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Novel therapeutic approaches based on the pathological role of gut dysbiosis on the link between nonalcoholic fatty liver disease and insulin resistance

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Abstract. – The growing global epidemic of obesity and type 2 diabetes mellitus has determined an increased prevalence of NAFLD (non-alcoholic fatty liver disease), making it the most common chronic liver disease in the Western world and a leading cause of liver transplantation. In the last few years, a rising number of studies conducted both on animal and human models have shown the existence of a close association between insulin resistance (IR), dysbiosis, and steatosis. However, all the mechanisms that lead to impaired permeability, inflammation, and fibrosis have not been fully clarified. Recently, new possible treatment modalities have received much attention.

To reach the review purpose, a broad-ranging literature search on multidisciplinary research databases was performed using the following terms alone or in combination: "NAFLD", "gut dysbiosis", "insulin resistance", "inflammation", "probiotics", "Chinese herbs".

The use of probiotics, prebiotics, symbiotics, postbiotics, fecal microbiota transplant (FMT), Chinese herbal medicine, antibiotics, diet (polyphenols and fasting diets), and minor therapies such as carbon nanoparticles, the MCJ protein, water rich in molecular hydrogen, seems to be able to improve the phenotypic pattern in NAFLD patients.

In this review, we provide an overview of how IR and dysbiosis contribute to the development and progression of NAFLD, as well as the therapeutic strategies currently in use.

Key Words:
NAFLD, Insulin resistance, Gut dysbiosis, Probiotics, Polyphenols, Endocannabinoid system, FMT, MCJ, Chinese herbs.

Abbreviations

ACC: Acetyl-CoA carboxylase; ADFM: modified intermittent fasting; AEA: N-arachidonoylethanolamine; BA: bile acid; BAMBI: BMP activing membrane-bound inhibitor homolog; BBR: berberine; BCCA: branched chain amino acid; CAT: catalase; ChREBP: carbohydrate response element binding protein; CKD: chronic kidney disease; eCB: endocannabinoid system; ECM: extracellular matrix; EE: endogenous ethanol; ER: endoplasmatic reticulum; FC: free cholesterol; FIAF: adipocytic factor induced by fasting; FMD: fasting-mimicking diet; FMO: flavin-containing monooxygenases; FOS:

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fructooligosaccharides; FXR: farnesoid X receptor; GalNAc: N-acetylgalactosamine; GLA: gut-liver axis; GOS: galacto-oligosaccharides; GPx: glutathione peroxidase; GRd: glutathione reductase; GSH: glutathione; HCC: hepatocellular carcinoma; HMGCR: HMG-CoA reductase; HSC: hepatic stellate cell; IMO: isomaltooligosaccharides; IR: insulin resistance; LPL: lipoprotein lipase; LPS: lipopolysaccharide; MAMPs: microbe-associated molecular pattern; MetS: metabolic syndrome; MMP: matrix metalloproteinase; NAC: N-acetyl-cysteine; NAFL: non-alcoholic fatty liver; NAFLD: non-alcoholic fatty liver disease; NAPE-PLD: N-acylphosphatidylethanolamine phospholipase-D; NASH: non-alcoholic steatohepatitis; NEFA: non esterified fatty acid; NRL: Nod-Like Receptors; OEA: oleoylethanolamine; OSAS: obstructive sleep apnea syndrome; PAMP: pathogen Associated Molecular Pattern; PCOS: polycystic ovary syndrome; PEA: palmitoylethanolamine; POS: pectic oligosaccharides; PUFA: polyunsatured fatty acid; ROS: reactive oxygen species; RS: resistant starch; SAM: S-adenosylmethionine; SBOS: soy oligosaccharides; SCFA: short chain fatty acid; SFA: long chain fatty acid; SOD: superoxide dismutase; SREBP1: sterol response element binding protein 1; T2DM: type 2 diabetes mellitus; TGF-β: transforming Growth Factor β; TMA: trimethylamine; TMAO: trimethylamine-N-oxide; TMP: tissue inhibitors of metalloproteinase; TOS: trans-galactooligosaccharides; TRL: Toll-like receptor; UDCA: ursodoxycholic acid; VLDL: very low density lipoprotein; XOS: xylooligosaccharides..

Introduction

Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of histological liver alterations resulting from hepatic steatosis, without any sign of secondary hepatic fat accumulation due to alcohol intake, hereditary conditions, or steatogenic medications[1,2]. It ranges from simple non-alcoholic fatty liver (NAFL) to its progressive form non-alcoholic steatohepatitis (NASH)[3]. NAFLD pathophysiology can evolve from hepatocyte ballooning, lobular inflammation, and/or fibrosis[4] up to cirrhosis, hepatocellular carcinoma (HCC)[5], and/or ultimately to death[6]. First described in 1980[7], it has gone from being defined as "a poorly understood and hitherto unnamed liver disease"[8] to being recognized as the most common chronic progressive liver disorder in Western countries[9], and the second leading cause of liver transplantation in the US and Europe[10]. NAFLD is a multisystem disease closely associated with the metabolic syndrome (MetS) and its components [obesity, insulin resistance (IR), hyperlipidemia, and hypertension][11], as well as with a wide range of further chronic extrahepatic conditions, such as type 2 diabetes (T2DM), cardiovascular diseases (CVD), chronic kidney disease (CDK), Polycystic Ovary Syndrome (PCOS), hypothyroidism, Obstructive Sleep Apnea Syndrome (OSAS), osteoporosis, colorectal cancer[12,13]. Among these, T2DM represents an emerging risk factor for the development and progression of NAFLD towards NASH, fibrosis, cirrhosis, and HCC[14]. Recent studies[15,16] have shown that the association between T2DM and NAFLD is bidirectional: as diabetes increases NAFLD severity, NAFLD is linked to a greater risk of T2DM. NAFLD improvement, on the contrary, is associated with a reduction of the risk of T2DM onset[16]. To further emphasize this strong connection between NAFLD and T2DM, the term MAFLD, or "fatty liver disease associated with metabolic dysfunction" was proposed in 2020[17]. In addition, therapeutic strategies used in T2DM have been suggested to improve hepatic steatosis, and it seems to be suitable for treating NASH and NAFLD subjects as well. Among them, Capuani et al[18] interestingly reported how glucagon-like peptide-1 receptor agonists (GLP-1 RAs) emerged as novel drug able to ameliorate liver steatosis. GLP-1 RAs are commonly used to regulate glycemic metabolism[19], owever, they have also been associated with improvements in lipid profile. NAFLD worldwide prevalence is estimated to be 25%[20] with remarkable differences due to age, gender and ethnicity[21,22]. Only 55.5% of this percentage is found in patients with T2DM[23] and there are some discrepancies based on the study population (75%) and the diagnostic tools used (18-33% with H-NMR spectroscopy and 72.8% with magnetic resonance spectroscopy)[24]. NAFLD pathogenesis, about which still little is known, is complex and multifactorial. It can currently be explained with the "*multiple hit"* model[25]: different genetic susceptibility variants such as *Snps* of *Pnpla3*, *Tm6sf2*, *Ncan*, *Gckr*, *Lyplal1*, *Ppp1r3b* genes[26] and environmental factors such as MetS, IR, Western diet, poor quality of sleep, sedentary lifestyle, and drug abuse make the liver more vulnerable and susceptible to steatosis[27,28]. As a consequence, lipotoxicity, liver damage, intestinal permeability impairment, endotoxemia, gut dysbiosis and an abnormal systemic immune response result in NASH, fibrosis, cirrhosis, and HCC[29]. Consequently, traditional treatment for NAFLD is merely palliative because of this complexity[30]. In the last few years, however, new potential therapies targeting the gut microbiota have been identified, the outcomes of which, i.e., improvement of steatosis, inflammation, dysbiosis, and liver function are promising

but still unsatisfying[31]. In this review, we firstly aim to summarize what we know about the role of IR as a promoter of gut dysbiosis, and then focus on the role and the dysbiosis-dependent modulation of the main molecular pathways involved in the development, progression and severity of NAFLD. Furthermore, we intend to illustrate new therapeutic approaches targeting dysbiosis, which aim to improve steatosis development and its progression to NASH.

