NAFLD and vitamin D: Evidence for intersection of microRNA-regulated pathways

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Abstract
Non-alcoholic fatty liver disease (NAFLD) is now the most common cause of chronic liver disease, worldwide. The molecular pathogenesis of NAFLD is complex, involving numerous signalling molecules, including microRNAs (miRNAs). Dysregulation of miRNA expression is associated with hepatic inflammation, fibrosis and hepatocellular carcinoma. Although miRNAs are also critical to the cellular response to vitamin D, mediating regulation of the vitamin D receptor and vitamin D’s anti-cancer effects, the role of vitamin-D-regulated miRNAs in NAFLD pathogenesis has been relatively unexplored. Therefore, this review aims to critically assess the evidence for a potential subset of miRNAs that are both dysregulated in NAFLD and modulated by vitamin D. Comprehensive review of eighty-nine human studies identified twenty-five miRNAs found dysregulated in more than one NAFLD study. In contrast, only seventeen studies, including a protocol for a trial in NAFLD, had examined miRNAs in relation to vitamin D status, response to supplementation, or vitamin D in the context of the liver. This paper summarises these data and reviews the biological roles of six miRNAs (miR-21, miR-30, miR-34, miR-122, miR-146, miR-200) found dysregulated in multiple independent NAFLD studies. While modulation of miRNAs by vitamin D has been understudied, integration of the data suggests seven vitamin-D-modulated miRNAs (miR-27, miR-125, miR-155, miR-192, miR-223, miR-375, miR-378) potentially relevant to NAFLD pathogenesis. Our summary tables provide a significant resource to underpin future hypothesis-driven research, and we conclude that the measurement of serum and hepatic miRNAs in response to vitamin D supplementation in larger trials is warranted.

Keywords: NAFLD: MAFLD: vitamin D: miRNA: obesity: type 2 diabetes

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Introduction
Non-alcoholic fatty liver disease (NAFLD) is now the most common cause of chronic liver disease and a significant public health problem worldwide. Closely associated with obesity, the global prevalence of NAFLD is estimated to be 24%, placing a significant clinical and economic burden on many countries, including the United Kingdom(1,2). Defined physiologically by excess accumulation of lipids in the liver, NAFLD is an umbrella term encompassing a range of histopathology from hepatic steatosis (non-alcoholic fatty liver, NAFL), to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. There is tremendous inter-individual variation both in disease phenotype and its progression, determined by dynamic interactions between genetic, metabolic and environmental factors that are not completely understood(3,4). Although several genetic variants have been identified that influence NAFLD susceptibility, dietary and lifestyle factors strongly influence disease progression, and weight loss is the mainstay of current clinical management guidelines(5–7).

The integral relationship between metabolic dysfunction and NAFLD, along with its complex and variable pathogenesis between individuals, prompted a consensus-driven proposal in April 2020 for a name change to ‘metabolic associated fatty liver disease’ (MAFLD)(4,8). More than a name change, this would change the diagnostic criteria from being exclusionary (absence of excess alcohol intake and other chronic liver diseases) to a positive diagnosis of: hepatic steatosis alongside either overweight/obesity, type 2 diabetes, or the presence of two metabolic risk factors in normal-weight individuals(8). Importantly, the recognition of the heterogeneity of NAFLD presentation and the disease as a continuum rather than a dichotomous stratification between NASH and non-NASH would permit the recognition of the coexistence of metabolic and other chronic liver diseases (including alcohol-related fatty liver), and potentially present opportunities for improved clinical trial designs(4,8).

Multiple positive endorsements for the proposal have been published in recent months, from patients(9), nurses(10), and professional associations(11–13). Nonetheless, time is required to update International Classification of Diseases (ICD) diagnostic codes. At the time of writing, the most recent codes (ICD-11, 09/2020) refer only to NAFLD and NASH; therefore, we use these terms here.

Individual dietary nutrients have been implicated in NAFLD pathogenesis and may differentially affect disease development

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and/or progression. For example, separate from their provision of energy, dietary macronutrients (saturated and n-3 fatty acids, fructose) appear to differentially effect lipid accumulation in the liver through multiple molecular and cellular mechanisms(14,15). Similarly, micronutrient deficiencies have been associated with NAFLD, and a mechanistic basis exists for their therapeutic targeting(16). However, to date, only a limited number of intervention trials have examined dietary supplements in patients with NAFLD, with mixed results(17). Vitamin D is of interest in part because it has anti-proliferative, anti-inflammatory and anti-fibrotic properties that have been shown to attenuate NAFLD progression in pre-clinical models(17,18). Moreover, low levels of dietary vitamin D intakes and poor vitamin D status are widespread and are notably observed in paediatric NAFLD(19–21). However, intervention trials of vitamin D supplementation in patients with NAFLD have been heterogeneous in their duration, dosing and outcome measurements, and questions remain about optimal regimens for efficacy in chronic liver disease(22).

The molecular pathogenesis of NAFLD is complex, involving numerous signalling molecules involved in hepatic metabolism, oxidative, inflammatory and fibrotic processes(23). These include microRNAs (miRNAs) that play an essential role in gene expression and regulatory networks involved in lipid and carbohydrate metabolism and cellular stress response pathways(24). Dysregulation of miRNA expression in the liver is associated with hepatic inflammation, fibrosis and the development and progression of multiple liver diseases, including hepatocellular carcinoma (HCC)(24,25). Separately, a body of evidence exists for miRNAs in mediating the cellular response to vitamin D, including the post-transcriptional regulation of the vitamin D receptor (VDR) and vitamin D’s anti-cancer properties(26). Interestingly, while the anti-cancer effects of vitamin D have been observed in liver(27,28), the potential role of vitamin-D-regulated miRNAs in NAFLD remains unexplored.

Therefore, the aims of the present paper were to first comprehensively review the data from human studies for involvement of miRNA in NAFLD pathogenesis. Secondly, we aimed to review serum miRNA profiling studies that have examined vitamin D status or response to supplementation, along with the limited research that has investigated miRNAs and vitamin D in the context of liver pathology. Finally, integrating these data, this paper aimed to critically assess the evidence for a potential subset of miRNAs that are both dysregulated in NAFLD and modulated by vitamin D.

MicroRNAs in health and disease

Typically only twenty-two nucleotides long(29), miRNAs are small, non-coding RNAs that are critical for development, regulate a variety of normal physiological processes, and are found dysregulated in cancer(30), liver(21) and metabolic diseases(31). They play a key role in the post-transcriptional regulation of gene expression, binding complementary sequences within mRNA transcripts and typically suppressing gene expression through mRNA degradation and translation repression. Recent estimates suggest there are approximately 2300 human miRNAs(32). However, miRNAs can have numerous mRNA targets, and individual mRNAs can be targeted by several miRNAs. Indeed, miRNAs regulate an estimated 60% of human protein coding genes impacting almost all biological processes(33,34).

Primary miRNAs (pri-miRNAs) are transcribed and processed in the nucleus into pre-miRNA molecules that contain a characteristic hairpin structure. After export into the cytoplasm, pre-miRNAs are processed into miRNA duplexes by the Dicer enzyme. During the subsequent multistep assembly of the RNA-induced silencing complex, only one of the miRNA strands (the guide strand) of these duplexes will be retained, becoming the mature miRNA targeting complimentary mRNA for repression(35). By current nomenclature conventions, mature miRNAs are given the prefix ‘miR’ and a number indicating identification order; family members with nearly identical sequences expressed from different precursors or genomic loci have lettered suffixes (e.g. miR-30a and miR-30c)(36). Mature miRNAs derived from the 5’ and 3’ arms of the pre-miRNA hairpin structure are annotated with the suffixes −5p and −3p, respectively. Although both the −5p and −3p forms may be functional depending on context, experimental data suggest the 5p form is more frequently found as mature miRNAs(37,38).

Similar to mRNA, the expression of miRNAs varies by cell and tissue type; and while some miRNAs are ubiquitously expressed, others are tissue specific(39). For example, miR-122 is specifically and highly expressed in the liver, representing a remarkable 70% of liver miRNA content(40). Present in blood, urine and other body fluids(41), miRNA levels have been shown to be altered in multiple diseases as a result of genomic events or alterations in miRNA biogenesis, prompting significant interest in miRNAs as clinical biomarkers and therapeutic targets(24,31,39). In the context of cancer in particular, functional and mechanistic studies have established that miRNA dysregulation can be causal, with miRNAs acting as tumour suppressors or oncogenes (oncomiRs)(30,31). Although still at an early stage of development, both miRNA mimics and inhibitors of miRNAs (anti-miRs) have been investigated as therapeutics for cancer, liver and other diseases in early phase clinical trials(42). Interestingly, miR-122 binds a region of the hepatitis C virus (HCV) genome promoting its accumulation(39), and Miraviren, an anti-miR-122 oligonucleotide, was the first miRNA-targeting drug administered in humans. Although found to be safe and specific in phase 2a studies in HCV patients, its use was superseded by effective direct-acting anti-viral drugs(43).

A limited number of miRNA-based diagnostic assays have already been brought to market(44), and considerable research has focused on profiling circulating miRNAs as biomarkers of obesity and metabolic disease, including NAFLD(28,31,42). However, the identification of miRNAs as clinical biomarkers or therapeutics is complicated by both technical and confounding factors related to miRNA biology(45,46). While a growing body of evidence suggests miRNAs secreted in extracellular vesicles are stable in circulation and act as paracrine and endocrine factors in metabolic diseases(31,42), this complicates the interpretation of miRNA expression and activity in any given cell, tissue or pathology. Age, sex and disease state all influence circulating miRNA profiles(30). Profiles vary between plasma and serum, and further variables include how long the sample was
stored, whether the donor was fed or fasted and what the donor’s activity levels are\textsuperscript{48,51,52}. Importantly, expression and regulatory mechanisms observed in experimental models are not always conserved in humans\textsuperscript{53,54}. Historically, authors have not consistently specified miRNA –5p and –3p forms or family member suffixes confounding data interpretation\textsuperscript{55}, and quantitative polymerase chain reaction (qPCR) primer sequences are also often omitted from methods sections, potentially contributing to reproducibility issues. Given the missing detailed information of mature miRNAs in most studies, we do not use this notation throughout the text, but do detail the reported family member names and mature form suffixes from the studies reviewed and summarised in our tables.

**The role of miRNAs in NAFLD pathogenesis**

NAFLD pathogenesis is now recognised to involve ‘multiple hits’ and crosstalk between multiple organs and the intestinal microbiome\textsuperscript{3,56}. Although ‘simple’ fatty liver (NAFL) is typically benign, NASH, defined by steatosis with inflammation, hepatocyte injury (ballooning) and possibly fibrosis, more frequently develops into advanced liver disease\textsuperscript{57}. In the liver at a cellular level, exacerbated by insulin resistance, lipid accumulation in the hepatocytes is driven by: increased lipid influx (from diet or adipose tissue lipolysis) and increased lipid synthesis (de novo lipogenesis), as well as impaired lipid oxidation and export. Notably, some of the miRNAs identified as altered in NAFLD play critical roles in hepatic lipid and carbohydrate metabolism and lipogenesis, regulating multiple transcription factors such as sterol regulatory element-binding protein 1 (SREBP-1), carbohydrate response element binding protein (ChREBP) and the peroxisome proliferator-activated receptors (PPARs)\textsuperscript{22,23}.

Progression from NAFL to NASH is a result of lipotoxicity and the over-production of reactive oxygen species (ROS), which leads to mitochondrial dysfunction, endoplasmic reticulum stress and the recruitment and signalling of immune cells. Adipokines from peripheral adipose tissue dysfunction and signalling molecules derived from the gut further drive this pro-inflammatory state systemically, as well as the resulting wound healing response and fibrosis locally in the liver\textsuperscript{58}. Although progression typically occurs on the order of decades, prognosis is dependent on severity of fibrosis on index biopsy\textsuperscript{59}, and NASH significantly increases risk of HCC and liver-specific mortality\textsuperscript{57}. At each stage of the progression of NAFLD, from inflammation to NASH-related HCC, miRNA-regulated pathways are also implicated, and the potential utility of miRNAs as either diagnostic biomarkers or therapeutic targets is of significant interest\textsuperscript{22,23,24}.

