

# The relationship between polyphenols and miRNAs: A novel therapeutic strategy for metabolic associated fatty liver disease

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## Abstract

Metabolic-associated fatty liver disease (MAFLD) is a public health problem that is increasingly recognized, currently affecting up to a quarter of the world's adult population. Although a biopsy is the current gold standard to diagnose MAFLD, there are potentially serious complications, making it inadequate. Thus far, noninvasive methods have not been able to determine the stage and the subtype of MAFLD. The development and prognosis of MAFLD are modulated by epigenetic factors, including microRNAs (miRNAs), which may be potential biomarkers for MAFLD. Polyphenols, found in many fruits and vegetables, may be useful, as they alter gene expression with epigenetic factors, such as miRNAs. This review presents an overview of the relationship between polyphenols and miRNAs in MAFLD. The literature suggests that miRNAs could be used as a diagnostic method for MAFLD, especially miRNA-122 and miRNA-34a. However, though it has been demonstrated that polyphenols may contribute to improving MAFLD, to our knowledge, no study to date has shown the relationship between polyphenols and miRNAs in MAFLD. The exact mechanisms of polyphenols on miRNAs in MAFLD remain unclear. Future studies may provide hope for diet therapy for MAFLD patients as well as the development of polyphenol-related foods or drugs that target miRNAs to treat MAFLD.

**Keywords:** Metabolic-associated fatty liver disease; microRNA; polyphenols.

## Introduction

Metabolic (dysfunction)-associated fatty liver disease (MAFLD), previously known as non-alcoholic fatty liver disease (NAFLD), is the most common chronic liver disorder worldwide, thought to affect more than one-third of the general population (estimated to be 30% of adults in industrialized countries).<sup>[1,2]</sup> The high prevalence of MAFLD has been associated with increasing levels of an unhealthy diet and low physical activity seen worldwide.<sup>[3]</sup>

**How to cite this article:** Bayram HM, Eren F, Gunes FE. The relationship between polyphenols and miRNAs: A novel therapeutic strategy for metabolic associated fatty liver disease. *Hepatology Forum* 2021; 2(3):128–136.

**Received:** September 04, 2021; **Accepted:** September 10, 2021; **Available online:** September 15, 2021

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MAFLD is a multisystem disorder with a complex pathophysiology.<sup>[1]</sup> It is recognized as the liver disease component of metabolic syndrome, and is associated with insulin resistance and genetic susceptibility. As the epidemic rates of obesity, type II diabetes mellitus, insulin resistance, and dyslipidemia continue to increase, the risk of MAFLD increases proportionately.<sup>[2,4]</sup>

In recent years, it has been demonstrated that epigenetic factors may cause the development of a wide range of diseases, including MAFLD, and miRNAs appear to have an important role.<sup>[5]</sup> Therefore, miRNAs have the potential for use in various clinical settings, such as early diagnosis and the monitoring of progression and response to treatment in various diseases.<sup>[6]</sup> Almost all genetic pathways, including transcription factors, secreted factors, receptors, and transporters, can be modulated by miRNAs.<sup>[7]</sup> Also, environmental conditions, such as stress and nutritional status, can modulate epigenetic factors, and so miRNAs may also be useful to assess the effects of diet and other lifestyle interventions.<sup>[8]</sup>

The value of polyphenols in functional foods is evident due to biological activity that includes antioxidant, anti-inflammatory, and anticancer behavior; regulation of lipid, carbohydrate, and amino acid metabolism; inhibition of platelet aggregation; and improvement of endothelial function.<sup>[9,10]</sup> Regular consumption of polyphenols has been associated with a reduction in the risk of several metabolic diseases, such as obesity, insulin resistance, hypertension, and cardiovascular disease.<sup>[11–13]</sup> Polyphenols may alter gene expression via epigenetic factors, such as miRNAs, by contributing to the modulation of key proteins.<sup>[14,15]</sup> Thus, they may contribute to the amelioration of MAFLD and other diseases, but the knowledge of the potential mechanisms of polyphenols and miRNAs in MAFLD remains limited. This review presents an overview of the known relationship between polyphenols and miRNAs in MAFLD.