IR – Gut Dysbiosis – NAFLD: the Relationship

Among the environmental factors, IR, i.e., the inability of insulin to perform its normal functions in skeletal muscle, adipose tissue, and liver[32], seems to have a close relationship with both dysbiosis and NAFLD development and progression[33] (Figure 1). IR development is strongly linked to excessive fat accumulation, nutrient overload and obesity[34,35]. IR and the consequent hyperinsulinemia are responsible for the increase in circulating free FFAs and TG levels, respectively, which is due to reduced lipogenesis and enhanced lipolysis in adipose tissue[36], decreased hepatic glycogen accumulation and increased gluconeogenesis[37]. All of these events result in higher hepatic FFA levels and hepatocyte exposure to lipotoxicity[38], or oxidative stress, ER stress[39], and lipotoxic lipids (saturated NEFA, SFA[40], LPC glycerophospholipids, FC, sphingolipids[41,42] and sphingosine 1-phosphate (S1P), PUFA and their derivatives, and oxysterols). These further exacerbate IR[29] and determine the release of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α[43,44], and/or chemokines in turn responsible for the onset of an inflammatory state[45] and an altered innate and adaptive immune response[46]. This is reflected in intracellular and tissue damage amplification, and in the progression from NAFL to NASH. The growing interest and the advanced knowledge in the gut-liver axis (GLA)[47,48] led to noticing the pro-inflammatory role of gut dysbiosis in NAFLD patients[49].

Gut dysbiosis is defined as the alteration of the composition, or loss of beneficial commensal such as *Odoribacter* genus (family *Porphyromonadaceae*), *Oscillibacter*, *Ruminococcus*, *Flavonifractoraceae* (family *Ruminococcipaceae*), *Rikenellaceae*, *Copococcus* (family *Lachnospiraceae*)[50], *Prevotella*, Anaerosporobacter, *Faecalibacterium*, and the overgrowth of pathogens[51] such as Proteobacteria (*E. Coli* and *Enterobacter*)[52], *Bacteroidetes* (Bacteroides)[53], *Lentisphaerae*, *Firmicutes* (*Clostridium XI, Lactobacillus* and *Anaerobacter*)[54], *Streptococcus* (*S. bovis* and *S. faecalis*), Allisonella and *Parabacteroides*. Gut dysbiosis leads to an impaired function (metabolic, trophic, and protective) of the entire intestinal ecosystem[55], and can feed hepatic and systemic inflammation by increasing intestinal permeability and endotoxemia and by exacerbating

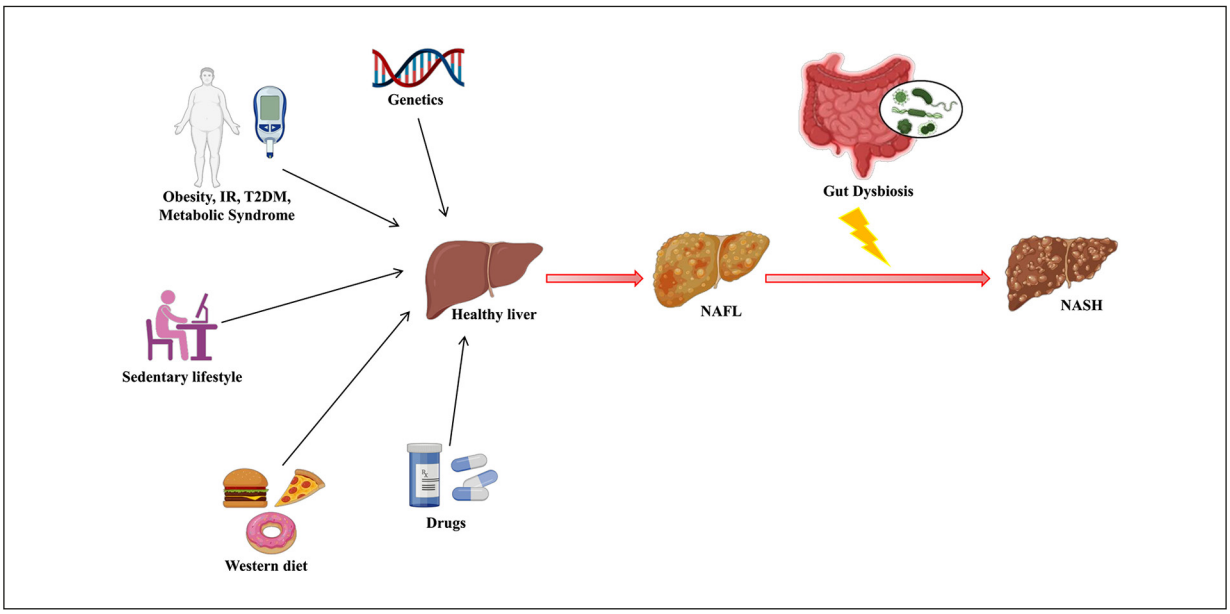

Figure 1. Genetic and environmental "multiple hits" impact on NAFLD. Created by using Biorender.com.

innate and adaptive immune responses[56]. Inflammation, together with injured or stressed hepatocytes and activated Kupffer cells (liver macrophages) activates hepatic stellate cells (HSCs) as myofibroblasts, which begin to secrete an excessive amount of extracellular matrix (collagen, glycoproteins, and glycans) faster than they degrade[57]. This results in the transition from NASH to the stage of fibrosis, which can, as the liver damage progresses, worsens to advanced fibrosis, characterized by the formation of fibrotic scars first and of regeneration nodules then, up to compensated liver cirrhosis, and eventually manifest liver failure, HCC, and death[58,59].

The Gut Dysbiosis

However, how does dysbiosis contribute to NAFLD pathogenesis? Recent findings[60,61] have found that it acts by modulating intestinal permeability and endotoxemia (a); energy homeostasis and fats storage (b); bile acids homeostasis (c); choline metabolism and toxic derivative TMAO production (d); endogenous ethanol and other toxic products such as acetaldehyde (e) synthesis; the production of other metabolites, such as phenylacetate and branched chain amino acids (BCAAs) (f); the endocannabinoid system (g), responsible for activating the pro-inflammatory and pro-fibrogenic pathways that determine their progression to NASH (Figure 2). Recently, Le Roy et al[62], De Minicis et al[63], and Wieland et al[64] have also hypothesized that NAFLD may be a communicable disease through the gut microbiota.

