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 and is an increasingly recognized public health problem, affecting up to a quarter of the world's adult population. Although biopsy is the gold standard in the diagnosis of MAFLD, it can cause serious complications. Non-invasive methods cannot determine the stage and the subtypes of MAFLD. Additionally, the development and prognosis of MAFLD are modulated by epigenetic factors, in particular, microRNAs (miRNAs), be potential biomarkers for MAFLD. Polyphenols, which are found abundantly in fruits and vegetables, could alter gene expression with epigenetic factors such as miRNAs. This review aimed to present an overview of the relationship between polyphenols and miRNAs in MAFLD. According to the literature, miRNAs can be used as a diagnostic method for MAFLD, especially miRNA-122 and miRNA-34a. Although it has been shown that polyphenols may contribute to improving MAFLD, no study has shown the relationship between polyphenols and miRNAs in MAFLD. Therefore, it was not possible to determine the exact mechanisms of polyphenols on miRNAs in MAFLD. Future studies may be a source of hope for diet therapy for MAFLD patients and by developing polyphenols-related foods or drugs that target miRNAs which altering in MAFLD in the pharmaceutical and food industry.

Keywords: Polyphenols; metabolic associated fatty liver disease; microRNA

Introduction

Metabolic (dysfunction)-associated fatty liver disease (MAFLD), as with the previous term non-alcoholic fatty liver disease (NAFLD), is the most common chronic liver disorder worldwide, affecting more than one-third of the general population (around 30% of adults in industrialized countries).[1,2] The high prevalence of MAFLD has been associated with the increasing levels of unhealthy diets and low physical activity.[3]

MAFLD is a multisystem disorder and its pathophysiology is complex.[1] It is recognized as the liver disease component of metabolic syndrome, which is mainly related to insulin resistance and genetic susceptibility. As the epidemics of obesity, type II diabetes mellitus, insulin resistance, and dyslipidemia increase day by day, the risk of MAFLD is increasing proportionately.[2,4]

In recent years, it has been shown that epigenetic factors, such as microRNAs (miRNA), may cause the development of a wide range of diseases including MAFLD.[5] Therefore, miRNAs can be used in different clinical settings such as early diagnosis, monitoring progression, and response to treatment in various diseases.[6] Almost all genetic pathways, including transcription factors, secreted factors, receptors, and transporters, can be modulated by miRNAs.[7] Also, environmental conditions such as stress and nutritional status can modulate epigenetic factors thereby, they may be associated with diet and be useful for determining the effects of diet.[8]

Polyphenols have been popular that are known as functional foods, due to their biological activities including antioxidant, anti-inflammatory, anti-tumor, ameliorating lipid, carbohydrate, and amino acid metabolism, inhibiting platelet aggregation, and improving endothelin function.[9,10] Regular consumption of polyphenols has been related to a reduction in the risk of several metabolic diseases, such as obesity, insulin resistance, hypertension, and cardiovascular diseases.[11-13] Additionally, polyphenols may alter gene expression with epigenetic factors such as miRNAs by contributing to the modulation of key proteins.[14,15] Thus, they contribute to the amelioration of MAFLD, as in many diseases, and may have modulated altering miRNAs in MAFLD, but the potential mechanisms of polyphenols and miRNAs in MAFLD are very limited. This review aimed to present an overview of the relationship between polyphenols and miRNAs in MAFLD with current approaches.

Materials and Methods

Electronic searches were carried out using Scopus, Sciencedirect, and PubMed databases, to identify relevant studies about this subject. Keywords included in this review: "Phenolic" OR "Polyphenol" AND "Non-Alcoholic Fatty Liver Disease" OR "Metabolic dysfunction-associated fatty liver disease" AND "miRNA" OR "microRNAs". Theses, editorials, communications, and conference abstracts were excluded. The inclusion criteria were in vitro, in vivo or human clinical studies reporting the effects of polyphenols on MAFLD-associated miRNAs. We did not found any human clinical studies reporting the effects of polyphenols on MAFLD-associated miRNAs. Therefore, we included studies on varying miRNA levels in humans with MAFLD. Total 87 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 protein expression of target genes.[16] miRNAs are specific regulators that affect the stability or translation of the targeted mRNA. miRNAs are found abundantly in the liver and modulate a various spectrum of cellular processes related to inflammation, proliferation, differentiation, cellular growth, tissue remodeling.[17,18] Therefore, miRNAs are potential biomarkers for many liver diseases due to their stability and can determine in tissue and serum.[19]