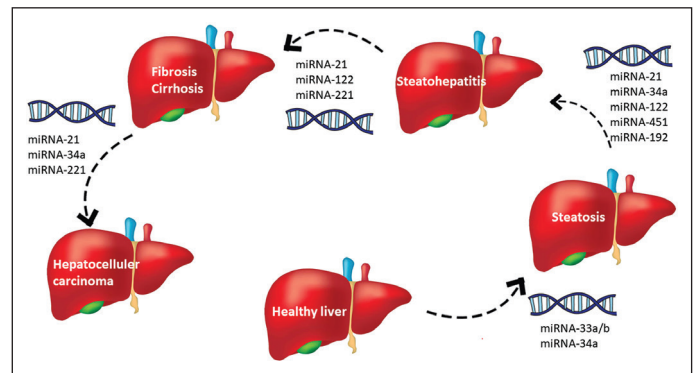
## Materials and Methods

A comprehensive electronic search of the Scopus, ScienceDirect, and PubMed databases was conducted to identify relevant studies. The keywords used were “phenolic” OR “polyphenol” AND “non-alcoholic fatty liver disease” OR “metabolic dysfunction-associated fatty liver disease” AND “miRNA” OR “microRNAs”. Theses, editorials, letters to editor, and conference abstracts were excluded. The inclusion criteria were *in vitro*, *in vivo*, or human clinical studies of the effects of polyphenols on MAFLD-associated miRNAs. We did not find any human clinical studies. A total of 85 studies were included in this review.

### The Role and Expression Level of miRNAs in MAFLD

miRNAs are endogenous, small non-coding RNAs that play a central role in regulating both mRNAs and the protein expression of target genes.<sup>[16]</sup> miRNAs are specific regulators that affect the stability or translation of the targeted mRNA. miRNAs are abundant in the liver and modulate a spectrum of cellular processes related to inflammation, proliferation, differentiation, cellular growth, tissue remodeling.<sup>[17,18]</sup> Therefore, since miRNAs can be detected in tissue and serum in a stable form, they are potential biomarkers for many liver diseases.<sup>[19]</sup>

Thousands of miRNAs have been identified, though their exact mechanisms remain unknown. Several miRNAs have shown anomalous expression in MAFLD (Fig. 1). One study identified 44 miRNAs with differential expression in MAFLD patients.<sup>[20]</sup> It has been reported that the serum level of miRNA-122, miRNA-34a, and miRNA-16 were upregulated in MAFLD patients compared with control groups, and that the level of miRNA-122 and miRNA-34a was correlated with the severity of MAFLD.<sup>[21]</sup> It was observed in another study that the serum level of miRNA-21, miRNA-34a, miRNA-122, and miRNA-451 were upregulated in MAFLD patients and that the serum level of miRNA-122 was positively associated with steatosis severity.<sup>[22]</sup> It has also been reported that hsa-miRNA-122-5p, hsa-miRNA-1290, hsa-miRNA-27b-3p, and hsa-miRNA-192-5p levels were higher in MAFLD patients and that miRNA levels were a more specific biomarker for MAFLD than alanine transaminase and fibrosis-4 index values.<sup>[23]</sup> An investigation of the serum level of miRNA-197, miRNA-146b, miRNA-10b, miRNA-181d, miRNA-34a, miRNA-122, miRNA-99a, and miRNA-29a in MAFLD patients yielded results indicating that the level of miRNA-181d, miRNA-99a, miRNA-197, and miRNA-146b were downregulated in MAFLD patients. Additionally, miRNA-197 and miRNA-10b were associated with the severity of inflammation while miRNA-181d and miRNA-99a levels were related to the serum level of gamma-glutamyltransferase in non-alcoholic steatohepatitis (NASH) patients.<sup>[24]</sup> It has also been noted that the serum level of miRNA-122 in mild steatosis patients was lower than that of severe steatosis patients, while the serum level of miRNA-122 in mild fibrosis patients was higher compared with that of severe fibrosis patients.<sup>[25]</sup> A study that analyzed 84 miRNAs in MAFLD patients showed that the serum level of miRNA-122, miRNA-192, miRNA-375, and miRNA-122 were upregulated in steatosis patients, and the serum level of miRNA-122 and miRNA-192 were significantly downregulated in NASH patients compared with the level observed in controls.<sup>[6]</sup> It was confirmed that the serum level of miRNA-21 was lower in NASH patients and that the serum level of miRNA-122 and miRNA-192 was differentially regulated in bland steatosis (NAFL) and NASH patients.<sup>[26]</sup> Similarly, the serum level of miRNA-21 was found to be lower in MAFLD patients.<sup>[27]</sup> It has been observed that the level of miRNA-122, miRNA-192, and miRNA-34a was associated with steatosis and inflammatory activity, and that only the miRNA-16 level was significantly correlated with fibrosis. It has also been reported that the serum level of miRNA-34a was lower in NASH patients than in MAFLD patients.<sup>[28]</sup> Furthermore, it was indicated that the serum level of miRNA-122 and miRNA-34a was higher, while the miRNA-331-3p and miRNA-30c levels were lower in MAFLD patients.<sup>[29]</sup> Another study found that the serum level of miRNA-122 and miRNA-34a was upregulated in MAFLD patients and strongly related with very-low-density lipoprotein and triglyceride (TG) levels.<sup>[30]</sup> The findings of other research confirmed that the serum level of miRNA-122 was upregulated in MAFLD patients.<sup>[31]</sup> It has also been reported that the



**Figure 1.** The role of miRNAs in key transitions of the pathogenesis of non-alcoholic fatty liver disease.

miRNA: MicroRNA.

