Review

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Review

The Many Faces of Metabolic Dysfunction-Associated Fatty Liver Disease Treatment: From the Mediterranean Diet to Fecal Microbiota Transplantation

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Abstract: The gastrointestinal tract is inhabited by the gut microbiota. The main phyla are *Firmicutes* and *Bacteroidetes*. In non-alcoholic fatty liver disease, now renamed metabolic dysfunction-associated fatty liver disease (MAFLD), an increase in *Firmicutes* and *Bacteroidetes* abundance promotes its pathogenesis and evolution into non-alcoholic steatohepatitis, liver cirrhosis and hepatocellular carcinoma. For this reason, an early treatment is necessary to disfavor its progression. The aim of the present narrative review is to evaluate the different therapeutic approaches to MAFLD. The most important treatment for MAFLD is lifestyle changes. In this regard, the Mediterranean diet could be considered the gold standard in the prevention and treatment of MAFLD. In contrast, a Western diet should be discouraged. Probiotics and fecal microbiota transplantation seem to be valid, safe, and effective alternatives for MAFLD treatment. However, more studies with a longer follow up and with a larger cohort of patients are needed to underline the more effective approaches to contrasting MAFLD.

Keywords: steatosis; probiotics; gut-liver axis; leaky gut

1. Introduction

The nomenclature non-alcoholic fatty liver disease (NAFLD), coined in 1980, indicates the presence of steatotic liver disease in the absence of other chronic liver diseases or alcohol consumption of more than 140 g/week for women and 210 g/week for men. However, due to the dysmetabolic comorbidities that commonly affect NAFLD patients, it was recently renamed to metabolic dysfunction-associated fatty liver disease (MAFLD) [1]. MAFLD is a clinical condition mainly characterized by the accumulation of fat in the liver parenchyma (>5% of hepatocytes). The pathological spectrum ranges from simple fatty liver to non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC). More advanced stages of the disease are associated with higher mortality, but all stages of MAFLD can significantly increase the risk of cardiovascular disease. MAFLD is a common cause of chronic liver disease worldwide. The histopathological sign of MAFLD is represented by hepatic steatosis, characterized by the accumulation of lipid droplets in hepatocytes. Signs of cell damage such as swelling, apoptotic changes and Mallory-Denk bodies are also typical, while portal and lobular inflammatory infiltrates are more characteristic of the NASH stage. The global incidence of MAFLD is 47 cases per 1,000 population [2]. In recent years, the global prevalence of the disease has been steadily increasing, from 25.3% in 1990-2006 to 38% in 2016-2019. In addition, the prevalence in men is higher than in women (40% and 26%, respectively) [2,3]. These data in South America are scarce. In Brazil, Chile, Mexico, and Colombia the prevalence was 35.2%,

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23%, 17% and 26.6% respectively [4]. The pathophysiological mechanisms underlying MAFLD are usually explained by the two-hit hypothesis, in which two damaging events occurring in sequence compromise the function and structure of the liver parenchyma: the accumulation of fatty acids in the liver, and subsequently the progressive appearance of oxidative stress and hepatocyte damage. This classic scheme is considered obsolete and has been replaced by the concept of multiple hits acting in parallel including insulin resistance, oxidative stress, genetic and epigenetic factors, the gut microbiota and environmental elements. The diagnosis of MAFLD is based on the presence of fatty liver detected by ultrasonography in the absence of the other causes (virus, alcohol, drugs), and the presence of dysmetabolic comorbidities such as overweight or obesity, hypertension and type 2 diabetes mellitus. In fact, the histological evaluation of the liver is not required for the diagnosis of MAFLD [5]. Proper management of these patients is necessary in preventing some liver complications, such as NASH, liver cirrhosis and HCC. There is considerable evidence of a link between MAFLD, dysbiosis and lifestyle: namely, that the synergy between the MD, physical activity and gut eubiosis promotes liver health. In this context, probiotics and fecal microbiota transplantation (FMT) have become the most promising treatments in clinical practice, based on the pivotal role of the "gut-liver axis" in the progression of MAFLD (Figure 1). The aim of the present narrative review is to evaluate the different therapeutic approaches in MAFLD.