In light of the aforementioned variables that may confound the reproducibility of miRNA research, we comprehensively surveyed the literature and examined the data from serum profiling or mechanistic studies involving liver tissues to identify miRNAs with good evidence for altered levels in humans with NAFLD. Specifically, we defined ‘good evidence’ as experimentally replicated beyond array or RNASeq profiling (Fig. 1A). Focusing on miRNAs found dysregulated in subjects with NAFLD in at least two independent studies, identified twelve miRNAs in liver and nineteen miRNAs in serum. Notably, six miRNAs (miR-21, miR-30, miR-34, miR-122, miR-146 and miR-200; Table 1) were found dysregulated in both liver and serum and in at least four independent studies (Fig. 1B). An additional six miRNAs were identified as altered in livers (miR-33, miR-141, miR-155, miR-199, miR-223 and miR-378; Supplementary Table 1), and a further thirteen (miR-16, miR-20, miR-22, miR-27, miR-29, miR-99, miR-125, miR-181, miR-192, miR-197, miR-375, miR-379 and miR-451; Supplementary Table 2) were found altered in serum from participants with NAFLD in more than one study.

Human NAFLD studies requiring a liver biopsy are typically done in single centres and are often limited in size, similar to miRNA biomarker discovery studies more generally\textsuperscript{48}. For this reason, the number of participants in each study group was noted in addition to summarising the direction of expression of the miRNAs and the stages of NAFLD examined. Unsurprisingly, liver sample sizes (\(n\) ranged from three to fifty-eight per group; Table 1 and Supplementary Table 1) were smaller than the number of observations in serum (\(n\) ranged from 8 to 392 per group Table 2 and Supplementary Table 2). While serum samples typically came from NAFLD case series, liver samples were often from bariatric surgery patients or tissue banks, and in one case\textsuperscript{61}, a post-mortem series. In spite of heterogeneous study designs, there was reasonably good concordance between studies in the (generally increased) direction of the miRNAs found dysregulated.

Given the diagnostic and therapeutic potential of miRNAs in NAFLD, we briefly outline relevant regulatory functions of the six miRNAs found altered in both liver and serum (Table 1) below. The limited data concerning functional and pathophysiological effects of their dysregulation arising from these studies, specifically in humans with NAFLD, are summarised in Table 2.

**MiR-21**

Originally described as an oncomiR dysregulated in multiple cancers, miR-21 is now recognised to play a role in numerous inflammatory and fibrotic diseases, including multiple liver diseases\textsuperscript{62}. In the context of NAFLD, miR-21 is up-regulated in both liver\textsuperscript{63–65} and serum\textsuperscript{66–68}, and circulating miR-21 levels have been positively correlated with serum ALT\textsuperscript{67,69}, steatosis, lobular inflammation\textsuperscript{67} and hepatic activity\textsuperscript{60} (Table 1). A single study found decreased hepatic miR-21 levels, but this was in post-mortem samples from people who had died of sudden cardiac death from severe coronary artery disease\textsuperscript{61}. The single study reporting decreased serum hepatic miR-21 levels had relatively fewer participants (twenty-five NAFLD versus twelve healthy controls (HCs)), and the diagnosis of NAFLD was not specified\textsuperscript{70}.

Experimental knockdown\textsuperscript{71} or deletion of miR-21\textsuperscript{72} in mice markedly reduces lipogenesis and hepatic steatosis in response
to high-fat feeding. Among the numerous targets of miR-21 are multiple metabolic and signalling pathways implicated in NAFLD pathogenesis (73, 74). These include the genes for several master transcriptional regulators involved in glucose and lipid metabolism, including the hepatocyte nuclear factor 4 alpha (HNF4α), forkhead box protein O1 (FOXO1) peroxisome proliferator-activated receptor alpha (PPARα) and sterol regulatory element-binding transcription protein 1c; (SREBP-1c) (71, 72), as well as 3-hydroxy-3-methylglutaryl-co-enzyme A reductase (HMGCR) (70). In addition, miR-21 is pro-fibrotic and, as the most up-regulated miRNA during hepatic stellate cell (HSC) activation (75), serves to amplify multiple genes involved in inflammation, fibrosis and NAFLD progression (73, 74). While the therapeutic targeting of miR-21 is considered to have potential for multiple fibrotic diseases (76), somewhat surprisingly the deletion of miR-21 did not inhibit fibrosis in mouse models of toxic and biliary liver injury (75). More research is required to resolve the likely differences in miR-21 targets between humans and mice, and the complexity of miR-21 actions in the context of NAFLD, as well as other chronic liver diseases.

**MiR-30**

Of the miRNAs profiled in Table 1, relatively fewer studies suggest a role for miR-30 in NAFLD. One challenge in interpreting the data is that the mir-30 family has five members (miR30a–e). Beyond the aforementioned issues of inconsistent specification of family member and 5′ and 3′ forms in the literature, the miR-30 family members have overlapping sequences that have been found to interfere with expression profiling, and it has been suggested that only high-throughput sequencing can clearly differentiate between miR-30 family members (77). While two studies described decreased hepatic miR-30b (78, 79) expression in NAFLD, along with decreased hepatic miR-30c in one of these reports (79), these data appear to be from the same bariatric surgery patients. Separately, two studies have reported a decrease in circulating miR-30c in NAFLD (69, 80), with one of these reporting decreased levels and negative correlations between miR-30c and multiple measures of disease severity (69). On the other hand, a single study has reported increased serum levels of miR-30a in a smaller number of participants (eleven NAFLD versus twelve HCs) (81).

Nonetheless, there are some experimental data to suggest a role for miR-30 in NAFLD pathogenesis. As recently reviewed (82), miR-30 has anti-fibrotic properties and has been found down-regulated in the context of HSC activation, hepatic fibrosis and cirrhosis in multiple models of liver injury, as well as human cirrhotic liver. Indeed, overexpression or restoration of miR-30a has been shown both in vitro and in vivo to suppress HSC activation by inhibiting epithelial-mesenchymal transition, reducing cell proliferation and migration, in line with its reported tumour suppressor activity (83). Separately, limited in vitro data suggest increased miR-30a-3p expression prompts triacylglycerol accumulation in hepatocytes via targeting and decreasing PPARα expression (81). Conversely, in separate experiments also in immortalised hepatocytes, fatty acid deposition triggered by both AMP-activated protein kinase (AMPK) disruption and Dicer knockdown was attenuated by overexpression of miR-30b and miR-30c associated with increased PPARα expression (79). These limited data nevertheless suggest that a biological role for miR-30 family members cannot be ruled out.

**MiR-34**

In contrast, many more studies have been in complete agreement in finding increased expression of miR-34 in liver (seven
| miRNA | Sample | Summary |
|-------|--------|---------|
| miR-21 | Liver | **Up (miR-21)** in NASH (n = 3) versus controls (n = 3) [NIH liver tissue repository][63]  
Up (miR-21) in NASH (n = 11) versus steatosis (n = 8) and HCs (n = 6) [pathology database][64]  
**Up (miR-21)** in NASH versus steatosis [N = 28, bariatric surgery patients][65]  
Down (miR-21-5p) in NAFLD (n = 12) versus non-NAFLD (n = 15) [postmortem samples, NCSD][66]  
Up (miR-21) in NASH (n = 87) versus NAFL (n = 50) and HCs (n = 61); positive correlation with ALT, steatosis and lobular inflammation[67]  
Up (miR-21) in NASH (n = 31) versus HCs (n = 37); positive correlation with hepatic activity[68]  
Up (miR-21) in NASH versus steatosis [N = 24, bariatric surgery patients][69]  
Down (miR-21-5p) in F > 2 (n = 29) versus F ≤ 2 (n = 46); positive correlation with ALT, AST, APRI (NAFL n = 25, NASH n = 50, HCs n = 17)[69]  
Down (miR-21) in NAFLD (n = 25) versus HCs (n = 12)[70] |
| miR-30 | Liver | Down (miR-30b-5p) in NASH (n = 17) and borderline NAFLD (n = 24) versus controls (n = 19); negative correlation with NAFLD [bariatric surgery patients][78]  
Down (miR-30b-c) in ≥5% steatosis (n = 25) versus <5% steatosis (n = 19) [bariatric surgery patients][79] |
| miR-34 | Serum | Up (miR-30a-3p) in NAFLD (n = 11) versus HCs (n = 10)[81]  
Down (miR-30c) in NAFLD (n = 18) versus HCs (n = 62)[80]  
Down (miR-30c-5p) in SAF ≥ 2 (n = 50) versus SAF < 2 (n = 25), down in NAS ≥ 5 (n = 38) versus NAS < 5 (n = 37), down in F > 2 (n = 29) versus F ≤ 2 (n = 46); negative correlation with FIB4, BARD, NAFLD_FS (NAFL n = 25, NASH n = 50, HCs n = 17)[80]  
Up (miR-34a) in NAFLD (n = 28) versus normal histology (n = 25) [participants with metabolic syndrome][114]  
Up (miR-34a) in NASH (n = 13) versus steatosis (n = 15) [bariatric surgery patients][96]  
Up (miR-34a) in NAS (n = 8) versus normal histology (n = 8) [liver tissue bank][110]  
Up (miR-34a-5p) in NASH (n = 42) versus steatosis (n = 18) and non-NAFLD (n = 37) [postmortem samples, CSD and NCSD][61]  
Up (miR-34a-5p) in NASH (n = 11) versus controls (n = 10) and NAFL (n = 12) [bariatric surgery patients][114]  
Up (miR-34a) in NASH (n = 5) versus non-steatosis (n = 3 CHB and PBC patients) [liver biopsy patients][132]  
Up (miR-34a-5p) in steatosis (n = 4) versus non-steatosis (n = 4) [liver tissue bank][120] |
| miR-34 | Liver | Up (miR-34a) in NAFLD (n = 34) versus HCs (n = 19), up in NASH (NAS > 5) versus steatosis, up in steatosis versus HCs discriminated NASH from steatosis (AUROC = 0.764)[80]  
Up (miR-34a) in NASH (n = 44) versus HCs (n = 221) [adult females], up in NAFLD (n = 48) versus HCs (n = 90) [adult males][86]  
Up (miR-34a) in NASH (n = 28) versus HCs (n = 36); discriminated NAFLD from HCs [AUROC = 0.781][86]  
Up (miR-34a) in NAFLD (n = 18) versus HCs (n = 62)[80]  
Up (miR-34a) in NASH (n = 31) versus NAFL (n = 17) and HCs (n = 37); positive correlation with histological severity but not fibrosis; discriminated NASH from non-NASH (AUROC = 0.811)[86]  
Up (miR-34a-5p) in SAF ≥ 2 (n = 50) versus SAF < 2 (n = 25), up in NAS ≥ 5 (n = 38) versus NAS < 5 (n = 37); positive correlation with ALT, AST, ferritin, APRI, FIB4 and total bilirubin (NAFL n = 25, NASH n = 50 and HCs n = 17); discriminated SAF ≥ 2 from SAF < 2 [AUROC = 0.76][86]  
Up (miR-34a) with inflammation versus non-inflammation (N = 116, post-transplant protocol biopsy in liver transplant recipients)[86]  
Up (miR-34a) in NAFLD (n = 210) versus HCs (n = 90), up in NASH (n = 86) versus steatosis (n = 124); positive correlation with ALT, AS and histological severity; discriminated NAFLD from HCs [AUROC = 0.77] and NASH from steatosis (AUROC = 0.84)[85]  
Up (miR-34a-5p) with increasing fibrosis severity [N = 132, NAFLD patients]; in multivariate analyses, positive correlation with steatosis, fibrosis, the PNPLA3 I148M and TM6SF2 E167K variants; discriminated fibrosis from no fibrosis [AUROC = 0.75, 0.73, 0.75 and 0.76 for stages 1, 2, 3 and 4][87] |
| miR-122 | Liver | Up (miR-34a-5p) in >33% steatosis versus <33% steatosis, down in severe fibrosis versus no or mild fibrosis [N = 52 biopsied NAFLD patients]; negative correlation with fibrosis, positive correlation with serum miR-122 levels[110]  
Up (miR-122-5p) in steatosis (n = 20) versus NNL (n = 14) and NASH (n = 31); negative correlation with AST [liver biopsy patients][111]  
Up (miR-122-5p) in NAFLD (n = 13) versus controls (n = 3) [female bariatric surgery patients][116]  
Down (miR-122) in NASH (n = 25) versus normal histology (n = 25) [participants with metabolic syndrome][114]  
Down (miR-122) in the more severe NAFL (n = 8) versus less severe NAFL (n = 5) and steatosis (n = 15) [bariatric surgery patients][96]  
Down (miR-122) in NASH versus non-steatosis [N = 14 non-tumour tissue from non-hepatitis B/C HCC resections][115]  
Down (miR-122-5p) in NASH (n = 42) versus non-NAFLD (n = 37) [postmortem samples, CSD and NCSD], positive correlation with NAFLD scoring[81]  
Down (miR-122-5p) in NAFLD (n = 17) and borderline NAFLD (n = 24) versus controls (n = 19); negative correlation with NAFLD [bariatric surgery patients][78] |
| miR-122 | Serum | Up (miR-122) in NAFLD (n = 34) versus HCs (n = 19), up in NASH versus steatosis, up in steatosis versus HCs; discriminated steatosis from HCs [AUROC = 0.927] and NASH from steatosis [AUROC = 0.698][84]  
Up (miR-122) in NASH (n = 20) versus HCs (n = 24)[183] |
### Table 1. (Continued)