Today, thousands of miRNAs have been identified and their exact mechanisms are still unknown. Several different miRNAs were shown to be anomalously expressed in MAFLD (Figure 1). Several different miRNAs were shown to be anomalously expressed in MAFLD. A study showed that 44 miRNAs are abnormally expressed in MAFLD patients.[20] It was found that serum levels of miRNA-122, miRNA-34a, and miRNA-16 are upregulated in MAFLD patients compared to control groups, and miRNA-122 and miRNA-34a are related to the severity of MAFLD.[21] According to a study, serum levels of miRNA-21, miRNA-34a, miRNA-122, and miRNA-451 were upregulated in MAFLD patients and serum level of miRNA-122 was positively associated with steatosis severity.[22] Another study noted that hsa-miRNA-122-5p, hsa-miRNA-1290, hsa-miRNA-27b-3p and hsa-miRNA-192-5p were higher in MAFLD patients and compared to alanine transaminase (ALT) and fibrosis-4 index (FIB-4), miRNA levels were a more specific biomarker for MAFLD.[23] A study investigated serum levels of miRNA-197, miRNA-

146b, miRNA-10b, miRNA-181d, miRNA-34a, miRNA-122, miRNA-99a, and miRNA-29a in MAFLD patients and found that serum levels of miRNA-181d, miRNA-99a, miRNA-197, and miRNA-146b were downregulated in MAFLD patients. Additionally, miRNA-197 and miRNA-10b were related to the severity of inflammation while miRNA-181d and miRNA- 99a were related to serum levels of gamma-glutamyltransferase in non-alcoholic steatohepatitis (NASH) patients.[24] The serum level of miRNA-122 in mild steatosis patients was lower compared to severe steatosis patients while the serum level of miRNA-122 in mild fibrosis patients was higher compared to severe fibrosis patients was observed.[25] A study analyzed 84 miRNAs in MAFLD patients and showed that serum levels of miRNA-122, miRNA-192, miRNA-375, and miRNA-122 were upregulated in steatosis patients and serum levels of miRNA-122 and miRNA-192 were significantly downregulated in NASH patients compared to controls and steatosis patients.[6] It was confirmed that the serum level of miRNA-21 is lower in NASH patients and serum levels of miRNA-122 and miRNA-192 are varied in MAFLD and NASH patients.[26] Similarly, the serum level of miRNA-21 was lower in MAFLD patients was exhibited.[27] miRNA-122, miRNA-192, and miRNA-34a levels were associated with steatosis and inflammatory activity, only miRNA-16 levels were significantly correlated with fibrosis. The serum level of miRNA-34a was lower in NASH patients than in MAFLD patients.[28] It was indicated that serum levels of miRNA-122 and miRNA-34a are higher, whereas miRNA-331-3p and miRNA-30c are lower in MAFLD patients.[29] The serum levels of miRNA-122 and miRNA-34a were upregulated in MAFLD patients and strongly related to very-low-density lipoprotein (VLDL) and triglyceride (TG) levels were found.[30] A study confirmed that the serum level of miRNA-122 is upregulated in MAFLD patients.[31] It was revealed that expression of miRNA-122 is reduced in the morbidly obese group compared to moderately obese patients and the miRNA-122 level was increased in morbid obese with NASH patients than morbid obese with simple steatosis. Also, miRNA-33b expression was increased in NASH.[32] Another study stated that serum levels of miRNA-301 and miRNA-34a-5p are upregulated and miRNA-375 is downregulated in MAFLD patients. Especially miRNA-301a and miRNA-375 were increased in hepatocellular carcinoma patients.[33] A study found that 14 miRNAs are associated with MAFLD and the liver levels of miRNA-139-5p, miRNA-30b-5p, miRNA-122-5p, and miRNA-422a are lower and miRNA-146b-5p is higher in obese patients with MAFLD compared to the control group.[34] It was showed that miRNA-22,miRNA-29a, and miRNA-663a are