expression of miRNA-122 was reduced in a morbidly obese group compared with moderately obese patients and that the miRNA-122 level was greater in morbid obese patients with NASH than in morbid obese patients with simple steatosis. The expression of miRNA-33b was greater in the NASH patients.<sup>[32]</sup> The findings of another study revealed that the serum level of miRNA-301 and miRNA-34a-5p were upregulated and miRNA-375 was downregulated in MAFLD patients. In addition, increased expression of miRNA-301a and miRNA-375 was noted in hepatocellular carcinoma patients.<sup>[33]</sup> Other researchers found that 14 miRNAs were associated with MAFLD and that the liver levels of miRNA-139-5p, miRNA-30b-5p, miRNA-122-5p, and miRNA-422a were lower and the level of miRNA-146b-5p was higher in obese patients with MAFLD compared with a control group.<sup>[34]</sup> It has also been demonstrated that miRNA-22, miRNA-29a, and miRNA-663a were upregulated in MAFLD patients.<sup>[35]</sup> Similarly, another study noted that miRNA-34a, miRNA-192, miRNA-27b, miRNA-122, miRNA-22, miRNA-21, miRNA-197, miRNA-30c, and miRNA-16 were correlated with MAFLD severity.<sup>[36]</sup> Table 1 provides details of studies about miRNA levels and Table 2 summarizes some possible miRNA pathways in MAFLD patients.

### The Relationship between Polyphenols and miRNAs

Polyphenols are secondary metabolites that are abundant in fruits and vegetables as well as other products, including coffee, tea, red wine, and dark chocolate.<sup>[14,37,38]</sup> Polyphenols are classified into 2 main groups: flavonoids and non-flavonoids. The non-flavonoids include subgroups of phenolic acids, stilbenes, and lignans. The main subgroups of flavonoids are flavanols, flavan-3-ols, isoflavones, and anthocyanidins, and minor flavonoid subgroups include flavan-3,4-diols, dihydroflavonols, chalcones, dihydrochalcones, coumarins, and auronones.<sup>[37]</sup> A dietary intake of polyphenols was estimated at 1-1.2 g per day, 40% of which were flavonoids.<sup>[9]</sup> Only 5% to 10% of polyphenols ingested are absorbed in the small intestine. During the process of absorption, polyphenols are often conjugated in the small intestine and later in the liver. The non-absorbable portion passes to the colon and is metabolized by intestinal microbiota.<sup>[14]</sup>

Polyphenols have been shown to have various therapeutic properties, such as antioxidant, anti-inflammatory, antidiabetic, antiallergic, antimicrobial, and anticancer effects, as well as improved lipid metabolism.<sup>[39]</sup> Most of the therapeutic effects of polyphenols have been linked to altering gene expression that encodes essential metabolic