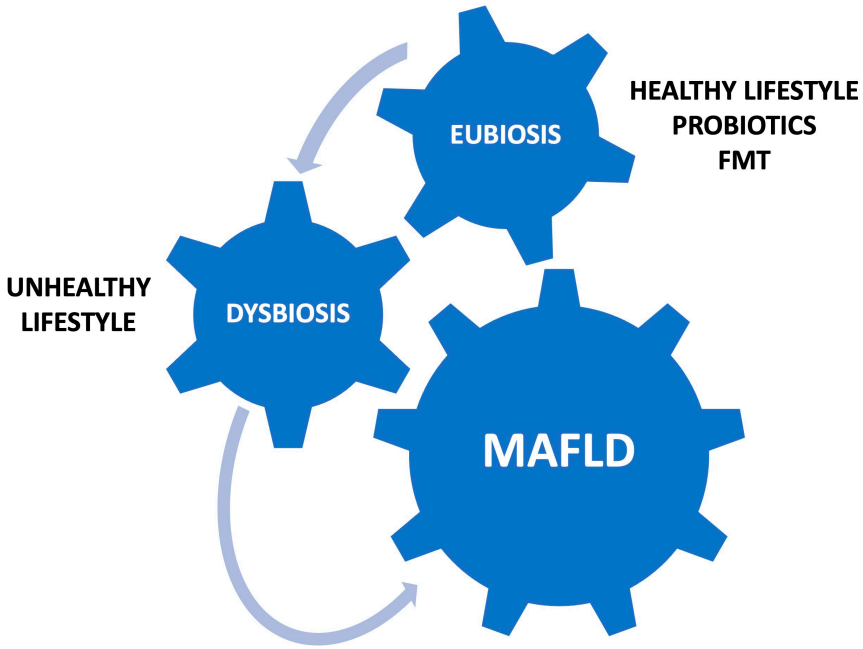

Figure 1. Schematic representation of the involvement of the gut-liver axis in MAFLD pathogenesis.

2. Gut Dysbiosis and MAFLD

The gastrointestinal tract is inhabited by the gut microbiota, a heterogeneous ecosystem of 10^{14} bacteria. The main phyla are Firmicutes and Bacteroidetes, followed by Actinobacteria, Cyanobacteria, Fusobacteria, Proteobacteria, and Verrucomicrobia [6]. Other components are fungi, archaea, phages, and viruses [7]. The microbiota begins to colonize the host at the moment of birth, although the paradigm of uterine sterility has recently been challenged. During and after birth, the neonatal gut is colonized by a variety of microbes. This process is conditioned by several factors: mode of birth, type of breastfeeding, hygienic conditions, exposure to antibiotic treatments. Usually, the gut microbial population takes on the configuration of an adult microflora during the first five years of life, even though it represents an ecosystem with a dynamic evolution. With a population of over 100 trillion microorganisms, the gastrointestinal tract is one of the most complex ecosystems found in nature. The gut microbiota is defined as a superorganism that is essential for host health

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and performs various functions such as immune homeostasis, which is essential in counteracting colonization by pathogenic bacteria and in maintaining the integrity of the intestinal barrier. In addition, it supports the health of the host by promoting the absorption of nutrients by providing enzymatic pathways that the host lacks. It also promotes the production of vitamins K and B, and short-chain fatty acids (SCFAs) [8]. The interaction between the gut microbiota, the immune system and the liver is defined as "gut-liver axis" [9]. Gut dysbiosis is an alteration in the structure and function of the gut microbiota, characterised by a decrease in "good" bacteria abundance and an increase in "bad" bacteria abundance, or a reduction of bacterial diversity. For this reason, it plays a central role in the pathogenesis of MAFLD [10]. In this way, the gut microbiota shows a reduced diversity at the phylum and family level. In patients with MAFLD, an increase in Proteobacteria at the phylum level, Enterobacteriaceae at the family level and *Escherichia*, *Dorea*, *Peptoniphilus* at the genus level was observed, compared to healthy individuals. At the same time, a decrease in Rikenellaceae and Ruminococcaceae at family level and in *Anaerosporobacter*, *Coprococcus*, *Eubacterium*, *Faecalibacterium* and *Prevotella* at the genus level was shown [11]. In a cross-sectional study, the gut microbiota of MAFLD patients was analysed using next-generation sequencing. As reported by the Authors, the Firmicutes/Bacteroidetes ratio was positively correlated with liver steatosis in the obese group [12]. Gut dysbiosis increases the production of SCFAs, leading to increased fat accumulation in the liver. SCFAs bind to G protein-coupled receptors 43 and 41, which are also expressed in adipocytes, inhibiting lipolysis and adipocyte differentiation. On the other hand, elevated levels of SCFAs stimulate the expression of carbohydrate response element binding protein (ChREBP). Monosaccharides from microbial fermentation activate hepatic ChREBP and consequently increase the levels of proteins involved in hepatic lipogenesis [13]. In addition, very-low-density lipoprotein synthesis is reduced with a consequent decrease in hepatic lipid export. Moreover, gut imbalance promotes hepatic inflammation by increasing intestinal permeability, known as "leaky gut" [14]. The translocation of bacteria and pathogen-associated molecular pattern molecules stimulates inflammatory response in the liver and subsequently steatosis [15]. In summary, in MAFLD there is a disequilibrium in Firmicutes/Bacteroidetes ratio, and this event promotes its pathogenesis and the development of NASH, liver cirrhosis and HCC [15].