Altered Intestinal Permeability and Endotoxemia

Dysbiosis is responsible for the impairment of the intestinal mucosal barrier structure (intestinal TJ proteins such as ZO-1 and occludin) and function, and therefore for its affected permeability ("leaky gut")[65] and metabolic endotoxemia (i.e., increased LPS le-

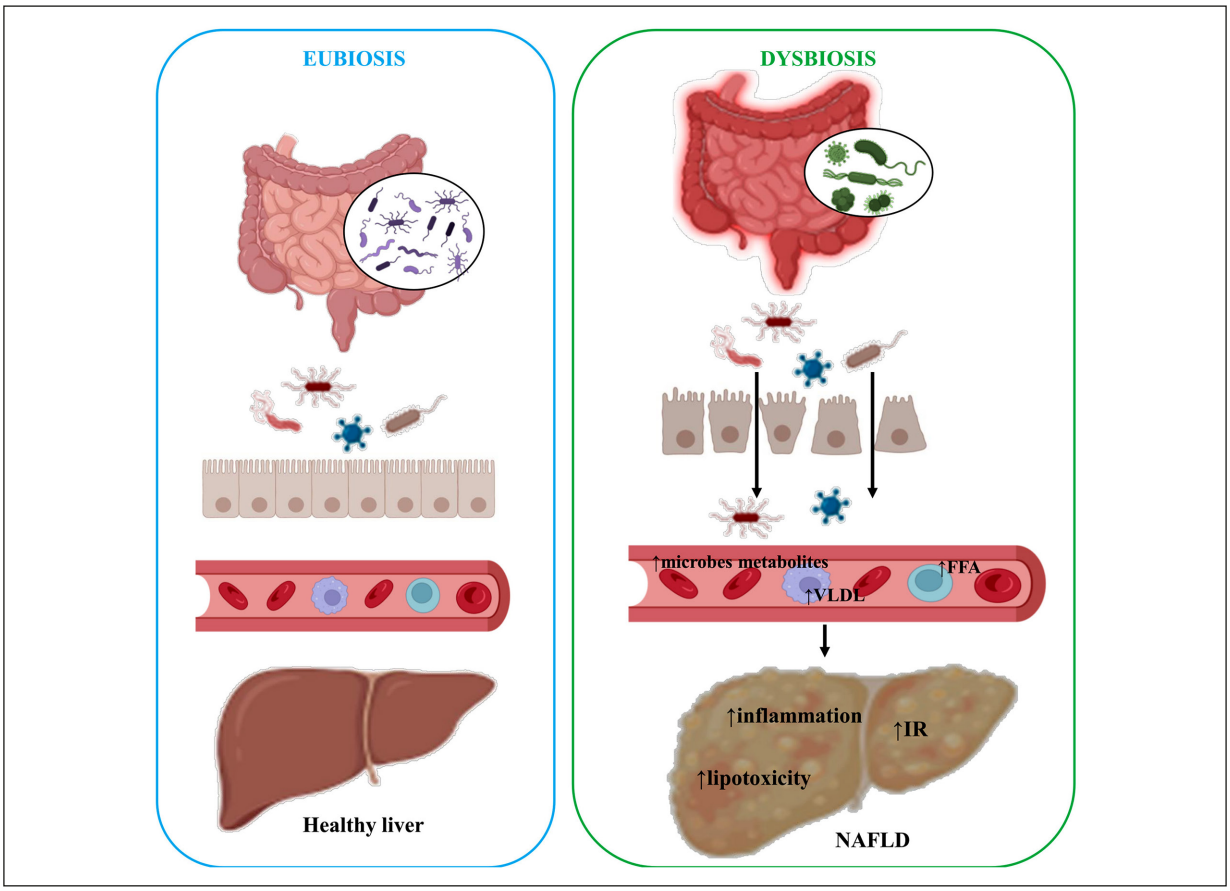

Figure 2. Recent hypothesis on the gut dysbiosis contribution to NAFLD pathogenesis and progression. Created by using Biorender.com.

vels)[66,67]. Irregular microvilli and spaced tight junctions allow the different exogenous and endogenous antigens, such as MAMP and PAMP, or their metabolic products[68] to reach the liver and recognize the PRR receptors NF-kβ (nuclear factor β), TRL-2/4/9 and NodLike Receptors (NRL) present on Kupffer cells/infiltrating macrophages and HSCs[69,70]. This link stimulates NF-kB activation, production and release of pro-inflammatory cytokines, chemokines, NO, ROS and protease[71], the down-regulation of bone morphogenic protein (BMP), and activation membrane-bound inhibitor homolog (BAMBI)[72]. This results in hepatic morpho-functional change, IR onset, oxidative stress and RE stress[73], the activation of an abnormal acute systemic inflammatory and immune response[74], characterized by an early accumulation of polymorphonuclear cells (CD4+ T cells, neutrophils, and monocytes)[75,76], and it is responsible for hepatocyte apoptosis and necrosis[77], the activation and proliferation of HSC and the production of TGF-β[57]. Simultaneously an overexpression of both matrix metalloproteinase (MMPs) and tissue inhibitors of metalloproteinases (TMPs) also occurs. These enzymes are respectively responsible for an intensified destruction of liver tissue, and for inhibiting - in particular TMP-1, collagen fibrogenesis degradation in the liver[78,79].

Energy Homeostasis and Fat Storage

Dysbiosis is able to influence both energy homeostasis and hepatic fat storage enhancing hepatic lipogenesis *de novo* and inhibiting FIAF (fasting-induced adipose factor) production and secretion in intestinal epithelial tissue[80]. Lipogenesis *de novo*, induced by ChREBP (carbohydrate response element binding protein) and SREBP-1c (Sterol response element binding protein 1c)[81,82], determines higher monosaccharide and TG hepatic content (approximately 2/3 times) which, however, are exported in the form of fat from the liver to ectopic storage (or futile cycle). The lack of FIAF expression, a member of the angiopoietin-like protein family that acts as a suppressor of LPL, induces a greater activity of the latter with a consequent increase and perpetuation of the intrahepatic accumulation of TG[83].

Microbiota Metabolites

Numerous studies[84,85,90] have observed a bidirectional relationship between the microbiota and the bile acids (Bas). Bile acids are known as the amphipathic molecules' steroid-derivative components of bile[84]. The microbiota modulates the size and composition of the BAs pool by acting on the expression of genes involved in synthesis, conjugation and reabsorption, while BAs maintain intestinal homeostasis by preventing bacterial overgrowth. However, an altered biliary homeostasis can determine a retention of bile salts and dysbiosis[85], characterized by reduced levels of *Bacteroidetes*, *Actinobacteria*, *Ruminococcaceae*, *Lachnospiraceae* and *Blautia* and of the genera *Sutterella* and *Allobaculum*[86] and elevated of *Enterobacteriaceae*[87]. Dysbiosis negatively affects serum concentrations of primary and secondary BAs and their ratio[88,89], hepatic bile acids synthesis and total fecal bile acids[90]. Hence, several studies[87] in patients with NAFLD[91,92], NASH and observed a perpetuation of the alteration of BAs homeostasis cirrhosis. These increases are reflected in the enhanced intestinal permeability associated with endotoxemia[93], metabolic alterations (IR) and inflammation[94], characterized by the release of pro-inflammatory cytokines, metabolic stress[95], activation of the cell death pathway[96], and a cascade of host immune responses that promote disease progression[97].

Gut dysbiosis can negatively modulate the metabolism of the amine choline first hydrolyzing it into trimethylamine (TMA), thanks to the enzyme choline TMA lyase, and subsequently oxidizing it into the toxic compound trimethylamine N-oxide (TMAO) by the FMO 1/3, with consequent steatogenic effects[98,99]. TMAO is able to alter the metabolism of bile acids[100] and to block hepatic insulin signaling pathway, exacerbating IR[101], hepatic steatosis[102] and its progression towards cirrhosis[103]. In patients with NAFLD/NASH, the close association between gut dysbiosis and endogenous ethanol (EE)[104] has long been known. Ethanol causes a dysbiosis in favor of the overgrowth of alcohol-producing bacteria[105], which in turn negatively induces an overexpression of hepatic ethanol metabolic enzymes required for the production or degradation of ethanol[106] and its toxic metabolites acetaldehyde and acetate[107]. The three metabolites (ethanol, acetaldehyde and acetate) act as hepatotoxins and have been independently linked with liver injury[108]. They are responsible for the activation of macrophages, the weakening of tight junctions with consequent increase in permeability and translocation of endotoxins into the portal circulation[109,110], as well as the down-regulation of intestinal AMP expression[111], the inhibi-

tion of TCA cycle with subsequent rising levels of acetate and intrahepatic accumulation of triglycerides[112], an exaggerated liver inflammatory response following the secretion of pro-inflammatory cytokines[113] and an enhanced activity of cytochrome P450 2E1 (CYP2E1)[114]. CYP2E1 is known as a powerful pro-fibrotic signal responsible for the oxidation of ethanol into acetaldehyde and the production of ROS and, therefore, for the oxidative liver injury, mitochondrial dysfunction, and hepatocyte necrosis[115].