**Table 1.** miRNA analysis in MAFLD patients

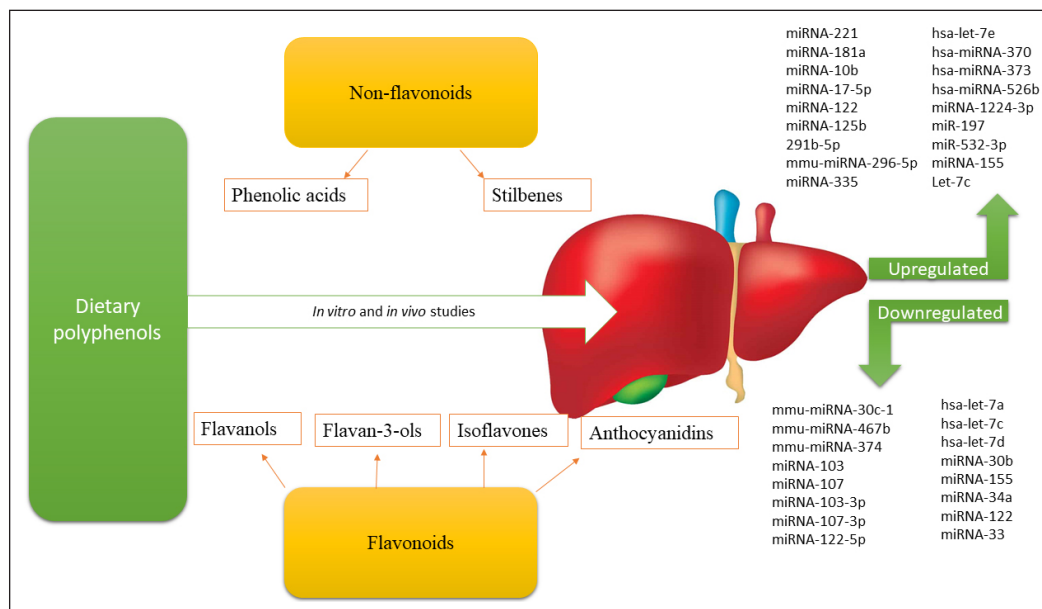
Study group	Biopsy	Method	Upregulated miRNAs	Downregulated miRNAs	References
Control group (n=19), MAFLD (n=34)	Yes	qRT-PCR	122, 34a (NASH), 16 (MAFLD)		Cermelli et al., 2011 <sup>[21]</sup>
Control group (n=31), MAFLD (n=92)	No	RT-PCR	21, 34a, 122, 451 (MAFLD)		Yamada et al., 2013 <sup>[22]</sup>
Control group (n=90+(80)), MAFLD (152+(103))	Yes	qRT-PCR	hsa-miRNA-122-5p, hsa-miRNA-1290, hsa-miRNA-27b-3p, hsa-miRNA-192-5p (MAFLD)		Tan et al., 2014 <sup>[23]</sup>
Control group (n=20), MAFLD (n=20)	Yes	qRT-PCR		181d, 99a, 197, 146b (MAFLD)	Celikbilek et al., 2014 <sup>[24]</sup>
MAFLD (n=52)	Yes	TaqMan Micro-RNA assays	122 (steatosis)	122 (fib.)	Miyaaki et al., 2014 <sup>[25]</sup>
Control group (16+(19)), MAFLD (16+(30)), NASH (16+(47))	Yes	RT-PCR	122, 192, 375 (NASH), 122 (Fib)		Piroia et al., 2015 <sup>[6]</sup>
Control group (n=61), MAFLD (n=50), NASH (n=87)	Yes	qRT-PCR	122, 192, 21 (NASH)		Becker et al., 2015 <sup>[26]</sup>
Control group (n=12), MAFLD (n=25)	No	qRT-PCR		21 (MAFLD)	Sun et al., 2015 <sup>[27]</sup>
Control group (n=37), MAFLD (n=17), NASH (n=31)	Yes	qRT-PCR	122, 192, 34a (NASH), 16, 21, 146 (MAFLD)		Liu et al., 2016 <sup>[28]</sup>
Control group (n=62), NALFD (n=18)	No	RT-PCR	122, 34a (MAFLD)	331-3p, 30c (MAFLD)	Zarrinpar et al., 2016 <sup>[29]</sup>
Control group (n=28), MAFLD (n=36)	Yes	qRT-PCR	122, 34a (MAFLD)		Salvoza et al., 2016 <sup>[30]</sup>
MAFLD (n=305)	Yes	TaqMan Micro-RNA assays	122 (steatosis, inf., ballooning and fib.)		Akuta et al., 2016 <sup>[31]</sup>
Control group (n=31), MAFLD (n=27), NASH (n=34)	Yes	RT-PCR	122 (NASH)		Auguet et al., 2016 <sup>[32]</sup>
Control group (n=10), MAFLD (n=12), NASH (n=1), cirrhosis (n=3)	Yes	RT-PCR	miR-301a-3p and miR-34a-5p	375	Guo et al., 2016 <sup>[33]</sup>
Control group (n=19), borderline (n=24), MAFLD (17),	No	RT-PCR	146-5p	139-5p, 30b-5p, 122-5p, 422a	Latorre et al., 2017 <sup>[34]</sup>
Control group (n=10), MAFLD (n=44)	Yes	RT-PCR	22, 29a (NASH), 663a (MAFLD)		López-Riera et al., 2017 <sup>[35]</sup>
Control group (n=17), MAFLD (n=46), NASH (n=50), fibrosis (n=29)	Yes	RT-PCR	34a-5p, 27b-3p, 22-3p, 122-5p, 192-5p (MAFLD and NASH) 27b-39, 21-5p, 122 (fib.)	30c-5p, 16-5p, 197-3p (MAFLD) 16-5p, 30c-5p (NASH and fib.)	López-Riera et al., 2018 <sup>[36]</sup>

MAFLD: Metabolic-associated fatty liver disease; MIRNA: Micro RNA; NASH: Non-alcoholic steatohepatitis; PCR: Polymerase chain reaction; qRT-PCR: Quantitative real-time PCR; RT-PCR: Real-time PCR.