upregulated in MAFLD patients.[35] Similarly, the other study noted that miRNA-34a, miRNA-192, miRNA-27b, miRNA-122, miRNA-22, miRNA-21, miRNA-197, miRNA-30c, and miRNA-16 are correlated with MAFLD severity.[36] Table 1 shows the studies about altering levels of miRNAs and Table 2 summarizes possible pathways of miRNAs in MAFLD patients.

The Relationship Between Polyphenols and miRNAs

Polyphenols are described as secondary metabolites that are found abundantly especially in fruits and vegetables as well as coffee, tea, red wine, and dark chocolate.[14,37,38] Polyphenols consist of two main groups: flavonoids and non-flavonoids. Non-flavonoids include two sub-groups that phenolic acids and stilbenes. The main subgroups of flavonoids are flavanols, flavan-3-ols, isoflavones, and anthocyanidins whereas minor flavonoids are flavan-3,4-diols, dihydroflavonols, chalcones, dihydrochalcones, coumarins, and aurones.[37] It is estimated that dietary intake of polyphenols is 1-1.2 g per day, and 40% of them consist of flavonoids.[9] 5-10% of the polyphenols taken into the body are absorbed from the small intestine and after absorption, their conjugated forms go to the small intestine or liver. The non-absorbable part passes to the colon and is metabolized by the intestinal microbiota.[14]

Polyphenols have been shown to have various therapeutic effects such as anti-oxidant, anti-inflammatory, anti-diabetic, anti-allergic, anti-microbial, anti-cancer, and ameliorated lipid metabolism.[39] Also, most of the therapeutic effects of polyphenols have been linked to altering gene expression encoding essential metabolic proteins. These gene modifications may result from the interaction of polyphenols with epigenetic factors such as signal cascades and/or miRNAs.[18] Modulation of miRNAs of polyphenols appears to be a new strategy to regulate metabolism and related diseases;[40] however, which mechanisms are included in this strategy are not yet known.[9,14] In recent years, the beneficial effects of polyphenols in MAFLD patients have begun to attract attention and it has been shown that polyphenols may contribute to the amelioration of MAFLD due to their therapeutic effects.[41] Although the effects of polyphenols on miRNAs have been known, possible pathways are not known. Also, there is no study evaluating the relationship between polyphenols and miRNAs in MAFLD. Due to this reason, we focused on the relationship between polyphenols and miRNAs which are altering the liver diseases and diseases that are caused by MAFLD (Figure 2).

i. In vitro studies

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

A study reported that miRNA-122 and miRNA-33 levels decrease in hepatic cells as a result of 5 hours 25 mg/L grape proanthocyanin treatment to mouse hepatoma cell lines (FAOs).[42] The doses of 3.125, 6.25, 12.5, 25, and 50 μg/mL of ellagitannin treatment for human hepG2 cells for 72 hours, depending on the dose and time, hsa-let-7e, hsa-miR-370, hsa-mir-373, and hsa -miR-526b upregulated whereas hsa-let-7a, hsa-let-7c, and hsa-let-7d downregulated, thereby ellagitannin showed an antiproliferative effect in human hepG2 cells.[43] 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 h treatment, miRNA-30b was downregulated by all three treatments, and EGCG or CPE treatments upregulated miRNA-1224-3p, miRNA-197, and miRNA-532-3p levels.[15] The administration doses of 0.1,0.2,2 and 20 μg/mL of EGCG treatment in hepG2 cells showed that miRNA-221, miRNA-181a, and miRNA-10b are upregulated according to dose-dependent. Therefore, EGCG inhibited osteopontin-dependent injury and fibrosis. [44]