Finally, the products of bacterial metabolism of phenylalanine, such as phenylacetate and branched chain amino acids (BCAA) are involved in NAFLD, NASH, and fibrosis pathogenesis[116,117]. Phenylacetate is related to an increased expression of genes coding for fats (*Lpl*, *Fas*), to higher hepatic TG levels and also to hepatic steatosis development. BCAAs are associated with mitochondrial dysfunction[118], and therefore with higher level of disease severity[119], and IR related to obesity and T2DM[120].

Endocannabinoid System (eCB)

The eCB endocannabinoid system is an intercellular lipid mediator with pleiotropic functions; it is composed of the cannabinoid receptor CB1, expressed in hepatocytes and endothelial cells of the liver, and CB2, expressed in Kupffer cells[121]. Microbiota-host interaction studies[122] in the colon of germ-free mice models have highlighted a tight association between gut dysbiosis, eCB, and NAFLD development and progression. Dysbiosis is associated with eCB hyperactivation in terms of high levels of eCB in plasma and adipocytes, and altered expression of eCB1 receptor. This is reflected in a change in the composition of the microbiota, an increase in intestinal permeability, endotoxemia, inflammatory states and body fat[123]. Indeed, hyperactivated CB1 promotes both hepatic expression of SREBP-1 and its target enzymes ACC1 and FAS, and *de novo* synthesis of fatty acids in the liver, or isolated hepatocytes expressing CB1[124]. Moreover, it inhibits adiponectin secretion, influencing the onset of MetS, which predisposes to the onset of NAFLD[125]. It also promotes energy conservation by stimulating its intake and inhibiting its expenditure with central and peripheral mechanisms, increases TNF-α, and reduces the synthesis of N-arachidonoylethanolamine (AEA), a close derivative of N-acylphosphatidylethanolamine phospholipase-D (NAPE-PLD) involved in the regulation of energy homeostasis, inflammation, obesity, IR, intestinal permeability, and dysbiosis[126]. The pharmacological inhibition of eCB-1 receptor, or the restoration of the microflora have been shown to reduce the tone of the peripheral eCB system, and consequently to improve the barrier function; endotoxemia seems to reduce IR and TNF-α levels, to delay the progression from steatosis to fibrosis and cirrhosis, and even to reverse hepatic steatosis[127].

Therapeutic Approaches: Current and Future

There are not currently specific therapeutic approaches for NAFLD and NASH treatment. Traditional therapy represents an effective line of intervention for the long-term (24 months) improvement of NAFLD histology[128], and it is commonly based on lifestyle modification, i.e. a combination of calorie-restricted diet and increased physical activity, administration of antidiabetic and lipid-lowering drugs, antioxidants and cytoprotective agents, such as vitamin E, vitamin C, betaine, SAM (S-adenosylmethionine), NAC (N-acetyl-cysteine) and UDCA (ursodoxycholic acid), vitamin D, vitamin J, and/or bariatric surgery[2,129,130]. However, it exhibits frequent failure rates due to reduced or absent compliance. The new knowledge on pathogenesis mechanisms and the poor compliance rates have led, in recent times, to the development of new potential therapeutic approaches, in particular those targeting the gut-liver axis and dysbiosis, such as probiotics, prebiotics, symbiotics, postbiotics, fecal microbiota transplantation (FMT), dietary approaches, antibiotics, Chinese herbal medicine, and other minor therapies (carbon nanoparticles, MCJ protein, and H_2- rich water) (Figure 3).

Probiotics, Prebiotics and Symbiotics

Probiotics, or "live microorganisms which, if taken in appropriate quantities, bring benefits for the host health"[131] (FAO/WHO) present in foods, dietary supplements, or drugs, represent a potential therapeutic strategy for NAFLD and NASH. Clinical and basic studies[132,137] have shown how a single probiotic, such as *Lactobacillus rhamnosus GG* (LGG), *Lactobacillus acidophilus L., L. plantarum WCFS1* and *Lactococcus lactis subsp.*

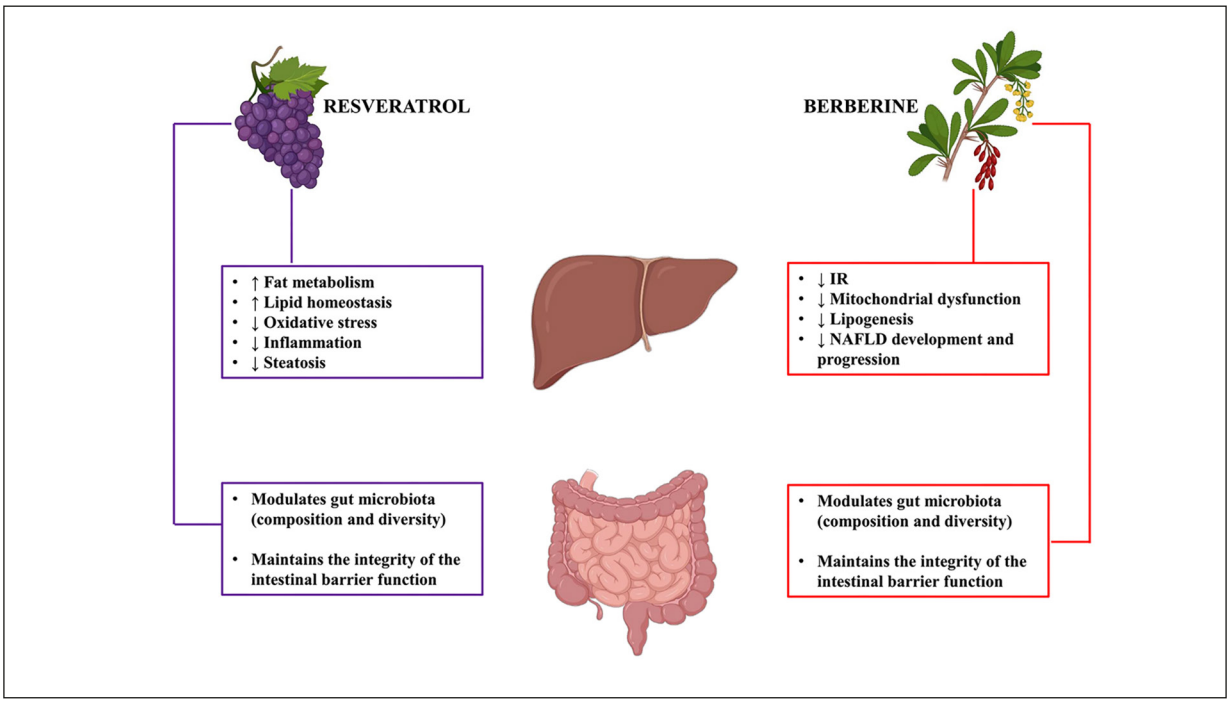

Figure 3. Schematic representation of the action, both in the liver and in the intestine, of three polyphenols silymarin, resveratrol and berberine in NAFLD pathogenesis. Created by using Biorender.com.