**Table 2.** Possible microRNA pathways in metabolic-associated fatty liver disease

miRNA	Pathway
miRNA-122 <sup>[61-64]</sup>	Lipid metabolism (cholesterol, VLDL, TG, HMGCR), carcinogenesis
miRNA -10b <sup>[65]</sup>	Lipid metabolism (PPAR- $\alpha$ )
miRNA -33 <sup>[66-68]</sup>	ABCA1 transport, ABCG1, Niemann Pick (NP) -C1, insulin signal pathway
miRNA -34 <sup>[6,21,61,69-71]</sup>	AMPK phosphorylation, miR-34a/SIRT1/p53 activation, MAFLD progression, lipid metabolism
miRNA -192 <sup>[72,73]</sup>	MAFLD progression
miRNA -214-5p <sup>[74]</sup>	MAFLD progression
miRNA -27a/b <sup>[75]</sup>	Lipid metabolism
miRNA -24 <sup>[76]</sup>	Lipid metabolism (Insig1)
miRNA -451 <sup>[77]</sup>	Inflammation
miRNA -1290, miRNA -27b-3p <sup>[78]</sup>	Variable
miRNA -192-5p <sup>[79]</sup>	Lipid metabolism (SCD-1)
miRNA -103/107 <sup>[8,80,81]</sup>	PANK1-3, lipid metabolism, development of insulin resistance, PPAR- $\alpha$ , caveolin-1
miRNA -155 <sup>[82,83]</sup>	Inflammation and liver injury, SREBP-1c, LXR - lipid metabolism
miRNA -29 <sup>[61]</sup>	SIRT1, HMGCR, LPL
miRNA -467b <sup>[84]</sup>	LDL metabolism
miRNA -143 <sup>[85]</sup>	FABP4, SLC2A4, PPAR $\gamma$ , and LIPE

ABCA1: member 1 of human transporter sub-family ABCA; ABCG1: Adenosine 5'-triphosphate-binding cassette subfamily G member 1; AMPK: Adenosine monophosphate-activated protein kinase; FABP4: Fatty acid-binding protein 4; HDL: High-density lipoprotein; HMGCR: 3-hydroxy-3-methyl-glutarylcoenzyme A reductase; LDL: Low-density lipoprotein; LIPE: Hormone-sensitive lipase; LPL: Lipoprotein lipase; LXR $\alpha$ : Liver X receptor R-alpha; MAFLD: Metabolic-associated fatty liver disease; miRNA: MicroRNA; NP-C1: Niemann Pick-C1; PANK1-3: Pantothenate kinase 1-3; PPAR- $\alpha$ : Peroxisome proliferator-activated receptor-alpha; PPAR $\gamma$ : Peroxisome proliferator-activated receptor  $\gamma$ ; SCD-1: Stearoyl-CoA desaturase 1; SIRT-1: Sirtuin-1; SLC2A4: Solute carrier family 2, facilitated glucose transporter member 4; SREBP-1c: Sterol regulatory element-binding transcription factor 1; TG: Triglycerides; VLDL: Very-low-density lipoprotein.

**Figure 2.** Possible effects of polyphenols on MAFLD-associated miRNAs.

MAFLD: Metabolic-associated fatty liver disease; miRNA: MicroRNA.

proteins. These gene modifications may result from the interaction of polyphenols with epigenetic factors, such as signal cascades and/or miRNAs.<sup>[18]</sup> Modulation of miRNAs by polyphenols appears to be a potential new strategy to regulate metabolism and related diseases;<sup>[40]</sup> however, the precise mechanisms are not yet known.<sup>[9,14]</sup> In recent years, the beneficial effects of polyphenols in MAFLD patients have begun to attract attention and it has been demonstrated that the ther-

apeutic effects of polyphenols may contribute to improvement of MAFLD.<sup>[41]</sup> Investigation of miRNA pathways continues.

Our review revealed no study evaluating the relationship between polyphenols and miRNAs in MAFLD. Therefore, we focused on the relationship between polyphenols and miRNAs that may have an impact on liver disease and diseases caused by MAFLD (Fig. 2).