3. Dietary Regimens in MAFLD

MAFLD is considered the hepatic manifestation of the metabolic syndrome, exacerbated by a high-calorie diet in genetically predisposed individuals [16]. Obesity plays a central role in the development of MAFLD: patients are mainly obese or overweight, with only a small part consisting of lean subjects [17]. Two of the main dietary approaches are Mediterranean diet (MD) and Western diet (WD). MD is a diet characterized by low saturated fat and high vegetable oils. MD contains several natural compounds with antioxidant, anti-inflammatory, antihypertensive, lipid-lowering, anti-diabetic, and anti-obesity effects [18]. For example, extra virgin olive oil with a high oleocanthal content is associated with a reduction in body mass index (BMI), transaminases and cytokine levels [17]. Tomatoes with the main component lycopene (LYC) reduce serum and hepatic fat levels, but the mechanism is still unclear. In addition, LYC induces the expression of cellular antioxidant enzymes and reduces the activity of reactive oxygen species-producing enzymes [18]. A prospective cohort study showed that the MD improved anthropometric parameters and lipid profile and reduced hepatic steatosis and liver stiffness. In addition, it underlined that the combination of antioxidant complex and diet improved insulin resistance, hepatic steatosis, and liver stiffness, comparing to a control diet [19]. Another study evaluated the clinical efficacy of MD in MAFLD patients. At the end of the treatment, BMI, waist circumference, waist-to-hip ratio, aspartate amino transferase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT), high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), serum glucose, total-cholesterol/HDL ratio, LDL/HDL ratio, TG/HDL ratio, homeostatic model assessment-insulin resistance (HOMA-IR), fatty liver index (FLI), Kotronen index and fatty liver score showed a significant improvement ($p<0.01$) [20]. On the other hand, WD is a dietary regimen rich in protein, fat and refined sugars characterised by overeating, frequent snacking, and a prolonged postprandial

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state. In a study performed by Bäckhed et al., the gut microbiota of high-fat diet-induced obese mice was transferred to germ-free mice. This transfer caused metabolic syndrome with alteration of the epithelial barrier [21]. This dietary approach has been linked to the promotion of dysbiosis and MAFLD [22]. In this regard, a large prospective cohort study evaluated the effects of the WD diet and a Prudent diet in 3527 patients with MAFLD, 1643 with liver cirrhosis and 669 patients with liver cancer. The dietary pattern was assessed with a food questionnaire. The Authors underlined the correlation between WD and increased risk of chronic liver diseases, while the Prudent diet was associated with a lower risk of liver cirrhosis [23]. These data showed the effect of lifestyle in the progression and prevention of MAFLD. In fact, the most important treatment for MAFLD has been shown to be lifestyle modification [24]. MD could be considered the gold standard in the prevention and treatment of MAFLD, and for this reason, a strict adherence to the traditional MD can help MAFLD patients in achieving a healthy state. On the contrary, WD should be discouraged [25]. Table 1 summarizes the studies about the use of different dietary regimens in MAFLD patients.

Table 1. Summary of studies about the use of different dietary regimens in MAFLD patients.

Study design	Study groups	Intervention	Outcomes
Randomized controlled trial [19]	Overweight-MAFLD group (*n*=50)	Moderately hypocaloric MD or MD diet and antioxidant supplementation or no treatment for six months	Significant improvement of anthropometric parameters, lipid profile, liver steatosis, and liver stiffness in group treated with MD diet and antioxidant supplementation
Uncontrolled trial [20]	MAFLD group (*n*=46)	MD and moderate physical activity for 6 months	Significant improvement of BMI, waist circumference, waist-to-hip ratio, AST, ALT, GGT, HDL, LDL, TG, serum glucose, total-cholesterol/HDL ratio, LDL/HDL ratio, TG/HDL ratio, HOMA-IR, FLI, Kotronen index, and fatty liver score
Prospective cohort study [23]	MAFLD group (n=3527) vs. liver cirrhosis group (*n*=1643) vs. liver cancer group (*n*=669)	WD or Prudent diet	WD was significantly associated with increased risk of chronic liver diseases; Prudent diet was significantly

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						associated with a lower risk of liver cirrhosis

Abbreviations: MAFLD, metabolic dysfunction-associated fatty liver disease; MD, Mediterranean diet; BMI, body mass index; AST, aspartate amino transferase; ALT, alanine amino transferase; GGT, gamma-glutamyl transferase; HDL, high-density lipoproteins, LDL, low-density lipoproteins; TG, triglycerides; HOMA-IR, homeostatic model assessment-insulin resistance; FLI, fatty liver index; WD, Western diet.