| miRNA       | Sample Summary                                                                                                                                                                                                 |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Up (miR-122)** | in NAFLD ($n = 44$) versus HCs ($n = 221$) [adult females], up in NAFLD ($n = 48$) versus HCs ($n = 90$) [adult males] positive correlation with steatosis severity$^{[60]}$                                          |
|             | Up (miR-122) in $>33\%$ steatosis versus $<33\%$ steatosis, down in severe fibrosis versus no or mild fibrosis [N = 67 NAFLD patients]; negative correlation with fibrosis, positive correlation with hepatic miR-122 levels; discriminated fibrosis (AUROC = 0.82)$^{[110]}$                      |
|             | Up (miR-122-5p) in NAFLD ($n = 103$) versus HCs ($n = 80$); discriminated NAFLD from HCs (AUROC = 0.759)$^{[108]}$                                                                                      |
|             | Up (miR-122-5p) in NASH ($n = 47$) versus steatosis ($n = 30$) and HCs ($n = 19$), up with histological severity; positive correlation with ALT, AST, GGT and serum CK-18 levels; discriminated histological severity (AUROC range 0.61–0.71)$^{[111]}$                         |
|             | Up (miR-122) in NASH ($n = 87$) versus NAFL (miR-50) and HCs ($n = 61$), up in NAFL versus HCs; positive correlation with ALT, steatosis, lobular inflammation and serum CK18-Asp396$^{[87]}$                                                      |
|             | Up (miR-122) with histological severity except fibrosis stage 4; positive correlation with ALT, AST, GGT and ferritin [N = 305 NAFLD]$^{[106]}$                                                                         |
|             | Up (miR-122) in NAFLD ($n = 28$) versus HCs ($n = 36$); discriminated NAFLD from HCs (AUROC = 0.858)$^{[88]}$                                                                                      |
|             | Up (miR-122) in NAFLD ($n = 18$) versus HCs ($n = 62$)$^{[88]}$                                                                                                                                             |
|             | Up (miR-122) in NASH ($n = 40$) versus controls ($n = 22$), up in NASH ($n = 22$) versus steatosis ($n = 18$) [MO women]; discriminated NAFLD from controls (AUROC = 0.82) and histological severity from mild disease (AUROC = 0.76)$^{[112]}$                   |
|             | Up (miR-122) in circulating exosomes in advanced stage NAFLD ($n = 3$) versus early stage NAFLD ($n = 3$)$^{[184]}$                                                                                           |
|             | Up (miR-122) in severe NAFLD ($n = 14$) and mild NAFLD ($n = 36$) versus HCs ($n = 61$), [independent European cohorts of children with obesity]; positive correlation with ALT, AST and serum CK16$^{[18]}$                                              |
|             | Up (miR-122) in NAFLD ($n = 58$) versus HCs ($n = 34$), up in NAS $>4$ ($n = 32$) versus NAS $<4$ ($n = 24$) and HC ($n = 34$), up in NAS $<4$ ($n = 24$) versus HC ($n = 34$); discriminated NAFLD from HCs (AUROC = 0.831)$^{[186]}$                                |
|             | Up (miR-122-5p) in NAFLD ($n = 52$) versus controls ($n = 48$); discriminated NAFLD from controls (AUROC = 0.774) [adults with T2DM]$^{[109]}$                                                                      |
|             | Up (miR-122) in NAFLD ($n = 210$) versus HCs ($n = 90$) and up in NASH ($n = 86$) versus steatosis ($n = 124$); positive correlation with ALT, AST and histological severity; discriminated NAFLD versus HCs (AUROC = 0.92) and NASH (AUROC = 0.81)$^{[88]}$    |
|             | Up (miR-122) in NASH ($n = 31$) versus NAFL ($n = 17$) and HCs ($n = 37$), up in NAFL ($n = 17$) versus HCs ($n = 37$); positive correlation with histological severity but not fibrosis$^{[88]}$                                                                 |
|             | Up (miR-122-5p) in SAF $\geq 2$ ($n = 50$) versus SAF $<2$ ($n = 25$), up in NAS $\geq 5$ ($n = 38$) versus NAS $<5$ ($n = 37$); positive correlation with ALT, AST, ferritin, APRI and BARD (NAFL $n = 25$, NASH $n = 50$ and NL $n = 17$)$^{[88]}$|
|             | Up (miR-122) with inflammation versus non-inflammation and ballooning versus non-ballooning (N = 116, post-transplant protocol biopsy in liver transplant recipients)$^{[86]}$                                         |
|             | Up (miR-122) with increasing fibrosis severity (N = 132 NAFLD patients); in multivariate analyses, positive correlation with steatosis, fibrosis, the PNPLA3 I148M and TM6SF2 E167K variants$^{[87]}$                        |
|             | Up (miR-122) in steatosis ($n = 120$) versus controls ($n = 60$) and fibrosis ($n = 120$) versus controls ($n = 60$); positive correlation with ALT, AST and GGT [obese patients]$^{[187]}$                                           |
|             | **Down (miR-122)** with improved histopathological feature; positive correlations between serum miR-122 ratio (ratio of level at second biopsy to that at first biopsy) and changes in histological scores as well as ALT, AST, GGT and ferritin [N = 36 NAFL patients with repeat biopsies]$^{[105]}$           |
|             | Down (miR-122) associated with risk of mortality (HR 4.39, P = 0.025) in multivariate analyses, and poor cumulative mortality rates over 10 years (N = 392 biopsy confirmed NAFLD patients with median 4.7 years follow-up)$^{[107]}$                                      |
| **miR-146** | **Liver**                                                                                                                                                                                                      |
|             | Up (miR-146b) in NASH ($n = 25$) versus normal histology ($n = 25$) [participants with metabolic syndrome]$^{[114]}$                                                                                           |
|             | Up (miR-146b-5p) in NAFLD ($n = 17$) versus controls ($n = 19$) and borderline NAFLD (n = 24); positive correlation with NAFLD [bariatric surgery patients]$^{[78]}$                                             |
|             | Up (miR-146) in steatosis ($n = 4$) versus non-steatosis ($n = 4$) [liver tissue bank]$^{[120]}$                                                                                                             |
| **Serum**   | Up (miR-146b) in NAFLD ($n = 20$) versus HCs ($n = 20$); discriminated NAFLD from HCs (AUROC = 0.75)$^{[121]}$                                                                                           |
|             | Up (miR-146b) in NASH ($n = 31$) versus HCs ($n = 37$)$^{[88]}$                                                                                                                                               |
| **miR-200** | **Liver**                                                                                                                                                                                                      |
|             | Up (miR-200c) in NASH fatty liver ($n = 20$) versus NASH non-fatty liver ($n = 15$) and normal histology ($n = 10$) [liver tissue bank]$^{[131]}$                                                               |
|             | Up (miR-200a/b/c) in steatosis ($n = 4$) versus non-steatosis ($n = 4$) [liver tissue bank]$^{[120]}$                                                                                                        |
| **Serum**   | Down (miR-200b/c) in NAFLD ($n = 11$) versus HCs ($n = 11$) [liver biopsy patients]$^{[132]}$                                                                                                              |
|             | Up (miR-200a) with increasing fibrosis severity (N = 132 NAFLD patients); in multivariate analyses, positive correlation with fibrosis and TM6SF2 E167K variants$^{[87]}$                                           |
|             | Up (miR-200) in NAFLD ($n = 57$) versus HCs ($n = 30$)$^{[133]}$                                                                                                                                              |

ALT, alanine aminotransferase; AST, aspartate transaminase; APTI, AST-to-platelet ratio index (fibrosis score); AUROC, the area under the receiver operating characteristic; CHB, chronic hepatitis B; CK18, cytokeratin-18; CSD, cardiac sudden death; CVD, cardiovascular disease; eLIP-IR, enhanced lipoprotein insulin-resistance index; F, fibrosis stage; FIB4, fibrosis 4; GGT, gamma-glutamyl transpeptidase; HC, healthy control; HCC, hepatocellular carcinoma; HR, hazard ratio; LFTs, liver function tests; MO, morbidly obese; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis; NCLS, non-cardiac sudden death; NNL, near-normal liver; PBC, primary biliary cirrhosis; PNPLA3, patatin-like phospholipase domain containing protein 3; SAF, steatosis, activity, and fibrosis score; T2DM, type 2 diabetes mellitus; TM6SF2, transmembrane 6 superfamily member 2.

† Activity is the sum of the score of lobular inflammation and hepatocellular ballooning.
Table 2. Functional and pathophysiologcal effects of dysregulated miRNA in NAFLD

| MicroRNA | Sample type | Sample member | Summary |
|----------|-------------|---------------|---------|
| miR-21   | Liver       | P47phox mRNA, 3-nitrotyrosine immunoreactivity, NF-κB activation and the immunoreactivity of TGF-β, CTGF, EDAFN, α-SMA and Col1α1, and SMAD2/3 and SMAD7 nuclear colocalisations: up in NASH (n = 3) versus HCs (n = 3); SMAD7 protein: down in NASH versus HCs(83) | Hepatic PPARα protein: down in NASH versus steatosis [N = 28, bariatric surgery patients](85) |
| miR-30   | Serum       | Hepatic ACSL1, Dicer and AMPK mRNA: down in >33% steatosis (n = 16) versus <5% steatosis (n = 19)(78) | Serum HMGCGR mRNA and protein: up NAFLD (n = 25) versus HCs (n = 12)(78) |
| miR-34   | Liver       | Hepatic SIRT1 protein: down in NASH (n = 13) versus steatosis (n = 15); p53 acetylation: up in NASH versus steatosis; TUNEL-positive cells: up in more severe NASH (n = 8) versus less severe NASH (n = 5) and steatosis [obese patients](96) | Hepatic ACSL1, Dicer and AMPK mRNA: down in >33% steatosis (n = 16) versus <5% steatosis (n = 19)(78) |
| miR-122  | Serum       | Not investigated | Not investigated |
| miR-146  | Liver       | Hepatic CTDNEp1 mRNA, lpin-1 mRNA and protein: up in NAFLD (n = 13) versus controls (n = 3) [female obese adults](96) | Not investigated |
| miR-200  | Liver       | Hepatic SREBP1 and FAS: up in NAFLD (n = 11) versus HCs (n = 11) [liver biopsy patients](130) | Not investigated |

ACSL1, acyl-CoA synthetase long-chain family member 1; Col1α1, collagen α1; CTGF, connective tissue growth factor; CTDNEp1, contactin-associated protein 1; EDAFN, extra domain A-fibronectin; HMGCGR, 3-hydroxy-3-methylglutaryl-co-enzyme A reductase; HNF4α, hepatocyte nuclear factor 4α; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NNL, near-normal liver; NS, not specified; PPARα, peroxisome proliferator-activated receptor α; PKAα1, AMP-activated protein kinase; SIRT1, sirtuin 1; α-SMA, α-smooth muscle actin; SMAD7, mothers against decapentaplegic homologue 7; SREBP1, sterol regulatory element-binding protein 1; TGF-β, transforming growth factor-β; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling.

studies) and increased serum levels (nine studies) in patients with NAFLD (Table 1). Higher serum miR-34 levels have been found in more advanced stages of NAFLD(86,88,89-87), and found to positively correlate with multiple markers of disease severity(90,89), including histological severity(90,85,87) and the PNPLA3 I148M and TM6SF2 E167K variants(87). Moderate diagnostic accuracy has been found in multiple studies for miR-34 in discriminating NAFLD(95,88), NASH(89,98,94,85,84,85) and fibrosis(95,87), with area under the receiver operating characteristics (AUROCs) ranging from 0.73 to 0.84.