cremori is able to improve liver function and related ALT and AST parameters, lipid (reducing hepatic β-oxidation of fatty acids), carbohydrate (insulin-sensitizing effect), bile acids, cholesterol, choline and ethanol metabolism, inflammatory status, intestinal barrier integrity and function, liver enzyme profile, as well as weight and body composition by reverting gut dysbiosis to eubiosis. In the same way, positive results on inflammation, steatosis, intestinal permeability and endotoxemia, serum lipid levels, liver damage biomarkers, and gut microbiota composition have been associated with combinations of multiple probiotic strains. In this contest, four RCTs, whose main characteristics and results are reported in meta-analysis by Ma et al[133], showed a significant reduction in aminotransferases ALT, AST and γ-GT levels, total cholesterol (TC), and TNF-α (Table I). Similar results were obtained with the 8-weeks integration of a formulation of multi-probiotic (14 probiotic bacteria *Bifidobacterium*, *Lactobacillus*, *Lactococcus* and *Propionibacterium*) ($p < 0.001$)[134] and after one year of a multi-strain oral probiotics (*L. paracasei, L. plantarum, L. acidophilus, L. delbrueckii subsp. bulgaricus, Bifidobacterium, B. infantis, B. short* and *S. thermophiles*)[135,136]. The probiotic formula based on *LGG, L. plantarum WCFS1* and anthraquinone from Cassia obtusifolia L. significantly reduced steatosis, TNF-α, endotoxemia ($p < 0.05$), improved permeability and attenuated gut dysbiosis, by increasing *Bacteroidetes* (*Bacteroides*, *Lactobacillus* and *Parabacteroides*) and reducing *Firmicutes* (*Oscillospira*)[137]. The use of probiotics also improves IR, in terms of fasting glucose, insulin, and HOMA-IR. A significant reduction of these parameters was actually observed after the integration of multi-strain probiotics (*Lactobacillus*, *Bifidobacterium* and *S. thermophilus*), and after the consumption of 220 gr of probiotic yogurt with *L. delbrueckii* subsp. *Bulgaricus*, and *S. thermophilus* for 24 weeks[138].

Prebiotics are defined as "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the beneficial gastrointestinal microbiota that gives benefits"[139]. They are represented by oligosaccharides fructooligosaccharides (FOS), galacto-oligosaccharides (GOS), xylooligosaccharides (XOS), isomaltooligosaccharides (IMO), trans- TOS (galactooligosaccharides), SBOS (soy oligosaccharides) and POS (pectic oligosaccharides), lactulose, fructans (inulin), resistant starch (RS) and alginate. All prebiotics are able to selectively

Table I. Characteristic and results of four RCTs reported in meta-analysis.

Type of studies	Population	Treatments	Time of treatments	Follow-up	Results
RCT in double blind	28 NAFLD patients	Mixture with 500 mln of Lactobacillus bulgaricus and Streptococcus thermophilus per day *vs.* placebo (120 mg of starch)	3 months	Yes	**ALT**: 67.7 +/- 25.1 *vs.* 60.4 +/- 30.4 UI/L ($p < 0.05$) **AST**: 41.3 +/- 15.5 *vs.* 35.6 +/- 10,4 UI/L ($p < 0.05$) **ɣ-GT**: 118.2 +/- 63.1 *vs.* 107.7 +/- 60.8 UI/L ($p < 0.05$
RCT in double blind	Children with obesity-related liver disease	2 billion CFU LGG/ 1 day *vs.* placebo	8 weeks	Yes	**ALT**: 61.6+/-31.80 *vs.* 40.1+/-22.37 UI/L (p =0.03)
RCT in double blind	66 NASH patients	Bifidobacterium longum + Fos + lifestyle modification *vs.* placebo	24 weeks	Yes	**AST**: -69.6 *vs.* -45.9 UI/mL (p <0.05) **LDL**: - 0.84 *vs.* -0.18 mmol/L ($p < 0.001$) **TNF-α**: -0.45 *vs.* -0.12 ng/mL ($p < 0.001$) **HOMA-IR**: -1.1 *vs.* -0.6 ($p < 0.001$) **Steatosis**: $p < 0.05$
RCT in double blind	20 NASH patients	Lepicol formula (*L. plantarum*, *L. deslbrueckii*, L.acidophilus, *L. rhamnosus* and *B. bifidum*) *vs.* usual care	6 months	Yes	**IHTG** (intrahepatic triglyceride): 16.0 +/- 6.6% *vs.* 14.9 +/- 7.0% ($p = 0.034$) **AST**: 37 +/- 40 *vs.* -16+/-39 IU/L ($p = 0.008$)

Modified by Ma et al[133].

stimulate the growth and activity of specific beneficial bacterial strains, to reduce intestinal pH, to resist to hydrolysis and gastrointestinal enzymes, to not be absorbed in the upper GI tract, to represent a selective substrate for one or more beneficial bacterial species in the colon, and to remain stable during food processing[140]. The few human studies[141] show how prebiotics are an effective adjuvant therapy for NAFLD and NASH by restoring dysbiosis. Among these, lactulose is able to stimulate the growth of *Lactobacilli*, *Bifidobacteria* and Gram-positive bacteria, and to protect from endotoxemia; the fungal prebiotic chitin-glucan (CG) (10%) has been proved to restore the number of bacteria from the clostridial cluster XIV including Roseburia spp[142]. Studies[143,144] on mice reported a reduction of inflammation, steatosis and an antidiabetic effect after administration, for 4 weeks, of oligofructose[143], and an improvement of liver function and reduction of BMI, AST, ALT, TC, TG, fatty liver, LPS-induced metabolic disturbances, inflammation, and steatosis in NASH after 1.5 gr of oat β-glucans for 12 weeks[144]. Akbarzadeh et al[145] observed a significant reduction in ALT (- 18.64 IU/L, p = 0.006), waist circumference (-4.6 cm, p=0.006) BMI (-1.3 Kg/m^2, p=0.006), weight (-3.5 Kg, p=0.03), body fat percentage (-2%, p=0.03), and caloric intake (-433.45 Kcal, p=0.02) in obese NAFLD patients after intake of 10 gr of psyllium (FOS)[145]. Resistant starch (RS) generally increases the turnover and laxation of bile salts and reduces postprandial blood glucose and blood fat levels[146]. However, the different beneficial effects on the metabolism and on the group of intestinal bacteria capable of responding to resistant starch are related to the different types of RS[147]. RS 4, for example, increases *Bacteroides* and *Parabacteroides spp.*[148], RS 2 *Ruminococcus bromii* and *Eubacterium rectale spp.*[149], and RS 3 the most resistant form[150] *E. rectale*, *Roseburia spp.* and *R. bromii*[151]. Alginate, a viscous algal polysaccharide used as a thickener, stabilizer or emulsifier thanks to its chemical-physical properties, was able to improve intestinal barrier function, and modify microbiota composition in favor of *Roseburia, Ruminococcus* and *Lachnospira*[152].

Symbiotics are a combination of prebiotics and probiotics in one formulation. Eslamparast et al[153] and Mofidi et al[154] observed improvement in inflammation, fibrosis score, and liver function, after lifestyle modification and simultaneous administration of symbiotic, containing 200 millions of seven different bacterial strains (*Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium longum,* and *Lactobacillus bulgaricus*), FOS, probiotics (magnesium stearate), and a vegetable capsule (hydroxypropyl methyl cellulose) over 28 weeks[153,154]. Scorletti et al[155] have shown an increase of *Bifidobacteria* and *Faecalibacterium fecal* and reduction of *Oscillibacter* and *Alistipes* with the symbiotic composed of FOS and *Bifidobacterium lactis*, while Bakhshimoghaddam et al[156] an improvement of the hepatic imaging ultrasound with the administration for 24 weeks of 300 gr/day of symbiotic yogurt, consisting of 10^8 CFU/Ml of *Bifidobacterium animalis* and 1.5 gr of inulin. The treatment with symbiotic *Bifidobacterium longum* and FOS associated with lifestyle modifications determined significant differences in the AST (- 23.7 IU/mL), LDL-C (- 0.66 mmol/L), TNF-α (-0.33 ng/mL), HOMA-IR (-0.5), steatosis, and the NASH activity index (p<0.05) after 24 weeks[138]. Recently, several other meta-analyses[157-158] have shown the efficacy of symbiotic therapy in improving LDL-C (-5 mg/dL), TC (-10.1 mg/dL) TAG (-10.1 mg/dL)[157], aminotransferases AST (-4.6 U/L), ALT (-6.9 U/L), γ-GT (-7.9 U/L), and TNF-α (-2.0 ng/mL) associated with reduced hepatic steatosis and hepatic rigidity, but no change in LPS levels and intestinal permeability[158].