4. Use of Probiotics in MAFLD

Probiotics are defined by the Food and Agriculture Organization of the United Nations and the World Health Organization as "live microorganisms that, when administered in sufficient quantities, confer a health benefit on the host". Probiotics manipulate the gut microbiota to improve its homeostasis [26]. In fact, recent evidences showed their efficacy in antibiotic-associated diarrhea, inflammatory bowel diseases (IBDs) and colorectal cancer [27]. The use of probiotics has been associated with beneficial effects in MAFLD in several studies [28]. In a double-blind, single-center clinical trial MAFLD patients were randomized to receive Symbiter or placebo. For 8-weeks, the Symbiter group received a concentrated biomass of 14 probiotic bacteria genera such as Bifidobacterium, Lactobacillus, Lactococcus, Propionibacterium every day, while the placebo group received placebo every day. The research team evaluated the changes in the FLI and liver stiffness measured by shear wave elastography. At the end of the administration, both placebo and probiotics were well tolerated. In the probiotic group, the FLI significantly decreased compared to the placebo group. In fact, it decreased from 84.33±2.23 to 78.73±2.58 in the probiotic group ($p<0.001$), whereas it did not change in the placebo group. However, there was no a significant difference in liver stiffness [29]. Another randomized controlled trial analysed the effect of the administration of Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus paracasei, Pediococcus pentosaceus, Bifidobacterium lactis, and Bifidobacterium breve in obese MAFLD patients for 12 weeks. At the end of the study, the intrahepatic fat fraction decreased from 16.3±15% to 14.1±7.7% in the probiotics group ($p=0.032$), while it did not change in the placebo group. In addition, the reduction in TG levels was also more significant in the probiotic group than in the placebo group [30]. A pilot study analysed the effect of a dosage of 500 milion of Lactobacillus bulgaricus and Streptococcus thermophilus in MAFLD patients. For three months, group 1 was treated with probiotics administration daily and group 2 received placebo. After treatment, in group 1 ALT, AST and GGT levels decreased from 67.7±25.1 to 60.4±30.4 UI/L ($p<0.05$), from 41.3±15.5 to 35.6±10.4 UI/L ($p<0.05$) and from 118.2±63.1 to 107.7±60.8 UI/L ($p<0.05$), respectively. Instead, in group 2 these parameters remained unchanged. In both groups no modifications of anthropometric parameters and cardiovascular risk factors took place [31]. Mohamad et al., showed that the use of six different Lactobacillus and Bifidobacterium species improved intestinal permeability with a reduction in fat absorption [32]. Clinical trials about the use of probiotics in patients with MAFLD are summarized in Table 2. Furthermore, the beneficial effect of probiotics has also been observed with pre-clinical and clinical studies in NASH models. A study performed in obese mice with NASH showed a reduction in histological liver steatosis and transaminase levels after administration of VSL#3 (containing Bifidobacterium, Lactobacillus, and Streptococcus genera) [33]. In an open-label trial on patients with NASH, one group received a probiotic cocktail (containing Lactobacillus, Bifidobacterium and Streptococcus genera) for 12 weeks. These patients showed a significant (>20%) reduction in serum ALT, liver stiffness, BMI and serum cholesterol levels compared to the control group [34]. In summary, the use of Bifidobacterium and Lactobacillus as probiotics improves gut dysbiosis, often associated with a WD [7]. The restoration of gut eubiosis seems to show a beneficial effect in MAFLD and NASH patients. However, new studies in a larger sample and a longer follow-up are necessary to confirm their use in clinical practice.

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Table 2. Summary of clinical trials about the use of probiotics in MAFLD patients.

Study design	Study groups	Intervention	Outcomes
Randomized controlled trial [29]	MAFLD group (*n*=59)	Administration of Symbiter or placebo for 8 weeks	FLI significantly decreased in probiotic group Probiotics significantly reduced the level of serum AST and GGT No significant difference in liver stiffness among groups
Randomized controlled trial [30]	Obese-MAFLD group (*n*=69)	Administration of probiotics or placebo for 12 weeks	Significant decrease of the intrahepatic fat fraction and in TG levels in the probiotics group
Randomized controlled trial [31]	MAFLD group (*n*=28)	One tablet per day with 500 millions of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* or with one placebo tablet (120 mg of starch) for 3 months	ALT, AST and GGT levels significant decreased in group treated with probiotics No significant changes in anthropometric parameters
Randomized controlled trial [32]	MAFLD group (*n*=46)	Administration of probiotics or placebo for 6 months	Significant improvement of intestinal permeability with a reduction in fat absorption after probiotics treatment

Abbreviations: MAFLD, metabolic dysfunction-associated fatty liver disease; FLI, fatty liver index; AST, aspartate amino transferase; GGT, gamma-glutamyl transferase, TG, triglycerides; ALT, alanine amino transferase.

5. FMT in MAFLD Patients

FMT consists in the transfer of stool from a healthy donor to a patient with gut dysbiosis [35]. The therapeutic benefit of FMT is determined by its capacity to restore the gut microflora composition