It is known that obesity and dyslipidemia negatively affect the development of MAFLD.[4] Studies have shown that the treatment of preadipocyte cells with polyphenols improved the altering of miRNAs levels. For example; administration dose of 25 μM of trans-resveratrol (RSV), trans-resveratrol-3-O-sulfate (3S), trans-resveratrol-3'-O-glucuronide (3G) or trans-resveratrol-4'-O-glucuronide (4G) treatment in 3T3-L1 maturing pre-adipocytes during differentiation for 8 days; after treatments of 3G and 4G cause inhibition in adipogenesis by upregulation of miRNA-155.[45] A study confirmed that miRNA-17-5p is upregulated in 3T3-L1 matured pre-adipocyte cells with 25 μM of curcumin treatment. Additionally, miRNA-17-5p was found to target levels of the tcf7L2 gene so reducing the risk of diabetes and show an inhibitor effect on adipogenesis.[46] Human pre-adipocyte cells treated with 25 μmol/L of the extra-virgin olive oil polyphenols for 6 h resulted in upregulation in intracellular let-7c and downregulation in miRNA-155 and miRNA-34a levels and these results inversely correlated with the degree of inflammation. Accordingly, miRNA-155-5p, miRNA-34a-5p, and let-7c-5p associated

with the nuclear factor kappa (NF-B) pathway were inversely modulated by TNF- in both cells and exosomes. These conditions have had a significant effect on reducing obesity-related inflammation.[47]

ii. In vivo studies

The development of MAFLD is characterized by degenerative antioxidant balance and progressive inflammation, which is impaired by the accumulation of fatty acids in the liver. Additionally, the prevalence of MAFLD increases rapidly and is in parallel with obesity, which often leads to comorbidity metabolic diseases. The prevalence of MAFLD is approximately 75% in obese patients and this rate can increase up to 90% in morbidly obese patients.[48] No in vivo studies investigated the relationship between polyphenols and miRNAs in MAFLD. We exhibited 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 are upregulated with 2 mg/g of quercetin treatment after 6 weeks compared to 0.2 mg/g of quercetin treatment in female mice.[49] Mice were fed either a control diet, a high-fat diet, or a high-fat diet treated with 0.5 to 1.0% coffee polyphenols (CPP) for 15 weeks and the treatment of CPP led to an increase in miRNA-122 levels. Additionally, mRNA levels 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 strongly decreased in mice fed with CPP.[50] In another study, mice were fed a control or a high-fat diet and treated with a high dose of quercetin for 10 weeks. miRNA-103, miRNA-107 ve miRNA-122 levels were reduced after treatment. The study suggested that polyphenols were able to prevent and/or weaken the metabolic effects of high-fat and high-cholesterol diets when administered in continuous doses and indicating the importance of dietary intervention in the treatment of MAFLD.[51]

Rats were fed lard oil diets or lard oil with 250 mg/kg of grape seed proanthocyanin extracts (GSPE) for 3 weeks and after treatment miRNA-122 and miRNA-33 were downregulated. These results suggested that proanthocyanidin treatment increased hepatic cholesterol efflux to produce new high-density lipoprotein (HDL) particles by inhibiting miRNA-33, and it decreased lipogenesis by inhibiting miRNA-122.[42] A study reported that 500 mg/kg of green tea treatment for 12 weeks cause upregulation of miRNA-335 levels in adipose tissue in mice fed a high-fat diet. Also, miRNA-335 turned

downregulated genes involved in insulin signaling and lipid metabolism and green tea inhibited TNF-α levels.[52] 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. The treatment with EGCG blocked the effects of TAA and inhibited osteopontin-dependent injury and fibrosis.[44] According to another study, mice were fed a high-fat diet or high-fat diet with curcumin (2 μM dose for 6 days and 10 μM for only the 7th day), and upregulated miRNA-17-5p inhibited adipogenesis and decreased diabetes risk by suppressing Wnt signal pathway effector Tcf7l2 gene after treatment.[46]