Notably, a recent meta-analysis published in 2020 that examined the utility of miRNAs as non-invasive biomarkers of NAFLD concluded that miR-34 had moderate diagnostic accuracy for total NAFLD with the lowest heterogeneity (I² = 5.73%, specificity I² = 33.16%, AUROC = 0.85) of the three miRNAs found most commonly studied (miR-34a, miR-99a, miR-122)(97,96). An earlier meta-analysis from 2018 had concluded (from three studies at the time) miR-34a to have the best diagnostic accuracy (AUROC = 0.7783) for discriminating NASH versus NAFLD, whereas, (from three studies) miR-122, discussed in detail below, was best at discriminating NAFLD from healthy controls (AUROC = 0.8174)(98). In addition, a recent systematic review has concluded that both miR-34a and miR-122 may be useful biomarkers in children with obesity and NAFLD(91).

As direct targets of the tumour suppressor gene p53, induction of miR-34 family members is associated with apoptosis and cell cycle arrest, and inactivation of the miR-34a and miR-34b/c genes is typically observed in cancer(92). Among multiple cell cycle and apoptosis-related direct gene targets of miR-34 is sirtuin 1 (SIRT1), which, in a fascinating regulatory loop, links cholesterol, lipid and energy homoeostasis to inflammation and p53-dependent apoptosis(92,93). An NAD-dependent deacetylase, SIRT1 directly deacetylates multiple metabolic regulators of relevance to NAFLD pathogenesis, including SREBP-1c, the PPARs (alpha and gamma), the farnesoid X receptor (FXR) and liver X receptors (LXR, alpha and beta), as well as nuclear factor-κB (NF-κB) and p53 itself(95-97). Data from experimental models suggest possible benefit from targeting and inhibiting the miR-34/p53 pathway(98,99), although some conflicting data exist around effects in HSCs(98,99). Interestingly, resveratrol, a naturally occurring dietary polyphenol that activates SIRT1, has been tested in a number of small clinical trials for benefit in NAFLD and other metabolic diseases(95,100). A recent meta-analysis synthesising data from six randomised controlled trials (RCTs) found that, although resveratrol significantly lowered tumour necrosis factor alpha (TNF-α) and high-sensitivity C-reactive protein (hs-CRP) levels, there were no changes in numerous other cardiometabolic risk markers(101). More potent, synthetic SIRT1 agonists are under development, and some are in early phase clinical trials(100).

**MiR-122**

In addition to its aforementioned role in HCV promotion, miR-122 is essential for lipid metabolism and has anti-inflammatory and anti-carcinogenic effects in the liver(102,103). The role and therapeutic targeting of miR-122 in liver disease has long been of considerable interest(99,104), and unsurprisingly, miR-122 has been the most explored miRNA in NAFLD. We identified twenty-three studies that measured miR-122 levels in serum, and eight studies that examined hepatic miR-122 expression in NAFLD (Table 1). Of the twenty-three serum studies, twenty-one found increased levels of miR-122 positively correlated with markers of NAFLD severity. Consistent with these findings, one study in a small number of patients in Japan reported decreased serum miR-122 levels with histological improvement on second
biopsy\(^{105}\), although, other data from the same group suggest that at stage 4 fibrosis miR-122 levels decrease\(^{106}\) and decreased levels of miR-122 (expressed relative to the median of the cohort) may be associated with risk of mortality\(^{107}\).

Multiple studies found miR-122 to have moderate diagnostic accuracy in discriminating either NAFLD\(^{84,98,108,109}\), NASH\(^{84,95}\) or histological severity\(^{86,110-112}\). Although a diagnostic assay for miR-122 is in pre-clinical development and has been tested in the context of drug-induced liver injury\(^{113}\), given that miR-122 is altered in multiple liver diseases, any potential utility as a biomarker for NAFLD will most likely be in combination with other miRNAs or biochemical markers\(^{67}\). Notably, the limited number of currently available miRNA-based diagnostics for other diseases are panels of ten or more miRNAs\(^{43}\).

On the other hand, the studies of miR-122 hepatic expression in aggregate were more inconclusive. Sample sizes were typically low, and participants and/or liver samples were heterogeneous in origin and variable in disease stage of NAFLD. Of the five studies reporting that miR-122 decreased in liver biopsies, three were staged as NASH\(^{61,96,114}\), one as NAFLD\(^{78}\) and one involved a small number of non-tumour HCC resected liver samples with steatosis\(^{141}\). Of the three studies reporting increased expression of hepatic miR-122 in steatosis, two found decreased expression in more advanced disease, such as NASH\(^{115}\) and fibrosis\(^{110}\), and the third specifically excluded NASH samples, only examining steatosis in bariatric surgery patients\(^{116}\).

The hepatic data perhaps suggest decreased expression of miR-122 in advanced disease, and the possibility of increased expression in steatosis. This fits the hypothesis of an early defensive response (in steatosis) and later causal factor in NASH progression\(^{24}\), and reconciles with several lines of experimental data highlighting the complexity of the dynamics of miR-122 expression and secretion in the regulation of lipid metabolism. While antisense oligonucleotide inhibition of miR-122 in vivo had beneficial effects on plasma cholesterol and hepatic steatosis in high-fat-fed (60% lard, for 19 weeks) mice\(^{102}\), genetic deletion of miR-122 causes steatohepatitis and tumourigenesis\(^{103,117}\). In addition, NEFAs have been demonstrated to increase the expression and secretion of miR-122 inhibiting triacylglycerol synthesis in both liver and muscle\(^{118}\). This mechanism would account for the increased serum levels of miR-122, but underscores that circulating miRNAs do not always reflect tissue expression or activity\(^{23}\). The question of whether humans with NAFLD might benefit from therapeutic targeting of miR-122 through either antagonists (anti-miRs) or miRNA mimics will require considerable more research and development and larger trials with careful staging of NAFLD.

**MiR-146**

Along with miR-155, miR-146 is recognised for multiple roles in the innate and adaptive immune responses and is considered an oncomiR\(^{119}\). However, knowledge regarding a role for miR-146 during NAFLD pathogenesis is limited. Three studies identified an increase in hepatic miR-146 in biopsies from participants with metabolic syndrome and NASH\(^{114}\), bariatric surgery patients with NAFLD\(^{78}\), and steatotic tissue bank biopsies\(^{120}\). Two studies detecting circulating miR-146 levels were conflicting, possibly relating to clinical stage of NAFLD or ethnic differences. Whereas one European cohort comparing histologically proven NAFLD patients with healthy age-matched participants \((n = 20\) per group\) found miR-146b decreased in NAFLD\(^{121}\), a separate Chinese cohort found miR-146b increased in serum from NASH patients (biopsy diagnosed, \(n = 51\)) versus healthy controls \((n = 37\)\(^{108}\).

In experimental models, decreased expression of miR-146 has been detected in dietary-induced NAFLD models\(^{122,123}\), and in vitro data show that miR-146 mimics can suppress lipid accumulation and inflammatory cytokines, such as tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interleukin-6 (IL-6)\(^{123,124}\). In addition, miR-146 is pro-fibrotic, and modulates fibrosis signalling pathways in HSCs\(^{92}\). In transforming growth factor-beta (TGF-\(\beta\))-stimulated cellular models, overexpression of miR-146 inhibited the proliferation and apoptosis of HSCs and the expression of pro-fibrogenic markers\(^{125,126}\). Furthermore, in a hepatic fibrosis rat model induced by CCl4, vein injection of miR-146a-expressing adenovirus increased the level of miR-146 and served to alleviate fibrogenesis\(^{127}\). Collectively, the data suggest that roles of the miR-146 family in NAFLD pathogenesis should be further explored, paying attention to the different isoforms and their unique regulatory functions as previously recommended\(^{55}\).

**MiR-200**

Key inhibitors of the epithelial-to-mesenchymal transition, the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) play critical roles in both normal development and cancer metastases. High expression of miR-200 is associated with an epithelial phenotype, and recent bioinformatic analyses suggest potential binding sites for miR-200b in the 3' untranslated regions of sixty different mRNAs involved in the epithelial-to-mesenchymal transition, the majority of which are associated with a mesenchymal phenotype that miR-200 likely inhibits\(^{129}\). Typically considered tumour suppressors, the miR-200 family have generally been reported down-regulated in multiple cancers, including HCC\(^{129}\). However, this may depend on tumour stage, with data suggesting the miR-200 family are down-regulated at the primary tumour site permitting intravasation, but up-regulated in distal metastases facilitating colonisation of metastatic breast cancer cells\(^{130}\).

In the context of NAFLD, to date, there are only limited data about the miR-200 family. While two studies have found miR-200a/b/c increased in steatotic and NASH liver samples from tissue banks\(^{120,131}\), a separate study using biopsies found miR-200b/c decreased in NAFLD compared with healthy controls\(^{122}\). An additional two studies have found miR-200 increased in serum from NAFLD patients\(^{97,133}\). Some evidence from animal models supports the idea that inhibition of miR-200 may attenuate steatosis, inflammation and fibrosis. For example, double deletion of miR-141 and miR-200 in mice with NASH induced from a methionine and choline deficient diet resulted in decreased steatosis and inflammation and alterations in multiple signalling pathways\(^{132}\). In a similar vein, high-fat-fed (20% lard for 4 weeks) mice transduced with an miR-200 inhibitor also exhibited reduced steatosis and fibrosis\(^{133}\). However, in a
separate study of high-fat-fed (45% fat for 10 weeks) mice, miR-200b and miR-200c, but not miR-200a, were found markedly decreased\(^{132}\). These data may reflect the use of different animal models, but could also relate to disease stage. While a rationale can be made for increased expression of miR-200 early in NAFLD, and decreased expression later during disease progression conferring risk of HCC, more data at greater resolution (e.g. from single-cell experiments discriminating between hepatocytes and HSCs) are required to confirm this.

**Intersection of miRNA pathways between NAFLD and vitamin D**

Vitamin D is a misnomer for a family of secosteroid hormones with varying degrees of activity\(^{155}\). The biological effects of the active form of vitamin D (calcitriol, 1,25-dihydroxycholecalciferol) are mediated through the interaction with the vitamin D receptor (VDR), a ligand-activated, nuclear receptor transcription factor\(^{135}\). A significant amount of experimental research shows vitamin D has anti-proliferative, anti-inflammatory and anti-fibrotic properties that might impact disease progression in chronic liver diseases, including NAFLD\(^{136,137}\). Polymorphisms in the vitamin D metabolic pathway are associated with histological severity of paediatric NAFLD\(^{20}\), and inadequate vitamin D status has been commonly observed in association with NAFLD severity in adults\(^{177}\). While vitamin D has been shown to improve insulin sensitivity and glycemic control in people with prediabetes and type 2 diabetes\(^{138,139}\), vitamin D supplementation trials in NAFLD have been typically small (twenty to thirty people per arm\(^{140}\)), single-centre trials and have been heterogeneous in duration, the form (e.g. vitamin D versus calcitriol), dose and mode of delivery of supplement, as well as the liver outcomes measured\(^{22}\). Only a minority have measured liver biomarkers by magnetic resonance\(^{141}\) or biopsy\(^{142}\). Multiple questions around dosing regimes, duration of intervention and ideal stage of NAFLD to intervene remain unanswered\(^{27,22}\), but potential benefits for younger people with earlier stages of NAFLD have been hypothesised\(^{137}\), and recently demonstrated\(^{142}\).

Interestingly, miRNAs have multiple essential roles in mediating the cellular response to vitamin D, including the post-transcriptional regulation of VDR\(^{143}\). Within the 3′ untranslated region of the VDR mRNA are binding sites for four miRNAs (miR-27, miR-125, miR-298, miR-346), which have been shown to decrease VDR protein levels\(^{144-147}\). Indeed, miR-125 inhibitors have been demonstrated to increase VDR expression and decrease proliferation and cell viability in vitro in HCC cells, while VDR levels were negatively correlated with miR-125 levels in tumour tissue from patients with HCC\(^{148}\). Moreover, multiple genes (CYP27B1, CYP24A1, RXRα) in the vitamin D pathway are regulated by miRNAs\(^{149-155}\), and VDR directly regulates the transcription of multiple miRNAs\(^{152}\). While a large body of pre-clinical research suggests the anti-cancer effects of vitamin D are mediated through miRNA regulation, data from human trials was no within-study experimental validation. Venn analysis of the twenty vitamin-D-associated miRNAs with the twenty-nine NAFLD-dysregulated miRNAs suggested that seven vitamin-D-regulated miRNAs (miR-27, miR-125, miR-155, miR-192, miR-223, miR-375 and miR-378; Fig. 1C) may be relevant to NAFLD pathogenesis. Notably, miR-27b directly targets and regulates VDR\(^{20}\), and has been found altered in serum from NAFLD patients in three studies\(^{69,108,159}\) summarised in Table S2. Also interesting among the intersecting miRNAs was miR-192, shown to decrease in the serum of adults with prediabetes supplemented with vitamin D (2000 IU [50 μg] cholecalciferol) for 4 months in correlation with favourable changes in fasting plasma glucose levels and disposition index (the product of insulin sensitivity and insulin secretion)\(^{160}\). As eight independent studies have shown miR-192 to be up-regulated in NAFLD patients (plus one outlier showing down-regulation; summarised in Table S2), in light of the data from Nunez Lopez and colleagues\(^{160}\) it remains tempting to speculate a benefit for vitamin D supplementation in NAFLD patients. Although trials of vitamin D supplementation in adults with NAFLD have been disappointing in terms of liver endpoints, as previously discussed, sufficient questions around trial design preclude completely rejecting vitamin D as having therapeutic benefit\(^{17,22,137}\), especially given its benefit to people with prediabetes and type 2 diabetes\(^{138,139}\).