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[36]. FMT can be administered by enema, upper gastrointestinal tract, colonoscopy, or oral capsules [37]. The requirements for FMT donors are age <60 years and healthy status, while exclusion criteria are risk of infectious disease, gastrointestinal comorbidities and factors that may affect the composition of the gut microbiota: systemic auto-inflammatory disease, atopic disease, metabolic syndrome, obesity, moderate/severe malnutrition, chronic pain syndromes, pregnancy, previous or planned gastrointestinal surgery, or a history of cancer [38]. FMT showed a high success rate in treating gastrointestinal infectious diseases, in particularly Clostridium difficile infection [39]. In addition, recent studies have shown that FMT is also effective in IBD patients [40]. However, it is less effective in IBD patients than in those patients colonized by Clostridium difficile. Therefore, the response could be due to differences in the gut microbiota composition between recipient and donor. In this competition, autologous FMT could be used [41]. In this way, autologous FMT is based on the use of collected feces in a state considered beneficial to restore gut microbial communities after perturbations. This approach is a better alternative to traditional FMT (defined as allogeneic FMT) [42]. As previously reported, probiotics improve intestinal permeability and have beneficial effects in MAFLD patients. However, there are no studies that have evaluated the correct dose and strain of probiotics and their adverse effects in MAFLD patients. Therefore, the use of live commensals from a healthy gut may be safer and more effective than probiotics. In MAFLD patients, few studies evaluated FMT efficacy. Xue et al., divided MAFLD patients into FMT group, non-FMT group, and healthy controls. The non-FMT group received oral probiotics (Bifidobacterium and Lactobacillus acidophilus, respectively), while the FMT group received 200 ml of bacterial cocktail from healthy donors for 3 days. This randomized controlled trial showed that FMT decreased the fat accumulation in the liver by improving the gut microbiota dysbiosis and the fatty liver disease. However, there were no statistical differences between the FMT and non-FMT groups in terms of liver function, hepatic fat accumulation and blood lipid levels. In addition, this study showed that FMT had a better effect in lean-MAFLD patients than in obese-MAFLD patients [43]. Another study compared the two different types of FMT in MAFLD patients. As reported by the Authors, allogenic FMT improved intestinal permeability better than autologous FMT. However, there were no significant statistical differences in insulin resistance and hepatic proton density fat fraction between autologous and allogeneic FMT [44]. Witjes et al., evaluated the effects of allogeneic FMT from a lean vegan donor via nasoduodenal tube in MAFLD/NASH patients. Liver biopsy and markers of steatohepatitis were assessed at baseline and after 24 weeks. At the end of the study, they showed that allogeneic FMT improved necro-inflammatory histology and bio-humoral liver profile [45]. Finally, a recent review underlined that FMT had good preclinical and clinical good results in MAFLD patients, especially in obese-MAFLD patients [46]. Clinical trials about the application of FMT in patients with MAFLD are summarized in Table 3.

Table 3. Summary of clinical trials about the application of FMT in MAFLD patients.

Study design	Study groups	Intervention	Outcomes
Randomized controlled trial [43]	FMT group (*n*=47) vs. non-FMT group (*n*=28) vs. healthy controls (*n*=10)	Administration of probiotics in non-FMT group Administration of 200 ml of bacterial cocktail from healthy donors for 3 days in FMT-group	Promotion of gut eubiosis after FMT Better efficacy of FMT among lean-MAFLD patients than obese-MAFLD patients

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Randomized controlled trial [44]	Allogenic FMT group (*n*=15) vs. autologous FMT group (*n*=6)	Allogenic or autologous FMT	Allogenic FMT significantly improved intestinal permeability better than autologous FMT No significant statistical differences in insulin resistance and hepatic proton density fat fraction between autologous and allogeneic FMT
Randomized controlled trial [45]	Autologous FMT (*n*=11) vs. allogenic FMT (*n*=10)	Allogenic or autologous FMT	Allogeneic FMT significantly improved necro-inflammatory histology and bio-humoral liver profile

Abbreviations: FMT, fecal microbiota transplantation; MAFLD, metabolic dysfunction-associated fatty liver disease.

6. Conclusions

NAFLD is a common cause of chronic liver disease worldwide. Due to several dysmetabolic comorbidities showed in patients with fatty liver, its nomenclature has been recent revised in MAFLD. A correct management of MAFLD-patients and the use of novel potential biomarkers are important to prevent MAFLD-related liver complications, such as NASH, liver cirrhosis and HCC [47]. Many evidences showed the correlation between MAFLD, gut dysbiosis and lifestyle. The gut microbiota improves health status by promoting nutrient absorption and supporting the immune system. The interaction between the gut microbiota, the immune system and the liver is defined as "gut-liver axis". Gut dysbiosis is the alteration of structure and function of gut bacteria and, consequently, of gut-liver-axis. This event plays a pivotal role in the pathogenesis of MAFLD and its progression. For this reason, probiotics and FMT have become promising treatments in clinical practice. Currently, the most important treatment of MAFLD is lifestyle changes. Obesity has a central role in the development of MAFLD. Indeed, MAFLD patients are mainly obese or overweight. MD could be considered the gold standard in prevention and therapeutic approach of MAFLD. The adherence to the MD can help MAFLD patients in improving the health status. On the other hand, WD was correlated with higher risk of chronic liver diseases, such as MAFLD, liver cirrhosis, and liver cancer. Therefore, WD should be discouraged. Other treatments are probiotics and FMT. Probiotics improved intestinal permeability with beneficial effects in MAFLD patients. However, there are no studies that evaluated the correct dose and strain of probiotics and their adverse effects in MAFLD patients. In this regard, there is a need for new evaluations on the role of probiotics in liver diseases [48–50]. For this reason, the use of live commensals from a healthy gut could be safer and more effective than probiotics. These issues have paved the way for the use of FMT in MAFLD patients. However, recent studies showed that there were no statistical differences between MAFLD patients treated with probiotics and FMT groups, in term of liver functions, fat accumulation and blood lipids levels. FMT consists in the transfer of stool in patient with alteration of gut microflora