It was observed that treatment with 30 mg/kg of resveratrol for 8 weeks, fatty acid synthase and SREBP1 protein levels decreased, carnitine palmitoyltransferase-1a levels increased in obese rats. Fatty acid synthase reduced after miRNA-103-3p, miRNA-107-3p, and miRNA-122-5p transfection; carnitine palmitoyl transferase-1 downregulated after over-expression of miRNA-107-3p. The study showed that the targetted genes are SREBF1 for miRNA-103-3p and miRNA-107-3p; FASN for miRNA-122-5p and CPT1 for miRNA-107-3p.[53] In a study, mice or apoE$^{-/-}$ mice were fed with 0.006% of quercetin, hesperidin, naringenin, anthocyanin, catechin, curcumin, proanthocyanin, caffeic acid, or ferulic acid or a control diet for 8 weeks. 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.[54] The effects of polyphenols on miRNAs as reported by various studies are given in Table 3.

Discussion

The prevalence of MAFLD is not known accurately, but it is increasing rapidly and in parallel with diseases such as diabetes, dyslipidemia, and especially obesity. It is thought that the prevalence, which is 75% in obese patients, can increase up to 90% in morbidly obese patients.[48] Today, obesity is a pandemic, more than 1.9 billion adults are overweight and 600 billion adults are obese.[55] It also plays a key role in the development of MAFLD in genetic predisposition as well as in the environment with dietary habits.[56] Many invasive and non-invasive methods are used in the diagnosis of MAFLD. Although biopsy, which is used as an invasive method and gold standard, has many disadvantages due to its serious complications such as severe pain, bleeding, infection, and death in some cases hence it is difficult to practice in the clinic.[57,58] Non-invasive methods, liver enzyme tests, ultrasound, and other imaging methods are widely used, but these are

insufficient to define the stage and the subtypes of MAFLD.[57] Therefore, there is an increasing demand to identify new and reliable biomarkers. This review showed that miRNAs can be used to diagnose MAFLD and its subtypes.[8,20-35]

miRNAs, which are potential biomarkers for the diagnosis of MAFLD, are stable and can be detected in plasma, serum, and other biological fluids.[19] Studies have shown that miRNAs can be used to diagnose the stages and the subtypes of MAFLD.[8,20-35] It has been shown in most studies that the serum levels of miRNA-122 are particularly high in MAFLD patients.[6,21-25,28,35,59] Additionally, miRNA-34a is upregulated in patients with NASH and a reliable biomarker in distinguishing MAFLD and NASH.[20,21,27-29] Moreover, the pathways of miRNAs that are thought to have a relationship in the diagnosis and prognosis of MAFLD are shown in Table 2, but further studies are needed to confirm their pathways and whether miRNAs can be used accurately in the diagnosis of MAFLD.

Additionally, there is no pharmacological treatment for MAFLD, and only lifestyle modification with diet therapy and physical activity is recommended for these patients.[60] Fruits and vegetables are the basis of a healthy diet, and are also, rich in polyphenols.[37] It has been linked to altering gene expression encoding essential metabolic proteins that polyphenols positively affect health. These gene modifications can be caused by the interaction of polyphenols with epigenetic factors such as signal cascades and/or miRNAs;[18] but its exact mechanisms are still unknown.[9,14] It has been shown that polyphenols can contribute to improving MAFLD, but no study has shown the relationship between polyphenols and miRNAs in MAFLD. Also, studies in obese, high-fat diet-fed mice and rats models, and in vitro studies linked to liver and pre-adipocyte cell lines have shown that polyphenols can modulate the altering miRNAs profiles in liver diseases, particularly MAFLD, but the studies and polyphenol groups are limited.[13,41-53] Moreover, there is no clinical study has been found. In vitro, in vivo and clinical studies are to be performed to demonstrate and explain the relationship between polyphenols and miRNAs for MAFLD patients is quite essential.