Postbiotics

Postbiotics, defined as "any substance released or produced through the metabolic activity of the microorganism, which directly or indirectly exerts a beneficial effect on the host"[159], represent a new potential alternative therapeutic approach to probiotics, prebiotics, and FMT for the treatment of NAFLD[160]. Their composition is variable and depends on the strain and their metabolic state; it includes SCFA, secondary bile acids, p40, HM0539 proteins, enzymes, peptides, bacteriocins, endo and exo-polysaccharides, bacterial lysates, cell wall fragments, cells-free supernatants, and branched-chain fatty acids. All play a role in gut microbiota modulation and homeostasis maintenance, where they act as inhibitors of growth and activity of pathogens[161], as signal molecules or as inducers of resilience. Postbiotics such as acetate, propionate and butyrate, alone or in combination[162] or p40[163] and HM0539 proteins

of the supernatant of *L. rhamnosus* GG[164] protect, maintain and enhance intestinal barrier function. *Bifidobacterium* exopolysaccharides act as local and systemic immunomodulators by increasing the secretion of anti-inflammatory cytokines[165]. Studies[166-168] have shown a role of postbiotic BSH (bacterial enzyme bile salt hydrolase), butyrate and propionate as modulators of systemic metabolic responses. BSH diversifies the bile acid pool as demonstrated in the filtered supernatant of the probiotic *Lactobacillus johnsonii*[166], butyrate reduces oxidative stress in the colon[167], and propionate inhibits the condensation of cholesterol precursors, and reduces total and intrahepatocellular adipose lipid content[168].

Antibiotics

Several studies[141] have confirmed a positive and therapeutic effect of short-term antibiotic treatment in NAFLD by promoting the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli* and by eliminating pathogens ("eubiotic" effect)[141]. This action, together with their properties (side effects, resistance, etc.) and possible failure to restore the microbiota after long-term treatment with antibiotics such as amoxicillin, metronidazole, bismuth, and vancomycin, makes their clinical use for NAFLD treatment cautious[169]. Non-absorbable antibiotics such as neomycin, cidomycin, and rifaximin are currently being tested. Neomycin, combined with polymyxin B, prevents fructose-induced intrahepatic fat accumulation by decreasing the translocation of intestinal toxins[170]. Cidomycin alleviates NASH severity, increases the rate of intestinal transit, and reduces serum levels of ALT, AST, and TNF-α when administered orally[171]. Rifaximin, a broad spectrum antibiotic insoluble in water and non-absorbable (<0.4%), with bactericidal and bacteriostatic activity against *Streptococcus*, *Bacteroides* and *Citrobacter*, significantly improves the clinical conditions of NAFLD patients by reducing inflammation[172], ALT, circulating endotoxins, IR, and NAFLD-fat score[173]. Opposite results on intrahepatic lipid content and insulin sensitivity were found in Cobbold et al[174] 's open-label pilot study, probably due to either the small sample size or the relatively low dose, or the short duration of the clinical study, or the influence of the antibiotic on both harmful and beneficial bacteria[141]. A multicenter, double-blind, placebo-controlled, phase III RCT study observed how the rifaximin-simvastatin combination, by modulating the gut microbiota, reduces the systemic inflammatory response, endotoxemia and plasma levels of secondary bile acids and fatty acids in patients with decompensated cirrhosis, preventing the development of high liver toxicity and/or chronic acute liver failure[175].

Fecal Microbial Transplantation

Considered a valid alternative to antibiotics, prebiotics and probiotics, fecal microbial transplantation (FMT) is a therapeutic approach that consists in the elaboration, standardization and transplantation of a fecal suspension of the entire gut microbial community of a healthy donor into the GI tract of a receiving patient[176]. Following FMT, a restoration of healthy gut environment occurs in these patients; it is characterized by a higher number of healthy gut bacteria and their metabolites, pathogenic factors reduction, and restoration of intestinal barrier structure and function[177]. Different studies[62,177,178,205] conducted on mice and humans have shown that FMT is able to attenuate liver disease through a beneficial effect on the gut microbiota. For example, NAFLD mice receiving the microbiota of donors fed with HDF, showed reduced fasting blood glucose, HOMA-IR, and NAS score[62], while mice with HFD-induced NASH and receiving FMT showed an increase of beneficial bacteria *Christensenellaceae, Lactobacillus* and *Prevotellaceae,* and a reduction of *Odoribacter* and *Oscillibacter* after 8 weeks. FMT also significantly increases the concentration of butyrate in cecal contents, reduces body weight, NAS score (from 6.90± 0.233 to 4.58 ± 0.260), transaminases, mRNA levels of TNF-α, MCP-1, IL-1β, IL-2, IL-6, IFN-γ e IL-17, and restores intestinal barrier function with consequent attenuation of endotoxemia, steatosis, lobular inflammation, and ballooning[178]. In humans, Bajaj et al[179] showed a restoration of microbial diversity and function with consequent improvement of dysbiosis and reduction of liver-related hospitalizations after oral administration in FMT capsules, while Craven et al[180] observed a reduction in small intestine permeability in NAFLD patients undergoing 6 weeks of allogeneic FMT, with no improvement in IR or hepatic fat fraction. Despite these different effects, safety, duration of effect, how FMT affects the GI microbial community and relative consequences, its physiological interactions with the patient and how long the modi-

fied microbiota is maintained in patients, remain to be clarified[181].

Dietary Approaches

Polyphenols

Polyphenols are water-soluble bioactive compounds of vegetable origin present in foods as fruit, tea, dark chocolate, coffee, red wine, and red berries[182,183]. Their anticoagulant, lipid-lowering, hypotensive, antioxidant, anti-inflammatory properties and their ability to interact bidirectionally with the gut microbiota[184], make them a great therapeutic strategy for NAFLD treatment[185].

Resveratrol (RSV; 3, 5 4′-trihydroxystilbene) is a natural phenolic compound present in grapes and wine with lipid-lowering, insulin-sensitizing, antioxidant, and anti-inflammatory properties. It can maintain the integrity of the intestinal barrier function, and reduce blood glucose, LDL-C, ALT significantly, BMI, and waist circumference[186,187], to modulate microbiota composition in favor of *Bacteroidetes*/*Firmicutes* ratio, *Lactobacilli* and *Bifidobacteria*[188] and a member of the nicotinamide adenine dinucleotide (NAD +) - dependent deacetylase family, known as SIRT1[189]. Once activated by resveratrol, SIRT1 plays a beneficial role in the liver against oxidative stress and inflammation, by promoting the deacetylation of PGC-1α and reducing the production of pro-inflammatory cytokines through the deacetylation of NF-κB. SIRT1 is also able to improve fat metabolism by inhibiting *de novo* lipogenesis *via* the deacetylation of SREBP-1c and ChREBP, and by increasing the β-oxidation of fatty acids *via* PPARα/PGC-1α deacetylation (transcriptional co-activator), rebalancing the hepatic lipid homeostasis[190]. Resveratrol is currently considered as an excellent candidate for NAFLD prevention, even if there are conflicting data on its efficacy.