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composition by healthy donors. The therapeutic benefit of FMT is determined by its capacity to restore the gut microbiota composition. It is more effective in lean patients than in obese MAFLD patients. Indeed, this novel approach was shown to be more effective in improving liver fat deposition and gut dysbiosis in obese MAFLD patients than in lean MAFLD patients. Another form of FMT is autologous FMT, based on the use of collected feces during a state considered beneficial in order to restore gut microbial communities after perturbations. Indeed, this approach is a better alternative to allogenic FMT. In conclusion, FMT seems to be a valid, safe, and effective alternative for the MAFLD treatment. However, since lean patients often do not respond to lifestyle changes, cholesterol-lowering agents and probiotics use, more studies with a longer follow up, especially in lean patients, are necessary in order to promote their use in real life contexts.

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References

1. Perazzo, H., Pacheco, A.G., Griep, R.H., Gracindo, R., Goulart, A.C., da Fonseca, M.D. Changing from NAFLD through MAFLD to MASLD: Similar prevalence and risk factors in a large Brazilian cohort. *J Hepatol* **2023**, *80*, e72, e74.
2. Teng, M.L., Ng, C.H., Huang, D.Q., Chan, K.E., Tan, D.J., Lim, W.H., Yang, J.D., Tan, E., Muthiah, M.D. Global incidence and prevalence of nonalcoholic fatty liver disease. *Clin Mol Hepatol* **2023**, *29*, S32, S42.
3. Younossi, Z.M., Golabi, P., Paik, J.M., Henry, A., Van Dongen, C., Henry, L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* **2023**, *77*, 1335, 1347.
4. Wong, V.W., Ekstedt, M., Wong, G.L., Hagström, H. Changing epidemiology, global trends and implications for outcomes of NAFLD. *J Hepatol* **2023**, *79*, 842, 852.
5. Abenavoli, L.; Spagnuolo, R.; Scarlata, G.G.M.; Scarpellini, E.; Boccuto, L.; Luzza, F. Ultrasound Prevalence and Clinical Features of Nonalcoholic Fatty Liver Disease in Patients with Inflammatory Bowel Diseases: A Real-Life Cross-Sectional Study. *Medicina* **2023**, *59*, 1935.
6. Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., Knight, R. Diversity, stability and resilience of the human gut microbiota. *Nature* **2012**, *489*, 220, 230.
7. Abenavoli, L., Scarpellini, E., Pellicano, R., Fagoonee, S., Larussa, T., Luzza, F. Mediterranean diet and probiotics supplementation to treat non-alcoholic fatty liver disease. **Minerva Med** *2020*, 111, 526, 528.
8. Abenavoli, L.; Scarpellini, E.; Colica, C.; Boccuto, L.; Salehi, B.; Sharifi-Rad, J.; Aiello, V.; Romano, B.; De Lorenzo, A.; Izzo, A.A.; et al. Gut Microbiota and Obesity: A Role for Probiotics. *Nutrients* **2019**, *11*, 2690.
9. Albillos, A., de Gottardi, A., Rescigno, M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J Hepatol* **2020**, *72*, 558, 577.
10. Carding, S., Verbeke, K., Vipond, D.T., Corfe, B.M., Owen, L.J. Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis* **2015**, *26*, 26191.
11. Aron-Wisnewsky, J., Vigliotti, C., Witjes, J., Le, P., Holleboom, A.G., Verheij, J., Nieuwdorp, M., Clément, K. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* **2020**, *17*, 279, 297.
12. Jasirwan, C.O.M., Muradi, A., Hasan, I., Simadibrata, M., Rinaldi, I. Correlation of gut Firmicutes/Bacteroidetes ratio with fibrosis and steatosis stratified by body mass index in patients with non-alcoholic fatty liver disease. *Biosci Microbiota Food Health* **2021**, *40*, 50, 58.