**Table 3.** Summary of in vitro and in vivo studies

Model	Study design	Polyphenols	Method	Upregulated miRNAs	Downregulated miRNAs	References
<i>In vitro</i>	Human hepG2 cells, 3.125, 6.25, 12.5, 25 and 50 µg/ml, 72 h	Ellagitannins	qRT-PCR	hsa-let-7e, hsa-miRNA-370, hsa-miRNA-373 and hsa-miRNA-526b	hsa-let-7a, hsa-let-7c and hsa-let-7d	Wen et al., 2009 <sup>[43]</sup>
<i>In vitro</i>	Human hepG2 cells, 50 mg/L of EGCG, 100 mg/L of GSPE or 100 mg/L of CPE, 5 h	GSPE, CPE or EGCG	qRT-PCR	miRNA-1224-3p, miR-197 and miR-532-3p (GSPE or CPE)	miRNA-30b (GSPE, CPE, EGCG)	Arola-Arnal et al., 2011 <sup>[15]</sup>
<i>In vitro</i>	3T3-L1 maturing pre-adipocytes, 25 µM, 8 days	Resveratrol	RT-PCR	miRNA-155		Eseberri et al., 2017 <sup>[45]</sup>
<i>In vitro</i>	Human pre-adipocytes, 25 µmol/L, 6 h	Extra-virgin olive oil polyphenols	qPCR	Let-7c	miRNA-155, miRNA-34a	Carpi et al., 2019 <sup>[47]</sup>
<i>In vitro</i>	FAO cells, 25 mg/L for 5 h	GSP	qRT-PCR		miRNA-122, miRNA-33	Baselga-Escudero et al., 2012 <sup>[42]</sup>
<i>In vitro</i>	Male Wistar rats, 250 mg/kg, 3 h					
<i>In vitro</i>	Human hepG2 cells, 0.02, 0.2, 2 and 20 µg/mL, 24 h	EGCG	qRT-PCR	miRNA-221, miRNA-181 °, miRNA-10b		Arffra et al., 2016 <sup>[44]</sup>
<i>In vivo</i>	Male Sprague Dawley rats, 20mg/kg, 16 weeks	Green tea				
<i>In vitro</i>	3T3-L1 maturing pre-adipocytes, 25 µM, 24 h	Curcumin	RT-PCR	miRNA-17-5p		Tian et al., 2017 <sup>[46]</sup>
<i>In vivo</i>	Male C57BL/6 mice, 2-10 µM, 7 days					
<i>In vivo</i>	Male C57BL/6J mice, 0.5 to 1.0% CPP, 15 weeks	Coffee polyphenols	RT-PCR	miRNA-122		Murase et al., 2011 <sup>[50]</sup>
<i>In vivo</i>	Female C57BL/6J mice, 0, 0.2 or 2 mg/g, 6 weeks	Quercetin	RT-PCR	miRNA-122 miRNA-125b		Boesch-Saadatmandi et al., 2012 <sup>[49]</sup>
<i>In vivo</i>	C57B6/J mice or apoE2/2 mice, 0.006% of the diet, 8 weeks	Quercetin, hesperidin, naringenin, anthocyanin, catechin, curcumin, proanthocyanin, caffeic acid, and ferulic acid	qRT-PCR	291b-5p, mmu-miRNA-296-5p	mmu-miRNA-30c-1, mmu-miRNA-467b and mmu-miRNA-374	Milenkovic et al., 2012 <sup>[52]</sup>
<i>In vivo</i>	Male mice deficient in LDL receptor in a C57BL/6J, high dose, 10 weeks	Quercetin	qRT-PCR		miRNA-103, miRNA-107, miRNA-122	Joven et al., 2012 <sup>[51]</sup>
<i>In vivo</i>	Male Sprague-Dawley rats, 30 mg/kg, 6 weeks	Resveratrol	RT-PCR		miRNA-103-3p, miRNA-107-3p, miRNA-122-5p	Gracia et al., 2017 <sup>[59]</sup>
<i>In vivo</i>	Mice, 500 mg/kg, 12 weeks	Green tea	qRT-PCR	miRNA-335		Otton et al., 2018 <sup>[52]</sup>

CPE: Cocoa proanthocyanidin extract; EGCG: Pure epigallocatechin gallate isolated from green tea; FAO: A rat hepatoma cell line; GSP: Grape seed proanthocyanidin; GSPE: Grape seed proanthocyanidin extract; MiRNA: MicroRNA; PCR: Polymerase chain reaction; qPCR: Real-time quantitative polymerase chain reaction; qRT-PCR: Quantitative real-time PCR; RT-PCR: Real-time PCR.



### i. *In Vitro* Studies

Polyphenols have been shown to improve lipid metabolism, inhibit adipogenesis and inflammation, and also provide antioxidant effects in cell line studies. Therefore, it is thought that the miRNAs in polyphenols may contribute to the amelioration of MAFLD patients.

A study reported that the miRNA-122 and miRNA-33 levels in hepatic cells decreased following 5 hours of a 25 mg/L grape proanthocyanin treatment in mouse hepatoma cell lines.<sup>[42]</sup> Ellagitannin doses of 3.125 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, and 50 µg/mL for 72 hours in human HepG2 cells demonstrated an antiproliferative effect; hsa-let-7e, hsa-miR-370, hsa-mir-373, and hsa-miR-526b were upregulated, whereas hsa-let-7a, hsa-let-7c, and hsa-let-7d were downregulated, depending on the dose and time.<sup>[43]</sup> In another study, HepG2 cells were treated with 50 mg/L of pure epigallocatechin gallate isolated from green tea (EGCG), 100 mg/L of grape seed proanthocyanin extract (GSPE) or 100 mg/L of cocoa proanthocyanidin extract (CPE). After 5 hours of treatment, miRNA-30b was downregulated by all 3 treatments, and EGCG or CPE treatments upregulated the level of miRNA-1224-3p, miRNA-197, and miRNA-532-3p.<sup>[15]</sup> In another study, the administration of 0.1 µg/mL, 0.2 µg/mL, 2 µg/mL, and 20 µg/mL of EGCG to HepG2 cells revealed that miRNA-221, miRNA-181a, and miRNA-10b were upregulated in a dose-dependent manner, indicating that EGCG inhibited osteopontin-dependent injury and fibrosis.<sup>[44]</sup>

