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Original Research Article

Investigation of MicroRNA-Mediated Regulation of Lipid Metabolism Enzymes in Non-Alcoholic Fatty Liver Disease

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Abstract

Background: MicroRNAs (miRNAs) regulate hepatic lipid biosynthesis and fatty-acid oxidation, but the overall connection between disease-associated miRNAs and their enzyme targets throughout the histological continuum of non-alcoholic fatty liver disease (NAFLD) is not fully understood.

Objective: To investigate the expression of miR-34a-5p, miR-33a-5p, and miR-122-5p and their functional effects in a hepatocyte steatosis model in relation to lipid-metabolism enzymes.

Methods: A simulated case-control dataset was created with 96 adults who were histologically normal controls (n = 24), simple steatosis (n = 36), or non-alcoholic steatohepatitis (NASH; n = 36). The expression of hepatic miRNAs and mRNAs was measured by reverse-transcription quantitative polymerase chain reaction (RT-qPCR), and the expression of proteins SIRT1, CPT1A, and FASN was assessed by immunoblotting. HepG2 cells were loaded with free fatty acids and treated with miRNA inhibition or replacement.

Results: Hepatic miR-34a-5p and miR-33a-5p increased progressively from controls to simple steatosis and NASH (1.00±0.29, 2.05±0.72, and 3.54±1.21-fold; and 1.00±0.25, 1.62±0.51, and 2.41±0.83-fold, respectively; both $p<0.001$), whereas miR-122-5p decreased (1.00±0.31, 0.74±0.22, and 0.49±0.18-fold; $p<0.001$). miR-34a-5p correlated inversely with SIRT1 ($r=-0.72$), miR-33a-5p with CPT1A ($r=-0.68$), and miR-122-5p with FASN ($r=-0.47$; all $p<0.001$). The miRNA model had an AUC of 0.91 when combined to differentiate NASH from non-NASH. The combination of anti-miR-34a/anti-miR-33a and miR-122 replacement decreased the levels of intracellular triglycerides by 41.6% relative to the fatty-acid loaded controls ($p<0.001$).

Conclusion: Coordinated miR-34a and miR-33a activation upon hepatic miR-122 depletion indicated a multi-miRNA regulatory network in NAFLD progression.

Keywords: microRNA; non-alcoholic fatty liver disease; lipid metabolism; SIRT1; CPT1A; FASN; steatohepatitis.

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Introduction

The most common chronic liver disease in the world is now considered to be non-alcoholic fatty liver disease (NAFLD), which is now part of the broader category of metabolic dysfunction-associated steatotic liver disease. A recent systematic review estimated that about 30% of adults have NAFLD, and that the prevalence of NAFLD increases as obesity, insulin resistance and type 2 diabetes increase [1]. Modern clinical recommendations acknowledge a spectrum of disease from simple steatosis to steatohepatitis, fibrosis, and cirrhosis and hepatocellular carcinoma [2]. The mechanisms leading to the development of excess calorie intake and insulin resistance are the first step in hepatic lipid accumulation, but the process is driven by interacting disturbances of lipid uptake, de novo lipogenesis, mitochondrial fatty-acid oxidation, oxidative stress, inflammation, and cell injury [3]. MicroRNAs are small non-coding RNAs that regulate gene expression by binding to target messenger RNAs by sequence complementarity. They are especially relevant for

complex disorders where multiple enzymes and transcriptional regulators are altered at the same time as they can affect several targets in the same metabolic pathway. In the liver, miRNAs control the synthesis of sterols, uptake of fatty acids, assembly of triglycerides, beta-oxidation, and secretion of lipoproteins, insulin signalling and inter-organ metabolic communication [4]. Several miRNAs have been repeatedly reported as dysregulated in NAFLD, such as miR-34a, miR-122, miR-192, miR-21 and miR-33 family, but with different directions and magnitudes depending on tissue compartment, disease stage and analytical method [5].

miR-122 is the most abundant liver-enriched miRNA and plays a dual function in maintaining liver identity and regulating lipid homeostasis. The expression of miR-122 is increased in the blood when the liver is injured, while it may be reduced as the steatosis and fibrosis increase. This compartment-specific behaviour has been shown in the studies that correlated serum miRNA signatures with liver histology [6] and that correlated hepatic and circulating miR-122 with the severity of steatosis and fibrosis [7]. So, the elevation of serum does not necessarily mean there is more regulatory activity inside cells.

miR-34a and miR-33 are more directly related to metabolic enzyme suppression. Experimental miR-34a inhibition leads to an increase in the expression of SIRT1 and peroxisome proliferator-activated receptor-alpha (PPARalpha), activation of AMP-activated protein kinase (AMPK), and decreases hepatocellular lipid accumulation [8]. In humans, miR-33a, which is encoded by SREBF2, and miR-33b, which is encoded by SREBF1, regulate the expression of carnitine palmitoyltransferase 1A (CPT1A), carnitine O-octanoyltransferase and hydroxyacyl-CoA dehydrogenase, which limit fatty-acid oxidation in the mitochondria and affect insulin signalling [9]. Independent work also confirmed that miR-33 inhibits cholesterol export and fatty-acid oxidation by acting in a coordinated fashion on ABCA1 and oxidative enzymes [10].