Out of the twenty miRNAs identified as altered by both NAFLD and vitamin D, only two, miR-125 and miR-155, were found in more than one vitamin D study (Fig. 1C). We identified
Table 3. Serum miRNA profiling studies examining vitamin D status

| Reference                      | Study design; Group (sample size); 25(OH)D status (nmol/L) | miRNAs related to 25(OH)D status | miRNA-related summary |
|--------------------------------|-------------------------------------------------------------|----------------------------------|-----------------------|
| Enquobahrie et al., 2011(188) | miRNA expression (microarray) in relation to 25(OH)D status† in early (16 weeks’ gestation) pregnancy (~34-year-old women); High 25(OH)D (n = 6): 98.05±15.3, Low 25(OH)D (n = 7): 57.10±5.0 | Microarray: miR-92b, –93, –138, –196a, –320d, –343-3p, –423-3p, –484, –573, –574-5p, –589, –601 | Microarray: Up in high 25(OH)D versus low 25(OH)D: miR-574-5p; Down in high 25(OH)D versus low 25(OH)D: miR-92b, –93, –138, –196a, –320d, –343-3p, –423-3p, –484, –573, –574-5p, –589, –601 |
| Lee et al., 2014(189)          | miRNA expression (microarray and qPCR) in relation to 25(OH)D status in AML patients (~60 years old); Normal vitamin D (n = 34): >80, Insufficient vitamin D (n = 34): 50–79-8; Deficient vitamin D (n = 29): <50 | Microarray: miR-96, –122, –125b-1, –134, –144, –182, –193b, –329, –451, –486-5p, –511, –595, –663, –886-3p, –1248 qPCR: Not significant | Microarray: Up in <50 (n = 10) versus >50 (n = 10): miR-96, –122, –144, –182, –193b, –329, –451, –486-5p, –595, –663, –886-3p, –1248 qPCR (N = 58): No miRNAs associated with 25(OH)D levels in validation cohort |
| Beckett et al., 2014(190)      | Circulating level of let-7 (qPCR) in relation to vitamin D intake in elderly cohort (~75 years old); Adequate intake† (n = 23): ns§; Inadequate intake (n = 177): ns§ | qPCR: let-7b-5p | qPCR: Down in adequate versus inadequate; negative correlation with vitamin D intake |
| Mohamadkhani et al., 2015(154) | miR-378 (qPCR) in relation to 25(OH)D status and HBV DNA level in CHB patients (~37 years old); CHB (n = 173): 55.5±20.7 | qPCR: miR-378 | qPCR: Positive correlation with plasma 25(OH)D status; negative correlation with viral load |
| Chen et al., 2017(181)         | miRNA expression (qPCR) in T cells of patients with SLE with 25(OH)D insufficiency (~36 years old); SLE patients (n = 42): 41.7±12.8 Normal vitamin D (n = 32): NR Insufficient vitamin D (n = 10): NR | qPCR: miR-10a, –125a, 342, –374b, –377, –410 | All miRNAs down in SLE versus controls except miR-377 and –410; all miRNAs were positively correlated with 25(OH)D levels |
| Ferrero et al., 2021(191)      | Circulating miRNome in healthy volunteers in relation to estimated vitamin D intake* (~40 years old) | RNAseq: ~348 miRNAs detected per sample | RNAseq: In GLM, miR-361-3p was positively correlated and let-7a-5p was negatively correlated with estimated vitamin D intake* |

AML, acute myeloid leukemia; CHB, chronic hepatitis B; FAS, fatty acid synthase; GLM, generalised linear regression model; ns, not specified; NR, not reported; HBV, hepatitis B virus; 25(OH)D, 25-hydroxycholecalciferol; qPCR, quantitative polymerase chain reaction; SLE, systemic lupus erythematosus.

† 25(OH)D status defined as high: ≥79.25 nmol/L, or low: <53.75 nmol/L;‡ The recommended adequate daily intake for vitamin D intake for vitamin D in Australia is 10 μg/d for 51–70 years old and 15 μg/d for those aged over 70 years; §Intake estimated by food frequency questionnaire 0–65 g/d; #Estimated from EPIC food frequency questionnaire.
Table 4. Serum miRNA profiling studies response to vitamin D supplementation

| Reference | Study design; Group (sample size) and vitamin D intake | Serum 25(OH)D status (nmol/l) | miRNAs related to 25(OH)D status§ | miRNA-related summary |
|-----------|-------------------------------------------------------|--------------------------------|----------------------------------|----------------------|
| Jorde et al., 2012<sup>192</sup> | Obese males (~60 years old) supplemented for 1 year; Vitamin D (n = 40); 20 000 or 40 000 IU cholecalciferol/wk Placebo (n = 37) | Vitamin D group: Baseline: 50.2 ± 14.2; 12 months: 101.7 ± 17.8; Placebo group: Baseline: 53.0 ± 19.1; 12 months: 49.6 ± 16.0 | miR-211, miR-532-3p | Down in 12-month versus baseline [in the placebo group]; up in vitamin D versus placebo; miR-532-3p: Positive correlated with serum 25(OH)D [at baseline] |
| Yu et al., 2017<sup>193</sup> | 60 allergic rhinitis (AR) patients and 20 healthy controls (HCs) (~35 years old) supplemented for 6 months; Vitamin D (n = 20); 2000 IU vitamin D<sub>3</sub>/d Placebo (n = 20) | All groups: <75 | miR-19a | Up in AR with vitamin D<sub>3</sub> versus HCs |
| Nunez Lopez et al., 2017<sup>160</sup> | Prediabetes adults (~59 years old) supplemented for 4 months; Vitamin D group (n = 40); 2000 IU cholecalciferol/d Placebo group (n = 21) | Vitamin D group: Baseline: 62.0 ± 14.8 4 months: 83.8 ± 19.5 Placebo group: Baseline: 66.5 ± 20.0 4 months: 43.3 ± 12.3 | miR-7, miR-23b, miR-152, miR-192-5p | Up in vitamin D versus placebo; miR-23b: Up in post versus pre [in vitamin D group]; miR-152: Up in post versus pre [in vitamin D group]; positiively correlated with serum 25(OH)D |
| Pastuszak-Lewandoska et al., 2020<sup>164</sup> | 20 male ultra-marathon (UM) runners (~38 years old) supplemented for 2 weeks; Vitamin D group (n = NS): 10 000 IU cholecalciferol/d Placebo group (n = NS) | NR | miR-155, miR-223 | miR-155: Up in both placebo and vitamin D groups [after UM] miR-223: Up in placebo group only [after UM] |

AR, allergic rhinitis; HC, healthy control; NS, not specified; NR, not reported; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)D, 1,25-dihydroxyvitamin D; PCa, prostate cancer; UM, ultra-marathon. § Jorde<sup>192</sup> used microarrays with quantitative polymerase chain reaction (qPCR) for validation; Nunez Lopez<sup>160</sup> and Pastuszak-Lewandoska<sup>164</sup> used qPCR alone.
### Table 5. Research studies characterising miRNA regulated by vitamin D involving liver pathology

| Reference | Liver pathology; Samples | Vitamin D treatment | Related miRNA | miRNA-related summary |
|-----------|--------------------------|---------------------|---------------|----------------------|
| Duan et al., 2015<sup>(194)</sup> | HCV; Human cell lines: Huh7.5 HCV Con1b replicon Huh7.5.1 infected with HCV J6/JFH1 | 1 μM calcitriol for 48 h | miR-130a | qPCR: Calcitriol potentiated miR-130a inhibition of HCV RNA replication, but calcitriol did not affect the expression of miR-130a |
| Mohamadkhani et al., 2015<sup>(154)</sup> | CHB; Serum n = 173; General population; Serum n = 28, Liver n = 20; | NR | miR-378 | qPCR: Positive correlation with plasma 25(OH)D status; qPCR: Negative correlation with CYP3A activity* in both liver and serum; no association with mRNA levels of CYP3A4, VDR and PPARγ [liver] |
| Ekstrom et al., 2015<sup>(158)</sup> | General population; Serum n = 28, Liver n = 20; | NR | miR-27b | qPCR: Up in PBCC versus PSC and controls in both liver and PBMCs; Positive correlation with hepatic VDR mRNA and SOCS1 protein level [liver] |
| Kemipinska-Podhorodecka et al., 2017<sup>(155)</sup> | PBC; Liver: PBC n = 22, PSC n = 13 and Controls n = 23 PBMCs from human: PBC n = 16, PSC n = 10 and Controls n = 11 | PBC patients were supplemented with vitamin D/calcium (amount NR) and had normal levels of serum vitamin D | miR155 | |
| Xu et al., 2018<sup>(148)</sup> | HCC; Liver: HCC n = 31 and NL n = 10; Human cell lines: HepG2 and SMMC-7221 | NR | miR-125a-5p | qPCR: Up in HCC versus NL, negative correlation with hepatic VDR mRNA [liver]; Down-regulation of miR-125a-5p increased VDR mRNA and protein expression in HepG2. |
| Provisiero et al., 2019<sup>(197)</sup> | HCC; Human cell lines: PLC/PRF/5, and JHH-6 | With or without 10⁻⁷ M 1,25(OH)₂D₃ for 12 h | miR-375 | Target: VDR (luciferase reporter assay) [in cells] qPCR: Up in vitamin D treated versus untreated; Target: c-MYC (luciferase reporter assay) qPCR: Up in cirrhosis versus NL [liver]; IHC: Up miR-125 expression with reduced VDR staining [liver]; Positive correlation with liver cirrhosis, negative correlation with hepatic VDR protein [liver] |
| He et al., 2021<sup>(156)</sup> | Liver cirrhosis | NR | miR-125 | Target: VDR [293T] |

CHB, chronic hepatitis B; CYP3A, cytochrome P450 3A; PBC, primary biliary cholangitis; PBMCs, peripheral blood mononuclear cells; PSC, primary sclerosing cholangitis; HC, healthy control; HCC, hepatocellular carcinoma; IHx, immunohistochemistry; NAFLD, non-alcoholic fatty liver disease; NL, normal liver; NR, not reported; qPCR, quantitative polymerase chain reaction; RCT, randomised clinical trial; SOCS1, suppressor of cytokine signalling 1; VDR, vitamin D receptor.

* CYP3A activity in serum measured by its endogenous marker 4β-hydroxycholesterol; CYP3A activity in liver measured by dextromethorphan N-demethylation.
three studies that reported dysregulation of miR-125 in the context of vitamin D modulated. Two of these miRNAs, miR-27 and miR-125, target vitamin D receptor (VDR) mRNA and decrease translation. The transcription of a third miRNA, miR-155, is inhibited by VDR, which directly interacts with IκB kinase (IKKβ), preventing nuclear factor (NFκB) activation and transrepression of the MIR155 host gene. Relevant to NAFLD, in the context of low vitamin D/VDR signalling, miR-155 lowers expression of the suppressor of cytokine signalling 1 (SOCS-1), increasing expression of pro-inflammatory cytokines.

**MiR-125**

The miR-125 family (miR-125a, miR-125b-1 and miR-125b-2) play essential roles in haematopoiesis and the normal function of immune cells and, perhaps unsurprisingly, have also been linked to a variety of cancers\(^\text{166}\). Their effects in cancer are dependent on cell type, and they have been shown to have both oncogenic and tumour suppressive activities. Along with the previously discussed miR-27, miR-125 is of note because it also targets and inhibits VDR translation\(^\text{26}\). We identified two studies where miR-125 was examined in relation to VDR in patients with HCC\(^\text{148}\) and liver cirrhosis\(^\text{156}\) (Table 5). In HCC tissues \((n = 31)\), miR-125a was found to be negatively correlated with VDR expression, and was expressed at much higher levels than in non-tumour controls \((n = 11)\)\(^\text{148}\). Similarly, in cirrhotic liver biopsies \((n = 60)\), miR-125a expression increased with severity of liver fibrosis in association with a corresponding decrease in VDR expression\(^\text{156}\).