13. Jasirwan, C.O.M., Lesmana, C.R.A., Hasan, I., Sulaiman, A.S., Gani, R.A. The role of gut microbiota in non-alcoholic fatty liver disease: pathways of mechanisms. *Biosci Microbiota Food Health*, **2019**, *38*, 81, 88.
14. Leung, C., Rivera, L., Furness, J.B., Angus, P.W. The Role of the Gut Microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol* **2016**, *13*, 412, 25.
15. Abenavoli, L.; Scarlata, G.G.M.; Scarpellini, E.; Boccuto, L.; Spagnuolo, R.; Tilocca, B.; Roncada, P.; Luzza, F. Metabolic-Dysfunction-Associated Fatty Liver Disease and Gut Microbiota: From Fatty Liver to Dysmetabolic Syndrome. *Medicina* **2023**, *59*, 594.
16. Abenavoli, L., Milic, N., Di Renzo, L., Preveden, T., Medić-Stojanoska, M., De Lorenzo, A. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. *World J Gastroenterol* **2016**, 22, 7006, 16.
17. Abenavoli, L., Luzza, F. Mediterranean diet and NAFLD: where are we now?. *Minerva Endocrinol (Torino)* **2021**, *46*, 371, 373.
18. Abenavoli, L.; Procopio, A.C.; Paravati, M.R.; Costa, G.; Milić, N.; Alcaro, S.; Luzza, F. Mediterranean Diet: The Beneficial Effects of Lycopene in Non-Alcoholic Fatty Liver Disease. *J. Clin. Med.* **2022**, *11*, 3477.
19. Abenavoli, L.; Greco, M.; Milic, N.; Accattato, F.; Foti, D.; Gulletta, E.; Luzza, F. Effect of Mediterranean Diet and Antioxidant Formulation in Non-Alcoholic Fatty Liver Disease: A Randomized Study. *Nutrients* **2017**, *9*, 870.
20. Gelli, C., Tarocchi, M., Abenavoli, L., Di Renzo, L., Galli, A., De Lorenzo, A. Effect of a counseling-supported treatment with the Mediterranean diet and physical activity on the severity of the non-alcoholic fatty liver disease. *World J Gastroenterol* **2017**, *23*, 3150, 3162.
21. Hamamah, S.; Amin, A.; Al-Kassir, A.L.; Chuang, J.; Covasa, M. Dietary Fat Modulation of Gut Microbiota and Impact on Regulatory Pathways Controlling Food Intake. *Nutrients* **2023**, *15*, 3365.
22. Abenavoli, L., Milic, N., Luzza, F., Boccuto, L., De Lorenzo, A. Polyphenols Treatment in Patients with Nonalcoholic Fatty Liver Disease. *J Transl Int Med* **2017**, *30*, 5.
23. Guo, W., Ge, X., Lu, J., Xu, X., Gao, J., Wang, Q., Song, C., Zhang, Q., Yu, C. Diet and Risk of Non-Alcoholic Fatty Liver Disease, Cirrhosis, and Liver Cancer: A Large Prospective Cohort Study in UK Biobank. *Nutrients* **2022**, *14*, 5335.
24. Pelusi, S., Valenti, L. Building Mass to Prevent non-Alcoholic Fatty Liver Disease?. *Hepatobiliary Surg Nutr* **2019**, *8*, 173, 176.
25. Abenavoli, L., Boccuto, L., Federico, A., Dallio, M., Loguercio, C., Di Renzo, L., De Lorenzo, A. Diet and Non-Alcoholic Fatty Liver Disease: The Mediterranean Way. *Int J Environ Res Public Health* **2019**, *16*, 3011.
26. Sanders, M.E., Heimbach, J.T., Pot, B., Tancredi, D.J., Lenoir-Wijnkoop, I., Lähteenmäki-Uutela, A., Gueimonde, M., Bañares, S. Health claims substantiation for probiotic and prebiotic products. *Gut Microbes* **2011**, *2*, 127, 33.
27. Kim, S.K., Guevarra, R.B., Kim, Y.T., Kwon, J., Kim, H., Cho, J.H., Kim, H.B., Lee, J.H. Role of Probiotics in Human Gut Microbiome-Associated Diseases. *J Microbiol Biotechnol* **2019**, *29*, 1335, 1340.
28. Cao, C., Shi, M., Wang, X., Yao, Y., Zeng, R. Effects of probiotics on non-alcoholic fatty liver disease: a review of human clinical trials. *Front Nutr* **2023** *10*, 1155306.
29. Kobyliak, N., Abenavoli, L., Mykhalchyshyn, G., Kononenko, L., Boccuto, L., Kyriienko, D., Dynnyk, O. A multi-strain probiotic reduces the fatty liver index, cytokines and aminotransferase levels in NAFLD patients: evidence from a randomized clinical trial. *J Gastrointestin Liver Dis* **2018**, *27*, 41, 49.
30. Ahn, S.B., Jun, D.W., Kang, B.K., Lim, J.H., Lim, S., Chung, M.J. Randomized, double-blind, placebo-controlled study of a multispecies probiotic mixture in nonalcoholic fatty liver disease. *Sci Rep* **2019**, *5*, 9, 5688.
31. Aller, R., De Luis, D.A., Izaola, O., Conde, R., Gonzalez Sagrado, M., Primo, D., De La Fuente, B., Gonzalez, J. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. *Eur Rev Med Pharmacol Sci* **2011**, *15*, 1090, 5.
32. Mohamad Nor, M.H.; Ayob, N.; Mokhtar, N.M.; Raja Ali, R.A.; Tan, G.C.; Wong, Z.; Shafiee, N.H.; Wong, Y.P.; Mustangin, M.; Nawawi, K.N.M. The Effect of Probiotics (MCP® BCMC® Strains) on Hepatic Steatosis, Small Intestinal Mucosal Immune Function, and Intestinal Barrier in Patients with Non-Alcoholic Fatty Liver Disease. *Nutrients* **2021**, *13*, 3192.
33. Li, Z., Yang, S., Lin, H., Huang, J., Watkins, P. A., Moser, A. B., Desimone, C., Song, X. Y., Diehl, A. M. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. *Hepatology* **2003,** *37*, 343, 50.