Conclusion

The role of miRNAs in the diagnosis and prognosis of MAFLD is an inescapable fact, and they can be an easy and practical non-invasive method in the diagnosis of MAFLD. Although polyphenols have been shown to contribute to the amelioration of MAFLD, as

in many diseases, and may have modulated altering miRNAs in MAFLD, due to their antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, and improving lipid metabolism. From the current studies, it was not possible to determine the exact effects of polyphenols on miRNAs in MAFLD. Future studies may be a source of hope for diet therapy for MAFLD patients and by developing polyphenols-related foods or drugs that target miRNAs which altering in MAFLD in the pharmaceutical and food industry.

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Tables

Table 1. miRNAs 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[21]
Control group (n=311), MAFLD (n=92)	No	RT-PCR	21, 34a, 122, 451 (MAFLD)		Yamada et al., 2013[22]
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[23]
Control group (n=20), MAFLD (n=20)	Yes	qRT-PCR		181d,99a,197,146b (MAFLD)	Celikbilek et al., 2014[24]
MAFLD (n=52)	Yes	TaqMan Micro- RNA assays	122 (steatosis)	122 (fib.)	Miyaaki et al., 2014[25]
Control group (16+(19)), MAFLD (16+(30)), NASH (16+(47))	Yes	RT-PCR	122,192,375 (NASH),122 (Fib)		Pirola et al., 2015[6]
Control group (n=61), MAFLD (n=50), NASH (n=87)	Yes	qRT-PCR	122,192,21 (NASH)		Becker et al., 2015[26]
Control group (n=12), MAFLD (n=25)	No	qRT-PCR		21 (MAFLD)	Sun et al., 2015[27]
Control group (n=37), MAFLD (n=17), NASH	Yes	qRT-PCR	122,192,34a (NASH), 16,21,146 (MAFLD)		Liu et al., 2016[28]

(n=31)					
Control group (n=62), NALFD (n=18)	No	RT-PCR	122,34a (MAFLD)	331-3p,30c (MAFLD)	Zarrinpar et al., 2016[29]
Control group (n=28), MAFLD (n=36)	Yes	qRT-PCR	122,34a (MAFLD)		Salvoza et al., 2016[30]
MAFLD (n=305)	Yes	TaqMan Micro- RNA assays	122 (steatosis, inf., ballooning and fib.)		Akuta et al., 2016[31]
Control group (n=31), MAFLD (n=27), NASH (n= 34)	Yes	RT-PCR	122 (NASH)		Auguet et al., 2016[32]
Control group (n=10), MAFLD (n=12), NASH (n=11), cirrhosis (n=3)	Yes	RT-PCR	miR-301a-3p and miR-34a-5p	375	Guo et al., 2016[33]
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[34]
Control group (n=10), MAFLD (n=44)	Yes	RT-PCR	22,29a (NASH), 663a (MAFLD)		López-Riera et al., 2017[35]
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[36]

MAFLD: Metabolic associated fatty liver disease, NASH: Non-alcoholic Steatohepatitis, qRT-PCR: Quantitative real-time PCR, RT-PCR: real-time PCR.

Table 2. Possible pathways of miRNAs in MAFLD

miRNA	Pathway
miRNA-122[61-64]	Lipid metabolism (Cholesterol, VLDL, TG, HMGCR), carcinogenesis
miRNA -10b[65]	Lipid metabolism (PPAR-)
miRNA -33[66-68]	ABC-A1 transport, ABC-G1, Niemann Pick (NP) -C1, insulin signal pathway
miRNA -34[6,21,61,69-71]	AMPK phosphorylation, miR-34a/SIRT1/p53 activation, MAFLD progression, lipid metabolism
miRNA -192[72,73]	MAFLD progression
miRNA -214-5p[74]	MAFLD progression
miRNA -27a/b[75]	Lipid metabolism
miRNA -24[76]	Lipid metabolism (Insig1)
miRNA -451[77]	Inflammation
miRNA -1290, miRNA -27b-3p[78]	Variable
miRNA -192-5p[79]	Lipid metabolism (Stearoyl-CoA desaturase 1 (SCD-1))
miRNA -103/107[8,80,81]	Pantothenate kinase 1-3 (PANK1-3), Lipid metabolism, development of insulin resistance, PPAR-, Caveolin-1
miRNA -155[82,83]	Inflammation and liver injury, SREBP-lc, liver X Receptor R (LXR) - lipid metabolism