To date, only a single observational study has evaluated miR-125 in relation to vitamin D status. Chen and colleagues reported a positive correlation between miR-125 expression in T cells and serum 25(OH)D levels in patients with systemic lupus erythematosus\(^\text{161}\) (Table 3). Separately, two studies have examined miR-125 in serum from NAFLD patients, with conflicting results\(^\text{162,163}\). Whereas Cai and colleagues found miR-125 decreased in serum from patients with ultrasound-diagnosed NAFLD \((n = 34)\) compared with non-NAFLD \((n = 20)\)\(^\text{162}\), a separate study reported the opposite, finding miR-125 increased in NAFLD \((n = 29)\) compared with healthy volunteers \((n = 24)\)\(^\text{163}\). Differences in NAFLD phenotype and/or qPCR methodologies employed may explain these contradictory findings. Notably, in the latter study, the diagnostic modality for NAFLD was unspecified and SYBR green staining was used for qPCR\(^\text{163}\). However, the associated experimental work of Cai and colleagues\(^\text{162}\), in combination with previous experimental work demonstrating that miR-125 targets fatty acid synthase\(^\text{167}\),
suggests miR-125 up-regulation is likely to be protective for NAFLD and liver fibrosis, and that miR-125 in relation to NAFLD is worthy of further investigation.

**MiR-155**

A notorious oncomiR, increased expression of miR-155 has been found in a host of different cancers, including HCC\(^{(168,169)}\). Transcription of the MIR155 host gene (MIRHG155\(^{27,28}\)), historically termed B-cell integration cluster, is regulated by numerous transcription factors involved in the inflammatory response, including nuclear factor-kappa B (NF-kB), interferon regulatory factors, TGF-β and hypoxia inducible factor 1 alpha, among others\(^{270}\). Therefore, the aberrant expression of miR-155 plays a vital role in multiple inflammatory molecule and signalling pathways. Critical to both innate and adaptive immune responses, miR-155 influences the immune inflammatory response in part through directly targeting suppressors of cytokine signalling 1 (SOCS1)\(^{171}\).

Interestingly, miR-155 is inhibited by VDR, which directly interacts with IkB kinase (IKKβ), preventing nuclear factor kB (NFkB) activation and transrepression of MIRHG155\(^{272}\). Calcitriol decreases miR-155 expression in human macrophages\(^{173,174}\) and adipocytes\(^{175}\). In mice, vitamin D supplementation ameliorated the increase in miR-155 in adipose tissue in response to high-fat feeding, in further support of an anti-inflammatory role of vitamin D in obesity\(^{176}\). Moreover, miR-155 has been observed to decrease in response to both dietary weight loss and bariatric surgery, and has been proposed as a biomarker of weight loss\(^{176,177}\). In the context of NAFLD, hepatic miR-155 expression was shown to be increased alongside miR-34a and miR-200a-c and other miRNAs in a small number (n = 4 per group) of tissue bank biopsies from patients with and without steatosis\(^{178}\). Hepatic expression of miR-155 has also been found elevated in cholestatic liver disease, and was related to decreased levels of VDR and SOCS1 protein in the peripheral blood mononuclear cells of patients\(^{179}\). The authors point out that the decreased VDR expression was observed in spite of patients being supplemented with vitamin D and having normal vitamin D status.

Perhaps counterintuitively, in 2016, Wang and colleagues\(^{180}\) reported significantly decreased circulating levels of miR-155 in fifty participants with NAFLD compared with fifty healthy controls, as well as decreased hepatic miR-155 levels in eleven biopsy samples from NAFLD patients compared with eleven control biopsies. However, in accompanying experimental work, they showed miR-155 directly targets LXRA, which targets SREBP-1c and fatty acid synthase (FAS) influencing lipid accumulation. In addition, high-fat-fed mice transfected with miR-155 mimics had significantly reduced hepatic steatosis, as well as decreased expression of LXRA, SREBP-1c and FAS\(^{181}\). Apart from the aforementioned study in cholestatic liver disease, we identified only one other study examining miR-155 response to vitamin D supplementation. Unusually, it involved very-high-dose vitamin D supplementation (10 000 IU/250 μg cholecalciferol) for 2 weeks prior to a 100 km ultra-marathon. In this small study done in a unique population, miR-155 levels increased in both groups after the ultra-marathon, but there was no difference between groups\(^{182}\).

Genome-wide analyses have demonstrated miR-155 has many hundreds of gene targets, and furthermore miR-155 binding and miR-155-dependent repression are regulated in a cell-context-dependent fashion\(^{178,179}\), which may explain these somewhat disparate results. However, the pre-clinical data and data from weight loss intervention studies suggest that the potential interactions between miR-155, vitamin D, and hepatic lipid metabolism and inflammation in the molecular pathogenesis of NAFLD, are worth pursuing.

**Conclusions**

This review critically assessed the evidence for a potential subset of miRNAs that are both dysregulated in NAFLD and modulated by vitamin D. Comprehensive review of the literature found numerous studies examining dysregulation of miRNA levels in humans with NAFLD. We identify twenty-nine miRNAs found dysregulated in more than one NAFLD study, including six (miR-21, miR-30, miR-34, miR-122, miR-146 and miR-200) found dysregulated in multiple independent NAFLD studies. On the other hand, a paucity of human studies were identified that had investigated miRNAs in relation to vitamin D status, response to supplementation, or vitamin D in the context of the liver. This is a notable gap in the evidence base, given that VDR mediates its cellular response in part by directly targeting miRNAs that regulate transcription factors involved in NAFLD pathogenesis, and considering that VDR expression is directly regulated by miRNAs likely disrupted in NAFLD.

Our critical review found evidence from human studies for seven vitamin-D-modulated miRNAs (miR-27, miR-125, miR-155, miR-192, miR-223, miR-375 and miR-378) potentially relevant to NAFLD pathogenesis (overall summary in Fig. 2). While we await the results of the ongoing trial of Ebrahimpour-Koujan and colleagues\(^{183}\) with interest, we believe that the measurement of serum and hepatic miRNAs in response to vitamin D supplementation in larger trials or biobanked samples is warranted. While miRNA analyses of liver tissue are unlikely to add diagnostic value to already informative, but invasive, liver biopsies, they may be key to further understanding pathobiology. On the other hand, the measurement of serum miRNAs is non-invasive. Given that current genetic risk factors for NAFLD are non-specific and predict severity of multiple liver diseases, a fascinating, unanswered question worthy of deliberate inquiry is whether serum miRNA signatures might yield diagnostic specificity for either chronic liver disease stage or aetiology. Although individual miRNAs alone seem unlikely to provide such specificity, for the earlier stages of NAFLD in particular, panels of diet-responsive miRNAs may be particularly intriguing. The summary tables within this review provide a significant resource to underpin future hypothesis-driven research to tackle such questions, including gene expression meta-analysis studies. We conclude that the modulation of miRNAs by
vitamin D has been understudied and that, based on the evidence to date, a therapeutic benefit for vitamin D supplementation in NAFLD can not be ruled out.

**Author contributions**

Z.Z., R.M., J.L.T. and J.B.M contributed to review concept and design. Z.Z. and J.B.M extracted data. R.M. and Z.Z. contributed to manuscript drafts. J.B.M. wrote the final manuscript. All authors critically reviewed the manuscript for intellectual content and approved the final version of the manuscript. Fig. 2 was created with BioRender.com.

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**Conflicts of interest**

All authors declare no conflicts of interest.

**Supplementary material**

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**References**

1. Younossi Z, Anstee QM, Marietti M, et al. (2018) Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* **15**, 11–20.
2. Younossi ZM, Blissard D, Blissard R, et al. (2016) The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology* **64**, 1577–1586.
3. Moore JB. (2019) From sugar to liver fat and public health: systems biology driven studies in understanding non-alcoholic fatty liver disease pathogenesis. *Proc. Nutr Soc* **78**, 290–304.
4. Eslam M, Sanyal AJ, George J. (2020) MAFLD: A consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology* **158**, 1999–2014.e1.
5. National Institute for Health and Care Excellence. Non-alcoholic fatty liver disease: assessment and management. *NICE Guideline NG49* 2016.
6. EASL-EASD-EASO. (2016) EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* **64**, 1388–1402.
7. Chalasani N, Younossi Z, Lavine JE, et al. (2018) The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* **67**, 328–357.
8. Eslam M, Newsome PN, Sarin SK, et al. (2020) A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. *J Hepatol* **73**, 202–209.
9. Shiha G, Korenjak M, Eskridge W, et al. (2021) Redefining fatty liver disease: an international patient perspective. *Lancet Gastroenterol Hepatol* **6**, 73–79.
10. Clayton M, Fabrellas N, Luo J, et al. (2021) From NAFLD to MAFLD: nurse and allied health perspective. *Liver Int.* **41**, 683–691.
11. Spearman CW, Desalegn H, Ocama P, et al. (2021) The sub-Saharan Africa position statement on the redefinition of fatty liver disease: from NAFLD to MAFLD. *J Hepatol.* **74**, 1256–1258.
12. Shiha G, Alswat K, Al Klhatry M, et al. (2021) Nomenclature and definition of metabolic-associated fatty liver disease: a consensus from the Middle East and north Africa. *Lancet Gastroenterol Hepatol* **6**, 57–64.
13. Mendez-Sánchez N, Arrese M, Gadano A, et al. (2021) The Latin American Association for the Study of the Liver (ALEH) position statement on the redefinition of fatty liver disease. *Lancet Gastroenterol Hepatol* **6**, 65–72.
14. Parry SA, Hodson L. (2017) Influence of dietary macronutrients on liver fat accumulation and metabolism. *J Investig Med* **65**, 1102–1115.
15. Yki-Järvinen H, Luukkonen PK, Hodson L, et al. (2021) Dietary carbohydrates and fats in nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* **18**, 770–786.
16. Pickett-Blakely O, Young K, Carr RM. (2018) Micronutrients in nonalcoholic fatty liver disease pathogenesis. *Cell Mol Gastroenterol Hepatol* **6**, 451–462.
17. Pacifico L, Osborn JF, Bonci E, et al. (2019) Association between vitamin D levels and nonalcoholic fatty liver disease: potential confounding variables. *Mini Rev Med Chem* **19**, 310–332.
18. Karatayli E, Stokes CS, Lamertt F. (2020) Vitamin D in preclinical models of fatty liver disease. *Anticancer Res* **40**, 527–534.
19. Gibson PS, Lang S, Gilbert M, et al. (2015) Assessment of diet and physical activity in paediatric non-alcoholic fatty liver disease patients: a United Kingdom case control study. *Nutrients* **7**, 9721–9733.
20. Gibson PS, Quaglia A, Dhawan A, et al. (2018) Vitamin D status and associated genetic polymorphisms in a cohort of UK children with non-alcoholic fatty liver disease. *Pediatr Obes* **13**, 433–441.
21. Zhu S, Wang Y, Luo F, et al. (2019) The level of vitamin D in children and adolescents with nonalcoholic fatty liver disease: a meta-analysis. *Biomed Res Int* **2019**, 7643542.
22. Zhang Z, Thorne JL, Moore JB. (2019) Vitamin D and nonalcoholic fatty liver disease. *Carr Opin Clin Nutr Metab Care* **22**, 449–458.
23. Gjorgjieva M, Sobolewski C, Dolicka D, et al. (2019) miRNAs and NAFLD: from pathophysiology to therapy. *Gut* **68**, 2065–2079.
24. Wang X, He Y, Mackowiak B, et al. (2020) MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. *Gut*.
25. Oura K, Morishita A, Masaki T. (2020) Molecular and functional roles of microRNAs in the progression of hepatocellular carcinoma – a review. *Int J Mol Sci* **21**.
26. Zeljic K, Supic G, Magic Z. (2017) New insights into vitamin D anticancer properties: focus on miRNA modulation. *Mol Genet Genomics* **292**, 511–524.
27. Chiang KC, Yeh CN, Chen MF, et al. (2011) Hepatocellular carcinoma and vitamin D: a review. *J Gastroenterol Hepatol* **26**, 1597–1603.
28. Wu DB, Wang ML, Chen EQ, et al. (2018) New insights into the role of vitamin D in hepatocellular carcinoma. *Expert Rev Gastroenterol Hepatol* **12**, 287–294.
29. Fang Z, Du R, Edwards A, et al. (2013) The sequence structures of human microRNA molecules and their implications. *PLoS One* **8**, e54215.
30. Goodall GJ, Wickramasinghe VO. (2021) RNA in cancer. *Nat Rev Cancer* **21**, 22–36.
40. Weber JA, Baxter DH, Zhang S, Rupaimoole R, Slack FJ. (2017) MicroRNA therapeutics.