34. Manzhalii, E., Virchenko, O., Falalyeyeva, T., Beregova, T., Stremmel, W. Treatment efficacy of a probiotic preparation for non-alcoholic steatohepatitis: A pilot trial. *J Dig Dis* **2017**, *18*, 698, 703.
35. Vindigni, S.M., Surawicz, C.M. Fecal Microbiota Transplantation. Gastroenterol Clin North Am **2017**, *46*, 171, 185.
36. Wang, J.W., Kuo, C. H., Kuo, F.C., Wang, Y.K., Hsu, W.H., Yu, F.J., Hu, H.M., Hsu, P.I., Wang, J.Y., et al. Fecal microbiota transplantation: Review and update. *J Formos Med Assoc* **2019**, *118*, S23, S31.
37. Allegretti, J.R., Mullish, B.H., Kelly, C., Fischer, M. The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. *Lancet* **2019**, *394*, 420, 431.
38. Del Barrio, M., Lavín, L., Santos-Laso, Á., Arias-Loste, M.T., Odriozola, A., Rodriguez-Duque, J.C., Rivas, C., Iruzubieta, P., Crespo, J. Faecal Microbiota Transplantation, Paving the Way to Treat Non-Alcoholic Fatty Liver Disease. *Int J Mol Sci* **2023**, *24*, 6123.
39. Rakotonirina, A., Galperine, T., Allémann, E. Fecal microbiota transplantation: a review on current formulations in Clostridioides difficile infection and future outlooks. *Expert Opin Biol Ther* **2022**, *22*, 929, 944.
40. Weingarden, A.R, Vaughn, B.P. Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. *Gut Microbes* **2017**, *8*, 238, 252.
41. Basson, A.R., Zhou, Y., Seo, B., Rodriguez-Palacios, A., Cominelli, F. Autologous fecal microbiota transplantation for the treatment of inflammatory bowel disease. *Transl Res* **2020**, *226*, 1, 11.
42. Rinott, E., Youngster, I., Meir, A.Y., Tsaban, G., Kaplan, A., Zelicha, H., Rubin, E., Koren, O., Shai, I.. Autologous fecal microbiota transplantation can retain the metabolic achievements of dietary interventions. *Eur J Intern Med* **2021**, *92*, 17, 23.
43. Xue, L., Deng, Z., Luo, W., He, X., Chen, Y. Effect of Fecal Microbiota Transplantation on Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial. *Front Cell Infect Microbiol* **2022**, *12*, 759306.
44. Craven, L., Rahman, A., Nair Parvathy, S., Beaton, M., Silverman, J., Qumosani, K., Hramiak, I., Hegele, R., Joy, T., Meddings, J., et al. Allogenic Fecal Microbiota Transplantation in Patients With Nonalcoholic Fatty Liver Disease Improves Abnormal Small Intestinal Permeability: A Randomized Control Trial. *Am J Gastroenterol* **2020**, *115*, 1055, 1065.
45. Witjes, J.J., Smits, L.P., Pekmez, C.T., Prodan, A., Meijnikman, A.S., Troelstra, M.A., Bouter, K.E.C., Herrema, H., Levin, E., Holleboom, A.G., et al. Donor fecal microbiota transplantation alters gut microbiota and metabolites in obese individuals with steatohepatitis. *Hepatol Commun* **2020**, *4*, 1578, 1590
46. Abenavoli, L.; Maurizi, V.; Rinninella, E.; Tack, J.; Di Berardino, A.; Santori, P.; Rasetti, C.; Procopio, A.C.; Boccuto, L.; Scarpellini, E. Fecal Microbiota Transplantation in NAFLD Treatment. *Medicina* **2022**, *58*, 1559.
47. Elsayed A, Ismaiel A, Procopio AC, Luzza F, Abenavoli L, Dumitrascu DL. Noninvasive biochemical markers and surrogate scores in evaluating nonalcoholic steatohepatitis. *Minerva Med* **2022**;113:864-874.
48. Abenavoli, L., Scarlata, GG., Scarpellini, E., Procopio, A.C., Ponziani, F.R., Boccuto, L., Cetkovic, N., Luzza, F. Therapeutic success in primary biliary cholangitis and gut microbiota: a safe highway?. *Minerva Gastroenterol (Torino)* **2024**, doi:10.23736/S2724-5985.23.03590-8.
49. Li, Z.J., Gou, H.Z., Zhang, Y.L., Song, X.J., Zhang, L. Role of intestinal flora in primary sclerosing cholangitis and its potential therapeutic value. *World J Gastroenterol* **2022**, *28*, 6213, 6229.
50. Abenavoli, L.; Giubilei, L.; Procopio, A.C.; Spagnuolo, R.; Luzza, F.; Boccuto, L.; Scarpellini, E. Gut Microbiota in Non-Alcoholic Fatty Liver Disease Patients with Inflammatory Bowel Diseases: A Complex Interplay. *Nutrients* **2022**, *14*, 5323.