It has been established that obesity and dyslipidemia negatively affect the development of MAFLD.<sup>[4]</sup> Studies have shown that the treatment of preadipocyte cells with polyphenols improved miRNA levels. For example, administration of 25 µM of trans-resveratrol, trans-resveratrol-3-O-sulfate, trans-resveratrol-3'-O-glucuronide (3G) or trans-resveratrol-4'-O-glucuronide (4G) treatment in 3T3-L1 to maturing pre-adipocytes during differentiation for 8 days resulted in 3G and 4G inhibition in adipogenesis through upregulation of miRNA-155.<sup>[45]</sup> Other research confirmed that miRNA-17-5p was upregulated in 3T3-L1 matured pre-adipocyte cells following 25 µM curcumin treatment. Additionally, miRNA-17-5p was found to target levels of the tcf7L2 gene, reducing the risk of diabetes, and had an inhibiting effect on adipogenesis.<sup>[46]</sup> Human pre-adipocyte cells treated with 25 µmol/L of extra-virgin olive oil polyphenols for 6 hours resulted in upregulation of intracellular let-7c levels and downregulation in miRNA-155 and miRNA-34a levels, which were inversely correlated with the degree of inflammation. Accordingly, the levels of miRNA-155-5p, miRNA-34a-5p, and let-7c-5p, associated with the nuclear factor kappa β (NF-κB) pathway, were inversely modulated by tumor necrosis factor alpha (TNF-α) in both cells and exosomes. It was suggested that these interactions could have a significant effect on reducing obesity-related inflammation.<sup>[47]</sup>

### ii. *In Vivo* Studies

The development of MAFLD is characterized by a degenerative antioxidant balance and progressive inflammation, and the accumulation of fatty acids in the liver. MAFLD increases with obesity, which is often associated with comorbid metabolic diseases. The prevalence of MAFLD is approximately 65% in obese patients and may be as high as 85% in the morbidly obese.<sup>[48]</sup> To our knowledge, there have been no *in vivo* studies that have investigated the relationship between polyphenols and miRNAs in MAFLD. We examined the relationship between polyphenols and miRNAs in obese mice or rat models fed with a high-fat diet due to the significant correlation between obesity and MAFLD. A study demonstrated that miRNA-122 and miRNA-125b in female

mice were upregulated with 2 mg/g quercetin treatment for 6 weeks compared with 0.2 mg/g quercetin treatment.<sup>[49]</sup> In other research, mice were fed a control diet, a high-fat diet, or a high-fat diet treated with 0.5% to 1.0% coffee polyphenols (CPP) for 2-15 weeks and increased miRNA-122 levels were seen. Additionally, the mRNA level of sterol regulatory element-binding protein (SREBP)-1c, acetyl-CoA carboxylase-1 and -2, stearoyl-CoA desaturase-1, and pyruvate dehydrogenase kinase-4 in the liver were significantly lower in mice fed with CPP.<sup>[50]</sup> In another study, mice were fed a control or a high-fat diet and treated with a high dose of quercetin for 10 weeks. The flavonoid treatment resulted in regulated expression of miRNA-103, miRNA-107, and miRNA-122. The study suggested that polyphenols may be able to prevent or weaken the metabolic effects of a high-fat and high-cholesterol diet when administered in a continuous dose, indicating the importance of dietary intervention in the treatment of MAFLD.<sup>[51]</sup>

In other research, rats were fed a lard oil diet or lard oil with 250 mg/kg of GSPE for 3 weeks and downregulation of miRNA-122 and miRNA-33 was observed. These results suggested that proanthocyanidin treatment increased hepatic cholesterol efflux to produce new high-density lipoprotein (HDL) particles by inhibiting miRNA-33, and decreased lipogenesis by inhibiting miRNA-122.<sup>[42]</sup> Another study reported that 500 mg/kg of green tea treatment for 12 weeks led to upregulation of miRNA-335 in adipose tissue in mice fed a high-fat diet. Consequently, miRNA-335 downregulated genes involved in insulin signaling and lipid metabolism. On the other hand, green tea inhibited TNF-α levels.<sup>[52]</sup> In addition, the upregulation of miR-221 was observed after 16 weeks with green tea treatment (20 mg/kg) treatment in rats with thioacetamide (TAA)-induced hepatic fibrosis. Treatment with EGCG blocked the effects of TAA and inhibited osteopontin-dependent injury and fibrosis.<sup>[44]</sup> According to another study, upregulated miRNA-17-5p inhibited adipogenesis and decreased diabetes risk by suppressing the Wnt signal pathway effector Tcf7L2 gene in mice fed a high-fat diet or high-fat diet with curcumin (2 µM dose for 6 days and 10 µM on day 7).<sup>[46]</sup>