41. Mori MA, Ludwig RG, Garcia-Martin R, Watt MJ, Miotto PM, De Nardo W, Backes C, Meese E, Keller A. (2016) Specific miRNA disease

42. Jopling C. (2012) Liver-specific microRNA-122: biogenesis and function.

43. Bonneau E, Neveu B, Kostantin E, van der Ree MH, van der Meer AJ, van Nuenen AC, Janssen HL, Reesink HW, Lawitz EJ, Bajan S, Hutvagner G. (2020) RNA-based therapeutics: from promise of being minimal invasive biomarkers in clinical set-

44. Chipman LB, Pasquinelli AE. (2019) miRNA targeting: growing beyond the seed. Trends Genet 35, 215–222.

45. Friedman RC, Farh KK, Burge CB, Kobayashi H, Tomari Y. (2016) RISC assembly: coordination between small RNAs and Argonaute proteins. Biochim Biophys Acta, 71–81.

46. Budak H, Bulut R, Kantar M, Dufourd T, Robil N, Mallet D, Chiang HR, Schoenfeld LW, Ruby JG, Ludwig N, Leidinger P, Becker K, Kobayashi H, Suzuki K, Ichino N, Tomari Y.

47. van der Ree MH, van der Meer AJ, van Nuenen AC, Janssen HL, Reesink HW, Lawitz EJ, Bajan S, Hutvagner G. (2020) RNA-based therapeutics: from promise of being minimal invasive biomarkers in clinical set-

48. Jopling C. (2012) Liver-specific microRNA-122: biogenesis and function. RNA Biol 9, 137–142.

49. Alles J, Fehlmann T, Fischer U, Ji C, Guo X, (2019) The clinical potential of circulating microRNAs in obesity. Nat Rev Endocrinol 15, 731–743.

50. Alles J, Fehlmann T, Fischer U, et al. (2019) An estimate of the total number of true human miRNAs. Nucleic Acids Res 47, 3553–3564.

51. Alles J, Fehlmann T, Fischer U, et al. (2019) An estimate of the total number of true human miRNAs. Nucleic Acids Res 47, 3553–3564.

52. Dufourd T, Robil N, Mallet D, Fehlmann T, Lehaller B, Schaum N, et al. (2020) Common diseases alter the physiological age-related blood microRNA profile. Nat Commun 11, 5958.

53. Roux J, Gonzalez-Porta M, Robinson-Rechavi M. (2012) Comparative analysis of human and mouse expression data

54. Schlosser K, Taha M, Deng Y, et al. (2015) Discordant regulation of microRNA between multiple experimental models and human pulmonary hypertension. Chest 148, 481–490.

55. Paterson MR, Krieger AJ. (2017) MiR-16a/b: a family with shared seeds and different roots. Physiol Genomics 49, 243–252.

56. Younossi ZM, Koenig AB, Abdelatif D, et al. (2016) Global epidemiology of nonalcoholic fatty liver disease meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–84.

57. Diehl AM, Day C. (2017) Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. N Engl J Med 377, 2063–2072.

58. Hagstrom H, Nasr P, Elskedt M, et al. (2017) Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 1265–1273.

59. Su Q, Kumar V, Sud N, et al. (2018) MicroRNAs in the pathogenesis and treatment of progressive liver injury in NAFLD and liver fibrosis. Adv Drug Deliv Rev 129, 54–63.

60. Younossi ZM, Koenig AB, Abdelatif D, et al. (2016) Global epidemiology of nonalcoholic fatty liver disease meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–84.

61. Diehl AM, Day C. (2017) Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. N Engl J Med 377, 2063–2072.
72. Calo N, Ramadori P, Sobolewski C, et al. (2016) Stress-activated miR-21/miR-21* in hepatocytes promotes lipid and glucose metabolic disorders associated with high-fat diet consumption. Gut 65, 1871–1881.

73. Benhamouche-Trouillet S, Postic C. (2016) Emerging role of miR-21 in non-alcoholic fatty liver disease. Gut 65, 1781–1783.

74. Zhang T, Yang Z, Kusumanchi P, et al. (2020) Critical role of microRNA-21 in the pathogenesis of liver diseases. Front Med 7, 7.

75. Caviglia JM, Yan J, Jang MK, et al. (2018) MicroRNA-21 and Dicer are dispensable for hepatic stellate cell activation and the development of liver fibrosis. Hepatology 67, 2414–2429.

76. Liu RH, Ning B, Ma XE, et al. (2016) Regulatory roles of microRNA-21 in fibrosis through interaction with diverse pathway reviews. Mol Med Rep 13, 2359–2366.

77. Stokowy T, Eszlinger M, Wiemniak M, et al. (2014) Analysis options for high-throughput sequencing in miRNA expression profiling. BMC Res Notes 7, 144.

78. Latorre J, Moreno-Navarrete JM, Mercader JM, et al. (2017) Decreased lipid metabolism but increased FA biosynthesis is coupled with changes in liver microRNAs in obese subjects with NAFLD. Int J Obes 41, 620–630.

79. Latorre J, Ortega PJ, Liñares-Pose L, et al. (2020) Compounds that modulate AMPK activity and hepatic steatosis impact the biosynthesis of microRNAs required to maintain lipid homeostasis in hepatocytes. ElBioMedicine 53, 102697.

80. Zarrinpar A, Gupta S, Maurya MR, et al. (2016) Serum microRNAs explain discordance of non-alcoholic fatty liver disease in monzygotic and dizygotic twins: a prospective study. Gut 65, 1546–1554.

81. Wang DR, Wang B, Yang M, et al. (2020) Suppression of miR-30a-3p attenuates hepatic steatosis in non-alcoholic fatty liver disease. Biochem Genet 58, 691–704.

82. Ezhilarasan D. (2020) MicroRNA interaction between hepatic stellate cell quiescence and activation. Eur J Pharmacol 885, 173507.

83. Zheng J, Wang W, Yu F, et al. (2018) MicroRNA-30a suppresses the activation of hepatic stellate cells by inhibiting epithelial-to-mesenchymal transition. Cell Physiol Biochem 46, 82–92.

84. Cermelli S, Ruggieri A, Marrero JA, et al. (2011) Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. PLoS ONE 6, e23937.

85. Hendy OM, Rahie H, El Fouly A, et al. (2019) The circulating micro-RNAs (-122, –34a and –99a) as predictive biomarkers for non-alcoholic fatty liver disease. Diabetes Metab Syndr Obes 12, 2715–2723.

86. Erhartova D, Cahova M, Dankova H, et al. (2019) Serum miR-34a is associated with steatosis and inflammation in patients with non-alcoholic fatty liver disease after liver transplantation. PLoS ONE 14, e0224820.

87. Ezaz G, Trivedi HD, Connelly MA, et al. (2020) Differential associations of circulating microRNAs with pathogenic factors in NAFLD. Hepatol Commun 4, 670–680.

88. Salvoza NC, Klinzing DC, Gomez-Cervantes J, et al. (2016) Association of circulating serum miR-34a and miR-122 with dyslipidemia among patients with non-alcoholic fatty liver disease. PLoS ONE 11.

89. Xin S, Zhan Q, Chen X, et al. (2020) Efficacy of serum miRNA test as a non-invasive method to diagnose nonalcoholic steatohepatitis: a systematic review and meta-analysis. BMC Gastroenterol 20, 186.

90. Liu CH, Ampuero J, Gil-Gómez A, et al. (2018) miRNAs in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol 69, 1335–1348.

91. Oses M, Margareto Sanchez J, Portillo MP, et al. (2019) Circulating microRNAs as biomarkers of obesity and obesity-associated comorbidities in children and adolescents: a systematic review. Nutrients 11, 2890.

92. Hermeking H. (2010) The miR-34 family in cancer and apoptosis. Cell Death Differ 17, 193–199.

93. Rottiers V, Näää AM. (2012) MicroRNAs in metabolism and metabolic disorders. Nat Rev Mol Cell Biol 13, 239–250.

94. Li X, Zhang S, Blander G, et al. (2007) SIRT1 deacetylates and positively regulates the nuclear receptor LXR. Mol Cell 28, 91–106.

95. Kosgei VJ, Coelho D, Guéant-Rodriguez RM, et al. (2020) Sirt1-PPAR cross-talk in complex metabolic diseases and inherited disorders of the one carbon metabolism. Cells 9, 1882.

96. Castro RE, Ferreira DM, Afonso MB, et al. (2013) miR-34a/SIRT1/p53 is suppressed by ursodeoxycholic acid in the rat liver and activated by disease severity in human non-alcoholic fatty liver disease. J Hepatol 58, 119–125.

97. Derdz K, Villegas KA, Harb R, et al. (2013) Inhibition of p53 attenuates steatosis and liver injury in a mouse model of non-alcoholic fatty liver disease. J Hepatol 58, 785–791.

98. Tian XF, Ji FJ, Zang HL, et al. (2016) Activation of the miR-34a/SIRT1/p53 signaling pathway contributes to the progress of liver fibrosis via inducing apoptosis in hepatocytes but not in HSCs. PLoS ONE 11, e0158657.

99. Feili X, Wu S, Ye W, et al. (2018) MicroRNA-34a–5p inhibits liver fibrosis by regulating TGF-β1/Smad3 pathway in hepatic stellate cells. Cell Biol Int 42, 1370–1376.

100. Ding RB, Bao J, Deng CX. (2017) Emerging roles of SIRT1 in fatty liver diseases. Int J Biol Sci 13, 852–867.

101. Rafie S, Mohammadi H, Ghavami A, et al. (2021) Efficacy of resveratrol supplementation in patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis of clinical trials. Complement Ther Clin Pract 42, 101281.

102. Essau C, Davis S, Murray SF, et al. (2006) miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 3, 87–98.

103. Hsu SH, Wang B, Kota J, et al. (2012) Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest 122, 2871–2883.

104. Bandiera S, Pfeffer S, Baumert TF, et al. (2016) miR-122–a key factor and therapeutic target in liver disease. J Hepatol 62, 448–457.

105. Akuta N, Kawamura Y, Suzuki F, et al. (2016) Analysis of association between circulating miR-122 and histopathological features of nonalcoholic fatty liver disease in patients free of hepatocellular carcinoma. BMC Gastroenterol 16, 141.

106. Akuta N, Kawamura Y, Suzuki F, et al. (2016) Impact of circulating miR-122 for histological features and hepatocellular carcinoma of nonalcoholic fatty liver disease in Japan. Hepatol Int 10, 647–656.

107. Akuta N, Kawamura Y, Arase Y, et al. (2020) Circulating microRNA-122 and fibrosis stage predict mortality of Japanese patients with histopathologically confirmed NAFLD. Hepatol Commun 4, 66–76.

108. Tan Y, Ge G, Pan T, et al. (2014) A pilot study of serum microRNAs panel as potential biomarkers for diagnosis of nonalcoholic fatty liver disease. PLoS ONE 9, e105192.

109. Ye D, Zhang T, Lou G, et al. (2018) Plasma miR-17, miR-20a, miR-20b and miR-122 as potential biomarkers for diagnosis of NAFLD in type 2 diabetes mellitus patients. Life Sci 208, 201–207.

110. Miyahira H, Ichikawa T, Kamo Y, et al. (2014) Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. Liver Int 34, e302–e307.
111. Pirola CJ, Fernández Gianotti T, Castaño GO, et al. (2015) Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 64, 800–812.

112. Auguet T, Aragonès G, Berlanga A, et al. (2016) miR353a/ miR353b and miR122 as possible contributors to hepatic lipid metabolism in obese women with nonalcoholic fatty liver disease. *Int J Mol Sci* 17, 1620.

113. Rissin DM, López-Longarela B, Pernagallo S, et al. (2012) Auguet T, Aragonès G, Berlanga A, et al. (2016) miR353a/ miR353b and miR122 as possible contributors to hepatic lipid metabolism in obese women with nonalcoholic fatty liver disease. *Int J Mol Sci* 17, 1620.

114. Cheung O, Pur N, Eicken C, et al. (2008) Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. *Hepatology* 48, 1810–1820.