The silymarin, a complex mixture of seven flavonoglignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid taxifolin from milk-thistle *Silybum marianum*, with antioxidant, anti-inflammatory, direct and indirect antifibrotic, and hepatoprotective properties[191], has also been shown to be helpful in improving NAFLD by reducing liver fibrosis and stiffness, oxidative stress, IR, steatosis, and mitochondrial disfunction, especially if associated with physical activity and a healthy diet[192]. However, studies on silymarin effect on gut dysbiosis are lacking.

In recent years, berberine (BBR), an isoquinine alkaloid present in various plants such as Berberidaceae, Ranunculaceae and Papaveraceae has aroused great interest. BBR is well-known for its numerous actions, such as inhibiting gluconeogenesis, IR and hepatic lipogenesis[193], regulating the MAPK pathway, improving mitochondrial function, and reducing the protein convertase subtilisin/kexin 9 (PCSK9) and the expression and methylation of DNA[194]. Moreover, BBR is able to improve NAFLD development and progression thanks to its hepatoprotective properties against different chemical insults, its influence on the composition and diversity of the gut microbiota, on the reduction of endotoxemia, and on the maintenance of integrity of the mucosal barrier[195].

Fasting-Mimicking Diet and Modified Intermittent Fasting

Recent studies[196] suggest how diets based on intermittent fasting DI (FMD and ADFM), i.e., regimes based on strong caloric restrictions for a limited period to a few days of the week or specific times of the day (CREA), have a positive effect on NAFLD dysbiosis and pathogenesis. The fasting-mimicking diet (FMD) is a cyclical nutritional approach consisting of mimicking the metabolic state of the host during fasting and aims to reduce the disadvantages of the more restrictive fasting diets, limited to the consumption of liquids only. A randomized and controlled clinical trial[197], divided into 2 phases of 3 months each on 100 patients, reported a significant reduction in weight (-2.6 ± 2.5 kg, $p<0.0001$), total body fat (−1.393 ± 1.786, $p=0.0002$), waist circumference (-4.1 ± 5.2 cm, $p=0.0035$), and IGF-1 levels (-21.7 ± 46.2 ng/ml, $p=0.0017$). Modified intermittent fasting (ADFM) consists of alternating between fasting days, in which a single midday meal is consumed with an intake of 25% of the daily energy requirement and an *ad libitum* diet. Johari et al[198] observed, after eight weeks of ADFM, a significant reduction in weight (-2.01 kg, $p=0.003$), BMI (-0.78 Kg/m^2, $p=0.003$), AST (-8.63 IU L, $p=0.004$), ALT (-25.16 IU/L, $p=0.001$), steatosis degree (-0.5, $p=0.001$) and fasting blood glucose (-0.75 mmol/L, $p=0.006$) in NAFLD patients. However, neither of the two diets led to a variation in total cholesterol, LDL-C, HDL, TG, and PRC. Further studies are necessary to understand all the mechanisms through which these diets work.

Chinese Phytotherapy

Chinese phytotherapy represents another potential therapeutic approach for NAFLD treatment. Herbs (246 of its common identified) and/or traditional Chinese formulas, taken as supplements and characterized by a multilevel and multitarget pharmacological activity, act through different molecular pathways (PPAR-α, SREBP -1c, NF-κB, PI3K, SIRT1, AMPK, p53, and Nrf2) on the pathogenic mechanisms, underlying the disease such as inflammation, lipogenesis, IR, mitochondrial disfunction, autophagy, oxidative stress, and gut microbiota[199,200].

Chinese Herbs

Almost all herbs [i.e., garlic, ginger, green tea, ulva prolifera, ginko biloba, Damask rose, Antrodia (antrodan), Sesame, Plumbago, Swertia, and loquat] reduce blood and hepatic TG, TC, AST, and ALT aminotransferases, except for sesame, citral (ginger) and diallyl disulfide (garlic) which also promote FFA blood levels reduction and their translocation to the liver. Many of these herbs improve insulin, body weight (ginger, green tea, anthrodia, sesame, plumbago, and loquat), fatty liver weight, and Fatty Liver Score (garlic, ginger, grape, psoralea, ginko biloba, antrodia, sesame, plumbago, swertia, and loquat). Only some of these reduce hepatic lipogenesis by down-regulating the expression of transcriptional factor SREBP-1c and lipogenic enzymes Fatty Acid Synthase (FAS), Acetyl-CoA Carboxylase (ACC), and Stearoyl-CoA desaturase (SCD), responsible for lipid storage under physiological conditions. The same herbs promote mitochondrial β-oxidation of long-chain fatty acids by up-regulating the expression of PPAR-α (peroxisome proliferator-activated receptor α) and the enzyme CPT-1 (carnitine palmitoyltransferase-1), which catalyzes the transfer of FFA from acyl -CoA to carnitine forming acyl-carnitine, subsequently translocated across mitochondrial membranes by CACT (carnitine acyl-carnitine translocase)[201]. Herbs such as garlic, ginger, psoralea, and swertia reduce pro-inflammatory cytokines expression (TNF-α, IL-1β, and IL-6); in particular, garlic, ginger, swertia, along with herbs such as sesame, plumbago, and loquat ameliorate oxidative function by reducing lipid peroxidation and CYP2E1 expression and by enhancing the activity of the antioxidant enzymes such as SOD, GSH, CAT, GPx, and GRd. Finally, only green tea catechins reduce endotoxemia and increase *Firmicutes*/*Bacteroidetes* ratio and SCFA-producing bacteria number[202,203] (Table II).

Carbon Nanoparticle

A synthetic porous carbon nanoparticle[204], highly absorbent, non-absorbable or degradable in the GI tract, excreted unaltered in feces and with

Table II. Most common Chinese herbs used for NAFLD treatment, their compounds and mechanism of action.

Chinese herbs compounds		Glucose	Insulin	Fatty liver score	FFA	Inflammation
Garlic	Essential oil	X	X	X	X	X
	Diallyl disulfide (essential oil)	X	X	X	X	X
Ginger	Citral		X	X	X	X
	Essential oil		X	X	X	X
	Zingerone	X		X		
Green tea	Polyphenols	X	X			X
Ulva prolifera	Sulfated polysaccharides			X		
Ginko	Ginkgolide A			X		
Damask rose	Ethanol extracts			X		
Antrodia cinnamomea fungus	Antrodan	X	X	X		
Sesame	Seeds ethanol extract	X	X	X	X	
Ceylon leadwort	Plumbagin		X	X	X	X
Swertia bimaculata	Swertiamarin	X		X		X
Loquat	Fruit extract	X		X		
Babchi	Seeds extract	X		X		X

Modified by Panyod et al[203].

a high absorption capacity, represents a new, safe, and non-antibiotic potential strategy for the treatment of NAFLD. Evidence has shown that this compound can counteract dysbiosis *in vivo,* reducing steatosis and liver inflammation and translocation, *in vitro,* of both hydrophobic and PM up to about 70kDa substances, such as intestinal toxins (ammonia, asymmetric dimethylarginine, acetaldehyde), cytokines (TNF-α and IL-6), hydrophobic bile acid, bacterial products (LPS, ROS) and exotoxins preventing their flow into the liver and systemic circulation[205].

