (11). Patients with NAFLD have increased intestinal permeability, and this was associated with changes in normal small bowel microbiota and increased prevalence of small intestinal bacterial overgrowth (54). This phenomenon may be associated with disruption of intercellular tight junctions of the intestine (54). Duca showed that mice on high fat feeding but not chow feeding, OP and the OP microbe recipient animals both exhibited altered tight junction protein levels indicating an impaired mucosal barrier (35). A recent study by Rahman et al performed in mice provided significant evidence that intestinal epithelial barrier dysfunction and microbial dysbiosis contribute to development of NASH (55). They showed that mice with disruption of the gene (F11r) encoding junctional adhesion molecule fed on diet high in saturated fat, fructose, and cholesterol (HFCD) for 8 weeks developed typical histologic features of severe NASH. In addition, this diet led to significant increase in inflammatory microbial taxa in F11r−/−, compared with control mice. Liver injury was also associated with significant increases in mucosal inflammation, tight junction disruption, and intestinal epithelial permeability to bacterial endotoxins (55). In this mouse model, a high calorie diet provided the first ‘hit’ favoring a pro-inflammatory gut microbial composition, which exacerbated gut permeability. In turn, enhanced gut leakiness resulted in microbial product translocation, which induced hepatic inflammation and injury ultimately resulting in the progression of NAFLD to NASH (55).
NAFLD and therapeutic interventions in gut microbiota

In view of a potentially beneficial role in NAFLD, therapeutic interventions in gut microbiota have attracted great research interest. As mentioned above, gut microbiota may influence energy harvest and affect satiety. Given these roles, randomized studies performed both in adults and children have investigated the role of probiotics and have shown promising results. Probiotics are live microorganisms that provide health benefit to the host when administered in adequate amounts by influencing the intestinal microbial ecology. A prebiotic is a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota, (i.e. a fiber). The synergistic combination of prebiotics and probiotics is described as synbiotic. Modulations of the gut microbiota with the use of probiotics and/or symbiotics can result in adaptations in regulating gut hormones and thereby reduce energy harvest, enhance the feeling of satiety, improve glucose metabolism and also improve gut barrier function and thereby ameliorate endotoxaemia and inflammation that are often found in obesity and type 2 diabetes.

A study by Cano et al performed in mice suggested that the administration of B. pseudocatenulatum in high fat diet-fed mice reduced hepatic steatosis. In addition, it reduced serum cholesterol, triglyceride, and glucose levels, decreased insulin resistance and improved glucose tolerance.

In a randomized trial including 20 patients with biopsy proven NASH, a 6-month course of a lactobacillus based formula improved steatosis and AST levels
The use of a probiotic yogurt containing Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12 for 28 weeks resulted in improved liver enzymes in a randomized trial of 38 patients with NAFLD (59). A small randomized trial of 30 patients with NAFLD evaluating a preparation of Lactobacillus bulgaricus and Streptococcus thermophiles administered for 3 months demonstrated an improvement in ALT levels (60). Similarly, a randomized controlled trial that included 38 subjects with metabolic syndrome, showed that a 28 week course of symbiotic therapy containing 200 million of seven strains of “friendly” bacteria resulted in significant improvement in liver biochemical tests and in various inflammatory markers (61).

A randomized trial in 48 children with histologically proven NAFLD, showed that a 4-month probiotic therapy with VSL#3 resulted in a significant improvement of ultrasonographic findings (17). Another randomized controlled study that included 22 children, showed that probiotic treatment with the Lactobacillus rhamnosus strain GG for 8 weeks improved ALT levels, but failed to improve ultrasonographic findings (62).

Although short term randomized trials have shown some promising results in the use of probiotics in the treatment of NAFLD, considering the longstanding course of the disease, larger long-term studies with appropriate histological outcomes are essential. Moreover, standardization of the probiotic composition and dose is required for meaningful conclusions.

**Conclusions**
Gut microbiota play a very important role in the homeostasis of human organism as they produce substances that serve as nutrient products, regulate energy harvest and contribute to vitamin biosynthesis and bile acid degradation. By affecting the secretion of gut hormones, microbiota can target multiple organs including the pancreas and the brain and thus contribute in insulin secretion, glucose regulation and satiety. Intestinal hormones are produced by the entero-endocrine cells, in response to nutritional and hormonal signals and regulate multiple biological functions. GLP-1 has a beneficial role in homeostasis by increasing insulin secretion and promoting euglucemia. Recent evidence suggests that GLP agonists could be beneficial in the treatment of NAFLD. Apart from GLP-1, GLP-2 and PYY are also intestinal hormones that improve insulin secretion and energy homeostasis whereas ghrelin has an opposite role by acting as a pro hunger hormone. The association of specific types of gut microbiota with obesity and NAFLD is still under investigation. Most studies indicate different populations of microbiota between lean and obese people as well as among different phenotypes of NAFLD. Available data from both animal and human studies suggest that a causative link of gut microbiota in NAFLD could be present though multiple mechanisms including increased energy harvest, affected intestinal barrier, production of ethanol and impaired choline metabolism. However, further long-term studies are necessary in order to confirm this conclusion, which in turn could further attract interest in the manipulation of the gut microbiome as a possible therapeutic target for the management of NAFLD. Available studies that mostly include probiotics have
shown encouraging results. However, larger long-term studies that would ideally include histologic confirmation of improvement in NAFLD are necessary in order to confirm the beneficial role of this approach.

Acknowledgments:

Dr Koukias received an educational scholarship from the Hellenic Association for the Study of the Liver. Dr Buzzetti was supported by the Andrew Burroughs Fellowship from AIGO (Associazione Italiana Gastroenterologi Ospedalieri) and M.I.M.I (Montecatini Interactive Medicine International).
References


5. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016.


32. Panasevich MR, Morris EM, Chintapalli SV, Wankhade UD, Shankar K, Britton SL, et al. Gut microbiota are linked to increased susceptibility to hepatic


Intestinal hormones are produced by the enteroendocrine (EEC) cells located in the gastro-intestinal system, in response to nutritional and hormonal signals. Products derived from intestinal bacterial metabolism can further influence their secretion. Some of these hormones act with an antagonistic effect: GLP-1 improves oral glucose tolerance and insulin sensitivity by increasing glucose-dependent insulin secretion and promoting augmentation of pancreatic β-cell mass. Conversely, ghrelin increases adiposity and decreases insulin while stimulating glucagon secretion in the pancreas. GLP-1 reduces gastric emptying and GI motility, mainly via vagal-mediated mechanisms, and targets the brain by stimulating satiety and thus decreasing food intake. Ghrelin also acts on the nervous system but has an orexigenic effect and stimulates gastric emptying.

There is evidence supporting that GLP-1 could have a beneficial effect on NAFLD development and progression, while products of the ghrelin gene may be involved in the pathogenesis of NASH and fibrosis. GLP-2 maintains the intestinal barrier by inducing intestinal epithelial cell proliferation and increasing the production of intestinal tight junction proteins.

Abbreviations: EEC, enteroendocrine cells; SCFA, short chain fatty acids; GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2.