115. Wang JM, Qiu Y, Yang Z, et al. (2013) Targeted delivery of miR-146a for ameliorating hepatic steatosis and inflammation by reprogramming multiple signaling pathways in NASH. *JCI Insight* 2.

116. Naderi M, Pazouki A, Arefan E, et al. (2017) Two triacylglycerol pathway genes, CTDNEP1 and LPIN1, are down-regulated by hsa-miR-122-5p in hepatocytes. *Arch Iran Med* 20, 165–171.

117. Tsi WC, Hsu SD, Hsu CS, et al. (2012) MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest* 122, 2884–2897.

118. Chai C, Rivkin M, Berkowitz L, et al. (2017) Metabolic circuit involving free fatty acids, microRNA 122, and triglyceride synthesis in liver and muscle tissues. *Gastroenterology* 153, 1404–1415.

119. Testa U, Pelosi E, Castelli G, et al. (2017) miR-146a and miR-155: two key modulators of immune response and tumor development. *Noncoding RNA* 3.

120. Wang JM, Qiu Y, Yang Z, et al. (2018) IRE1α prevents hepatic steatosis by processing and promoting the degradation of select microRNAs. *Sci Signal* 11.

121. Celikkale M, Baskol M, Taheri S, et al. (2014) Circulating microRNAs in patients with non-alcoholic fatty liver disease. *World J Hepatol* 6, 613–620.

122. Jin X, Liu J, Chen YP, et al. (2017) Effect of miR-146 targeted HDMCP up-regulation in the pathogenesis of nonalcoholic steatohepatitis. *Plos ONE* 12, e0174218.

123. He S, Guo W, Deng F, et al. (2018) Targeted delivery of microRNA 146b mimic to hepatocytes by lactosylated PDMAEMA nanoparticles for the treatment of NAFLD. *Artif Cells Nematol Biotechnol* 46, 217–228.

124. Jiang W, Liu J, Dai Y, et al. (2015) MiR-146b attenuates high-fat diet-induced non-alcoholic steatohepatitis in mice. *J Gastroenterol Hepatol* 30, 933–943.

125. He Y, Huang C, Sun X, et al. (2012) MicroRNA-146a modulates TGF-beta1-induced hepatic stellate cell proliferation by targeting SMAD4. *Cell Signal* 24, 1923–1930.

126. Yuan BY, Chen YH, Wu ZF, et al. (2019) MicroRNA-146a-5p attenuates fibrosis-related molecules in irradiated and TGF-beta1-treated human hepatic stellate cells by regulating PTEN/PI3K/AKT signaling. *Radiat Res* 192, 621–629.

127. Zou Y, Li S, Li Z, et al. (2019) MiR-146a attenuates liver fibrosis by inhibiting transforming growth factor-β1 mediated epithelial-mesenchymal transition in hepatocytes. *Cell Signal* 58, 1–8.

128. Görecki T, Rabin B. (2021) The role of microRNAs in epithelial to mesenchymal transition and cancers; focusing on mir-200 family. *Cancer Treat Res Commun* 28, 100385.

129. Mao Y, Chen W, Wu H, et al. (2020) Mechanisms and functions of mir-200 family in hepatocellular carcinoma. *Onco Targets Ther* 13, 15479–15490.

130. Hilmarsdottir B, Brieum E, Berghorsson JT, et al. (2014) Functional role of the microRNA-200 family in breast morphogenesis and neoplasia. *Genes* 5, 804–820.

131. Tran M, Lee SM, Shin DJ, et al. (2017) Loss of miR-141/200c ameliorates hepatic steatosis and inflammation by reprogramming multiple signaling pathways in NASH. *JCI Insight* 2.

132. Guo J, Fang W, Sun L, et al. (2016) Reduced miR-200b and miR-200c expression contributes to abnormal hepatic lipid accumulation by stimulating JUN expression and activating the transcription of srebp1. *Oncotarget* 7, 36207–36219.

133. Wang Y, Zeng Z, Guan L, et al. (2020) GRHL2 induces liver fibrosis and intestinal mucosal barrier dysfunction in non-alcoholic fatty liver disease via microRNA-200 and the MAPK pathway. *J Cell Mol Med* 24, 6107–6119.

134. Demet L, Hsu JJ, Tintut Y. (2018) Steroid hormone vitamin D: implications for cardiovascular disease. *Circ Res* 122, 1576–1585.

135. Moore DD, Kato S, Xie W, et al. (2006) International Union of Pharmacology. LXII. The NR1H and NR1I receptors: constitutive androstane receptor, pregnene X receptor, farnesoid X receptor alpha, farnesoid X receptor beta, liver X receptor alpha, liver X receptor beta, and vitamin D receptor. *Pharmacol Res* 58, 742–759.

136. Vession MT, Robotham SK. (2012) D-livering the message: the importance of vitamin D status in chronic liver disease. *J Hepatol* 57, 897–909.

137. Barchetta I, Cimini FA, Cavollo MG. (2020) Vitamin D and metabolic dysfunction-associated fatty liver disease (MAFLD): an update. *Nutrients* 12.

138. Mirhosseini N, Vatanparast H, Mazidi M, et al. (2018) Vitamin D supplementation, glycemic control, and insulin resistance in prediabetics: a meta-analysis. *J Endocr Soc* 2, 687–709.

139. Li X, Liu Y, Zheng Y, et al. (2018) The effect of vitamin D supplementation on glycemic control in type 2 diabetes patients: a systematic review and meta-analysis. *Nutrients* 10, 375.

140. Guo XF, Wang C, Yang T, et al. (2020) Vitamin D and non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Food Funct* 11, 7389–7399.

141. Barchetta I, Del Ben M, Angelico F, et al. (2016) No effects of oral vitamin D supplementation on non-alcoholic fatty liver disease in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *BMC Med* 14, 92.

142. El Amrousy D, Abdelhai D, Shawky D. (2021) Vitamin D and non-alcoholic fatty liver disease in children: a randomized controlled clinical trial. *Eur J Pediatr*: doi: 10.1007/s00431-021-04243-4.

143. Zenata O, Vrzal R. (2017) Fine tuning of vitamin D receptor (VDR) activity by post-transcriptional and post-translational modifications. *Oncotarget* 8, 35390–35402.

144. Pan YZ, Gao W, Yu AM. (2009) MicroRNAs regulate CYP3A4 expression via direct and indirect targeting. *Drug Metab Dispos* 37, 2112–2117.

145. Mohri T, Nakajima M, Takagi S, et al. (2009) MicroRNA regulates human vitamin D receptor. *Int J Cancer* 125, 1328–1333.

146. Essa S, Denzer N, Mahlknecht U, et al. (2010) VDR microRNA expression and epigenetic silencing of vitamin D signaling in melanoma cells. *J Steroid Biochem Mol Biol* 121, 110–113.

147. Chen Y, Du J, Zhang Z, et al. (2014) MicroRNA-346 mediates tumor necrosis factor α-induced downregulation of gut epithelial vitamin D receptor in inflammatory bowel diseases. *Inflamm Bowel Dis* 20, 1910–1918.

148. Xu J, Wang Y, Zhang Y, et al. (2018) Astemizole promotes the anti-tumor effect of vitamin D through inhibiting miR-125a-5p-mediated regulation of VDR in HCC. *Biomed Pharmacother* 107, 1682–1691.
Circulating levels of miR-7, miR-152 and miR-192 respond targeting TNFAIP3. NF-κB-mediated inflammatory response in NAFLD via directly targeting microRNA-155-SOCS1 in macrophages infected with dengue virus: implications for the cytokine response. 

Karkeni E, Bonnet L, Marcotorchino J, et al. (2018) Vitamin D limits inflammation-linked microRNA expression in adipocytes in vitro and in vivo: a new mechanism for the regulation of inflammation by vitamin D. Epigenetics 13, 156–162.

Langi G, Szczersinski L, Kretowski A. (2019) Meta-analysis of differential miRNA expression after bariatric surgery. J Clin Med 8.

Catanzaro G, Filardi T, Sabato C, et al. (2016) A micro-RNA expression signature for human NAFLD progression. J Gastroenterol Hepatol 31, 1022–1030.

Guo XY, Chen JM, Sun F, et al. (2017) circRNA_0046367 prevents hepatotoxicity of lipid peroxidation: an inhibitory role against hepatic steatosis. Oxid Med Cell Longev 2017, 3695–3703.

Lee YS, Kim SY, Ko E, et al. (2017) Exosomes derived from adipose tissue can specifically predict liver injury of chronic hepatitis B. J Med Virol 89, 2239–2249.

Arboleda JF, Fernandez GJ, Urcuqui-Inchima S. (2019) MicroRNA-mediated attenuation of miR-155 in human macrophages infected with dengue virus: implications for the cytokine response. Infect Genet Evol 69, 12–21.

Langi G, Szczersinski L, Kretowski A. (2019) Meta-analysis of differential miRNA expression after bariatric surgery. J Clin Med 8.

Catanzaro G, Filardi T, Sabato C, et al. (2020) Tissue and circulating microRNAs as biomarkers of response to obesity treatment strategies. J Endocrinol Invest 44, 159–1174.

Nam JW, Rissland OS, Koppstein D, et al. (2014) Global analyses of the effects of different cellular contexts on microRNA targeting. Mol Cell 53, 1051–1063.

Hsin JP, Lu Y, Loeb GB, et al. (2018) The effect of cellular context on microRNA-directed gene regulation in four major immune cell types. Nat Immunol 19, 1137–1145.

Xu Y, Zalzala M, Xu J, et al. (2015) A metabolic stress inducible miR-34a-HNF4α pathway regulates lipid and lipoprotein metabolism. Nat Commun 6, 7466.

Guo Y, Xiong Y, Sheng Q, et al. (2016) A microRNA expression signature for human NAFLD progression. J Gastroenterol 51, 1022–1030.

Guo XY, Chen JM, Sun F, et al. (2017) circRNA_0046367 prevents hepatotoxicity of lipid peroxidation: an inhibitory role against hepatic steatosis. Oxid Med Cell Longev 2017, 3695–3703.

Lee YS, Kim SY, Ko E, et al. (2017) Exosomes derived from palmitic acid-treated hepatocytes induce fibrotic activation of hepatic stellate cells. Sci Rep 7, 3710.

Branch D, Roos J, Inzaghi E, et al. (2018) Circulating levels of miR-122 and nonalcoholic fatty liver disease in pre-pubertal obese children. Pediatr Obes 13, 175–182.

Jampoka K, Muangpaisarn P, Khongnonman K, et al. (2018) Serum miR-29a and miR-122 as potential biomarkers for...
non-alcoholic fatty liver disease (NAFLD). MicroRNA 7, 215–222.

187. Hegazy MA, Abd AI, Abuel Fadl S, et al. (2021) Serum micro-RNA-122 level as a simple noninvasive marker of NAFLD severity. Diabetes Metab Syndr Obes 14, 2247–2254.

188. Enquobahrie DA, Williams MA, Qiu C, et al. (2011) Global maternal early pregnancy peripheral blood mRNA and miRNA expression profiles according to plasma 25-hydroxy-vitamin D concentrations. J Matern Fetal Neonatal Med 24, 1002–1012.

189. Lee HJ, Muindi JR, Tan W, et al. (2014) Low 25(OH) vitamin D3 levels are associated with adverse outcome in newly diagnosed, intensively treated adult acute myeloid leukemia. Cancer 120, 521–529.

190. Beckett EL, Martin C, Duesing K, et al. (2014) Vitamin D receptor genotype modulates the correlation between vitamin D and circulating levels of let-7a/b and vitamin D intake in an elderly cohort. J Nutrigenet Nutrigenomics 7, 264–273.

191. Ferrero G, Carpi S, Polini B, et al. (2020) Intake of natural compounds and circulating microRNA expression levels: their relationship investigated in healthy subjects with different dietary habits. Front Pharmacol 11, 619200.

192. Jorde R, Svartberg J, Joakimsen RM, et al. (2012) Plasma profile of microRNA after supplementation with high doses of vitamin D3 for 12 months. BMC Res Notes 5, 245.

193. Yu ZJ, Zeng L, Luo XQ, et al. (2017) Vitamin D3 inhibits microRNA-17-92 to promote specific immunotherapy in allergic rhinitis. Sci Rep 7, 546.

194. Duan X, Guan Y, Li Y, et al. (2015) Vitamin D potentiates the inhibitory effect of microRNA-130a in hepatitis C virus replication independent of type I interferon signaling pathway. Mediators Inflamm 2015, 508989.