MCJ

MCJ (or DNAJC15) is a small insoluble transmembrane protein of 147 amino acids encoded by the nuclear gene *Dnajc15,* mainly located in the internal mitochondrial membrane of hepatocytes. Recent studies[206,207,208] showed as MCJ, present at high levels in patients with steatosis, acts on gut dysbiosis affecting NAFLD/NASH severity, as an essential endogenous negative regulator of mitochondrial metabolism. MCJ is involved in the formation of respirasomes, supercomplexes responsible for triggering apoptosis, inflammation and fibrosis. Therefore, the inhibition of its expression and/or function could represent a potential safe alternative strategy to treat NAFLD. Hatle et al[206] observed how the loss of MCJ leads to increased activity of complex I and in the membrane potential and, therefore, in mitochondrial respiration. The absence of MCJ, prevent the pathological accumulation of lipids in the liver in altered metabolic conditions, such as fasting and high cholesterol diet. The same result was observed by Barbier-Torres et al[207] using si-MCJ (silencing MCJ) combined with lipid nanoparticles and with two GalNAc-siRNA sequences (si381 e si393). In addition to both increased mitochondrial respiration and hepatic catabolism and the expression of genes involved in hepatic mitochondrial β-oxidation of fatty acids (*Cpt1*, *Acadm*, *Acadl*, *Fatp2*, *Abcd1*, *Pgc1α*, and *Nrf2*), si-MCJ is able to enhance glycolysis, hepatic glycogenesis and insulin response and to reduce weight gain, with no increase in ROS[208] or ketosis after treatment. This results in decreased apoptosis rates, steatosis and inflammation of the liver, which in turn results in a milder liver damage (as demonstrated by the lower levels of serum AST), fibrosis, NASH development, and progression to cirrhosis or HCC.

H2-rich Water

The liver is an organ that shows a high capacity to accumulate exogenous H_2, supplied through several routes of administration. Among these, Otha[209] described oral intake with water, obtained by dissolving a concentration up to 0.8 mM (1.6 mg/L) of H_2 in water (HRW, H_2-rich water) at atmospheric pressure and room temperature or by reacting metallic magnesium and water. Thanks to its antioxidant, anti-inflammatory and anti-apoptotic properties, H_2 represents an essential regulator of hepatic homeostasis and therefore, a potential therapeutic mean to protect the liver from acute or chronic damage, and to prevent and treat NAFLD[210]. H_2 is able to directly and selectively remove the highly oxidizing compounds OH and ONOO– in the mitochondria of cultured cells, suppress lipid peroxidation associated with free radical chain reactions, and regulate negatively NF-κB and pro-inflammatory cytokines[211,212]. In mouse models, Kawai et al[213] observed ALT, TNF-α, IL-6, oxidative stress, balloning, fibrosis, apoptosis, FFA absorption and β-oxidation reduction ($p<0.05$), while Lin et al[214] TNF-α (-6.4%, $p<0.05$), IL-6 (-10%, $p>0.05$), ALT (-11.5%, $p<0.05$), AST (-10.9%, $p>0.05$), TG (-13.9%, $p<0.05$), TC (10.3%, $p<0.01$), and ROS reduction. In humans, intake of 900 mL/day of HRW for 8 weeks significantly reduced small and dense LDL by 15.5% ($p<0.01$), oxidized LDL, and FFA, and increased plasma SOD levels in patients with T2DM[215]; the intake of 1.2 ppm HRW for 28 days reduced steatosis ($p<0.05$) and AST (-10%) in twelve overweight patients with NAFLD[216]. H_2 also seems to be able to protect intestinal barrier integrity, modulate the microbiota, and ameliorate clinical characteristics of gut microbiota disorders including diarrhea, weight, and fluid loss[217]. A relatively small quantity of HRW effectively determines an increase in the abundance of hydrogenotrophic bacteria (methanogens, acetogens, and sulfate-reducing bacteria) and their metabolites (methane, acetate, and H_2S) and a growth of anaerobic and butyrate-producing bacteria. In 2019, Sha et al[218] confirmed such beneficial effect on gut microbiota on thirty-eight youth soccer players, who showed a greater abundance and diversity of the intestinal flora after taking 1.5-2.0 l/day of HRW for 2 months[131].

Conclusions

NAFLD is the most common chronic liver disease in the Western world and a leading cause of liver transplantation. The pathophysiological mechanisms underlying NAFLD, and related

therapeutic strategies are currently the subject of worldwide research; new knowledge on these is still growing. NAFLD mechanism of pathogenesis is complex and growing evidence indicates that both different genetic and environmental factors (such as T2DM, IR, metabolic syndrome, Western diet, poor quality of sleep, sedentary lifestyle, and drugs) contribute to its development and progression. IR plays a predominant role since it firstly exposes hepatocytes to steatosis and lipotoxicity. Hence, a worsening of IR and the release of pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and/or chemokines, resulting in inflammation and an impaired immune response. Moreover, the amplification of cell and tissue damage and in the progression to NASH occurs. Recently, the hypothesis that the gut-liver axis, particularly the dysbiosis, is involved in NAFLD development and progression, is increasingly accepted. This can exacerbate immune responses, and both hepatic and systemic inflammation by modulating intestinal permeability, endotoxemia, energy and BAs homeostasis, choline metabolism, its toxic derivative TMAO, endogenous ethanol and its derivatives acetaldehyde and acetate, phenylacetate and BCAAs and the endocannabinoid system. Inflammation, along with injured hepatocytes and activated Kupffer cells, leads HSCs with myofibroblastic activity to secrete an excessive amount of extracellular matrix, thus determining the transition to fibrosis. Continuous liver damage promotes progression to advanced fibrosis, liver cirrhosis, and HCC. Despite this, further studies are needed to fully understand all the molecular targets and signaling pathways involved. The absence of a gold standard treatment for NAFLD has led research towards new potential therapeutic strategies aimed above all to restore the condition of eubiosis. In this review, we have examined different approaches for NAFLD treatment like probiotics, prebiotics, symbiotics, postbiotics, non-absorbable antibiotics (neomycin, cidomycin, and rifaximin), dietary approaches (polyphenols and diets based on intermittent fasting DI), Chinese herbs, FMT, and minor therapies such as carbon nanoparticle, MCJ protein and H2-rich water. Several studies[132,141,184,205] on animal and cellular models have shown how these therapies are able to improve the pathological condition by modulating the gut microbiota, or by reverting the condition of dysbiosis into eubiosis. In general, mentioned therapies are able to improve IR, steatosis, permeability, endotoxemia, and oxidative function, and reduce the levels of serum transaminases ALT and AST, pro-inflammatory cytokines TNF-α, IL-6 and the lipid profile (TC, TG) inhibiting hepatic lipogenesis. Many of these approaches also result in a significant reduction in body weight, body fat and waist circumference. Among the minor therapies, carbon nanoparticle can prevent the translocation of toxins, cytokines, bile acids, bacterial products and exotoxins into the liver and systemic circulation *in vitro*, while the inhibition of MCJ expression and/or function is essential to improve mitochondrial respiration and insulin response, and induce hepatic mitochondrial β-oxidation, glycolysis, and glycogenesis. Finally, hydrogen-rich water is able to directly and selectively remove the highly oxidizing compounds OH and $ONOO^-$ in the mitochondria, to suppress lipid peroxidation associated with free radical chain reactions, and to modulate the microbiota and consequently conditions such as diarrhea, weight gain, and fluid loss. Therefore, cited therapies determine in different ways an improvement in steatosis, lipotoxicity, inflammation and apoptosis, which in turn result in a reduction in liver damage and in the progression to NASH, fibrosis, cirrhosis, and HCC, and in an improvement in symptomatology. However, their application in clinical practice requires further investigation due to the small number of clinical studies on humans.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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FG, MI and VR critically review the project. AN, MT and NDD provided critical feedback to help revise the manuscript. DDM and DP edited and supervised the review. All authors have read and approved the final version of the manuscript to be published.

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