It has also been observed that treatment with 30 mg/kg of resveratrol for 8 weeks resulted in decreased fatty acid synthase and SREBP1 protein levels and increased carnitine palmitoyltransferase-1a levels in obese rats. Fatty acid synthase was reduced after miRNA-122-5p transfection; miRNA-122-5p transfection; carnitine palmitoyl transferase-1a was downregulated by the over-expression of miRNA-107-3p. The study showed that SREBF1 is a target gene for miRNA-103-3p and miRNA-107-3p, FASN is a target for miRNA-122-5p, and CPT1A is a target for miRNA-107-3p.<sup>[53]</sup> It has also been noted that in mice or apoE<sup>-/-</sup> mice fed with 0.006% quercetin, hesperidin, naringenin, anthocyanin, catechin, curcumin, proanthocyanin, caffeic acid, or ferulic acid or a control diet, mmu-miRNA-291b-5p and mmu-miRNA-296-5p were upregulated, while mmu-miRNA-30c-1, mmu-miRNA-467b and mmu-miRNA-374 were downregulated after 8 weeks.<sup>[54]</sup> The effects of polyphenols on miRNAs as reported in various studies are provided in Table 3.

### Discussion

The precise prevalence of MAFLD is not known, but it is increasing rapidly alongside diseases such as diabetes, dyslipidemia, and particularly obesity. The prevalence among obese individuals has been estimated at 65%, and it can be as much as 85% in the morbidly obese.<sup>[48]</sup> Today, obesity is a pandemic; worldwide, more than 1.9 billion adults are overweight and 600 billion adults are obese.<sup>[55]</sup> Obesity plays a key role in the development of MAFLD, as well as genetic predisposition and environmental factors, such as dietary habits.<sup>[56]</sup> Several invasive and

noninvasive methods are used in the diagnosis of MAFLD. Although a biopsy, an invasive method, is the current gold standard, it has many disadvantages due to the potential for serious complications, such as severe pain, bleeding, infection, and even death, and it can therefore be difficult to apply in the clinic.<sup>[57,58]</sup> Noninvasive methods, such as liver enzyme tests, ultrasound, and other imaging methods are widely used, but these are not sufficient to define the stage and subtype of MAFLD.<sup>[57]</sup> There is a growing need to identify new and reliable biomarkers.

miRNAs are stable and can be detected in plasma, serum, and other biological fluids.<sup>[19]</sup> Several studies have shown that miRNAs may represent a useful tool to diagnose the stage and the subtype of MAFLD.<sup>[8,20-35]</sup> Research has demonstrated that the serum level of miRNA-122 is particularly high in MAFLD patients.<sup>[6,21-25,28,35,59]</sup> Additionally, miRNA-34a is upregulated in patients with NASH and can serve as a reliable biomarker to distinguish between MAFLD and NASH.<sup>[20,21,27-29]</sup> The miRNA pathways thought to have a relationship in the diagnosis and prognosis of MAFLD are shown in Table 2, but further studies are needed to confirm the pathways and whether miRNAs can be used accurately in the diagnosis of MAFLD.

As yet, there is no pharmacological treatment for MAFLD; only lifestyle modification with diet therapy and physical activity is recommended.<sup>[60]</sup> Fruits and vegetables are the basis of a healthy diet, and are also rich in polyphenols.<sup>[37]</sup> A link has been established that polyphenols positively affect health through action to alter gene expression encoding essential metabolic proteins. These gene modifications may be the result of the interaction of polyphenols with epigenetic factors, such as signal cascades and/or miRNAs,<sup>[18]</sup> but the exact mechanisms are still unknown.<sup>[9,14]</sup> It has been demonstrated that polyphenols can contribute to improving MAFLD, but, to our knowledge, no study has yet shown the relationship between polyphenols and miRNAs in MAFLD. Studies of obese, high-fat diet-fed mice and rats, and *in vitro* studies linked to liver and pre-adipocyte cell lines have shown that polyphenols can modulate the miRNA profiles in liver disease, particularly MAFLD, but the studies and polyphenol groups examined are limited.<sup>[13,41-53]</sup> Moreover, there has been no clinical study to date. *In vitro*, *in vivo*, and clinical studies are urgently needed to further demonstrate and explain the relationship between polyphenols and miRNAs for MAFLD patients.

## Conclusion

It is fairly certain that miRNAs can have a role in the diagnosis and prognosis of MAFLD and could provide an easy and practical noninvasive method for the diagnosis of MAFLD. Polyphenols have been shown to contribute to the amelioration of MAFLD, as in many diseases, and may modulate miRNAs in MAFLD due to their antioxidant, anti-inflammatory, antidiabetic, anticancer properties, as well as improve lipid metabolism, it is not yet possible to determine the exact effects of polyphenols on miRNAs in MAFLD. Future studies may be a source of hope for diet therapy recommendations for MAFLD patients and the development of polyphenol-related foods or drugs that target miRNAs.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – HMB, FE, FEG; Design – HMB, FE, FEG; Supervision – HMB, FE, FEG; Literature review – HMB; Writing – HMB; Critical review – FE, FEG.

**Conflict of Interest:** The authors have no conflict of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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