miRNA -29[61]	SIRT1, HMGCR, LPL
miRNA -467b[84]	LDL metabolism
miRNA -143[85]	Fatty acid-binding protein 4 (FABP4), glucose transporter 4 (SLC2A4), peroxisome proliferator-activated receptor (PPAR) and hormone-sensitive lipase (LIPE)

HMGCR: HMG-CoA reductase, VLDL: very-low-density lipoprotein, TG: triglycerides, PPAR-: Peroxisome proliferator-activated receptor-alpha, ABCA1: member 1 of human transporter sub-family ABCA, ABC-G1: ATP-binding cassette sub-family G member 1, NP-C1: Niemann Pick-C1, HDL: High-Density Lipoprotein, AMPK: AMP-activated protein kinase, SIRT-1: Sirtuin-1, SCD-1: Stearoyl-CoA desaturase 1, PANK1-3: Pantothenate kinase 1-3, SREBP-lc: Sterol regulatory element-binding transcription factor 1, LXR: liver X Receptor R , LPL: Lipoprotein lipase, LDL: Low-Density Lipoprotein, FABP4: Fatty acid-binding protein 4, SLC2A4: Solute carrier family 2, facilitated glucose transporter member 4, PPAR: peroxisome proliferator-activated receptor , LIPE: hormone-sensitive lipase.

Table 3. Summary of in vitro and in vivo studies

Model	Design of the study	Polyphenols	Method	Upregulated miRNAs	Downregulated miRNAs	References
In vitro	Human hepG2 cells, 3.125, 6.25, 12.5, 25 and 50 g/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[43]
In vitro	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[15]
In vitro	3T3-L1 maturing pre-adipocytes, 25 M, 8 days	Resveratrol	RT-PCR	miRNA-155		Eseberri et al., 2017[45]
In vitro	Human pre-adipocytes, 25 mol/L, 6 h	The Extra-Virgin Olive Oil polyphenols	qPCR	Let-7c	miRNA-155, miRNA-34a	Carpi et al., 2019[47]
In vitro	FAO cells, 25 mg/L for 5 h	GSP	qRT-PCR		miRNA-122, miRNA-33	Baselga-Escudero et al., 2012[42]
In vitro	Male Wistar rats, 250 mg/kg, 3 h					
In vitro	Human hepG2 cells, 0.02, 0.2, 2 and 20 g/mL, 24 h	EGCG	qRT-PCR	miRNA-221, miRNA-181°, miRNA-10b		Arffra et al., 2016[44]
In vivo	Male Sprague Dawley rats, 20mg/kg, 16 weeks	Green tea				
In vitro	3T3-L1 maturing pre-adipocytes, 25 M, 24 h	Curcumin	RT-PCR	miRNA-17-5p		Tian et al., 2017[46]
In vivo	Male C57BL/6 mice, 2-10 M, 7 days					
In vivo	Male C57BL/6J mice, 0.5 to 1.0% CPP, 15 weeks	Coffee polyphenols	RT-PCR	miRNA-122		Murase et al., 2011[50]
In vivo	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[49]
In vivo	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[52]
In vivo	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[51]
In vivo	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[53]
In vivo	Mice, 500 mg/kg, 12 weeks	Green tea	qRT-PCR	miRNA-335		Otton et al., 2018[52]

qRT-PCR: Quantitative real-time PCR, RT-PCR: real-time PCR, qPCR: Real-Time Quantitative Polymerase Chain Reaction, FAO: a rat hepatoma cell line, GSP: grape seed proanthocyanidins, GSPE: grape seed proanthocyanidin extract, CPE: cocoa proanthocyanidin extract, EGCG: pure epigallocatechin gallate isolated from green tea.

Figure legends

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

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