Other miRNAs regulate the other lipogenic arm. miR-27a has been reported to inhibit fatty-acid synthase (FASN) and stearoyl-CoA desaturase 1 (SCD1) and miR-21 has been associated with regulation of 3-hydroxy-3-methylglutaryl-CoA reductase, which is involved in triglyceride and cholesterol metabolism [11,12].

Most studies, however, focus on a single miRNA or target, often in animal or cell models. There are few human studies combining histological, miRNA expression, enzyme level changes, and functional validation. The current simulated study aimed to explore the coordinated expression of miR-34a-5p, miR-33a-5p and miR-122-5p in normal liver, simple steatosis and NASH; identify their relationships with SIRT1, PPARA, CPT1A, FASN, SCD1 and ACACA; and assess the ability of targeted modulation of these miRNAs to reverse lipid accumulation in fatty-acid-loaded hepatocytes.

Materials and Methods

Study design and sample size: This model of the academic was a simulated comparative translational design. The dataset consisted of 96 adults, aged 18-65 years, who were divided into three groups: histologically normal controls (n=24), simple steatosis without ballooning (n=36), and NASH (n=36). The minimum sample size for a one-way analysis of variance with three groups, with an effect size of f=0.35, a power of 90%, and an alpha of 0.05 was estimated to be 90, while 96 observations were modelled to allow for incomplete molecular measurements.

Eligibility criteria and histological classification: The simulated inclusion criteria included availability of liver tissue, fasting serum, complete metabolic data, liver biochemical data, and the length of the biopsy, which was at least 15 mm and contained 10 or more portal tracts. Macrovesicular steatosis of at least 5% of the hepatocytes, excluding secondary causes, was used to define NAFLD. Alcohol consumption > 20 g/day in women and > 30 g/day in men, viral hepatitis, autoimmune or cholestatic liver disease, Wilson disease, hemochromatosis, pregnancy, malignancy, treatment with steatogenic drugs, decompensated cirrhosis and recent weight change > 5% were exclusion criteria. The NAFLD activity score (NAS) was assigned based on steatosis, lobular inflammation and ballooning. Steatosis and lobular inflammation with hepatocyte ballooning were required by NASH.

Clinical and biochemical assessment

The following factors were documented: age, sex, body mass index (BMI), waist circumference, diabetes status, and medication use. Standardized automated methods were used to measure fasting glucose, insulin, triglycerides, total cholesterol, high-density lipoprotein cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase, and gamma-glutamyl transferase.

When glucose was measured in mg/dL, insulin resistance was estimated as HOMA-IR by multiplying the fasting insulin level by the fasting glucose level and dividing by 405.

RNA extraction and quantitative expression analysis: The total RNA, including small RNA, was simulated as being extracted from frozen liver tissue by phenol-column purification. The integrity and concentration of RNA were assessed

spectrophotometrically and by microfluidic electrophoresis. The expression of miR-34a-5p, miR-33a-5p and miR-122-5p was determined by stem-loop reverse transcription and TaqMan assays. After stability testing, RNU6B and miR-16-5p were used as reference controls. SYBR Green assays were used to quantify the expression of messenger RNA (mRNA) of SIRT1, PPARA, CPT1A, FASN, SCD1, and ACACA, with normalization to GAPDH and RPLP0. The relative expression was determined by the 2^-delta-delta Ct method using the control group as calibrator.

Protein quantification: Immunoblotting was used to measure the levels of SIRT1, CPT1A and FASN proteins. Equal amounts of total protein were separated by SDS-PAGE, transferred to PVDF membranes and then incubated with validated primary antibodies. Chemiluminescence was used to visualize bands, densitometry to quantify bands, and normalization to beta-actin and expressed as fold of the control mean.

Functional hepatocyte validation: HepG2 cells were grown in high glucose Dulbecco modified Eagle medium containing 10% fetal bovine serum. Steatosis was induced with 2:1 oleate-palmitate mixture at a final concentration of 0.5 mmol/L for 24 hours. Cells were transfected with anti-miR-34a, anti-miR-33a, miR-122 mimic, a combination of anti-miR-34a and anti-miR-33a, and sequence-matched negative controls. Intracellular triglycerides were normalised to total protein. Lipid accumulation and target restoration were evaluated using oil red O staining, quantitative polymerase chain reaction, and immunoblotting. For each condition six independent experiments were run.

Statistical Analysis: Data were presented as mean ± SD, median (IQR) or number and percentage, depending on the distribution. One-way analysis of variance with Tukey post-hoc testing, Kruskal-Wallis testing with Dunn correction, or chi-square testing were used to evaluate group differences. The Benjamini-Hochberg false-discovery rate was used for molecular comparisons. Spearman correlations were used to evaluate the relationship between miRNAs, enzyme targets, metabolic parameters and NAS. After controlling for age, sex, BMI, HOMA-IR, and ALT, multivariable logistic regression revealed independent associations with NASH. Discrimination was assessed using receiver operating characteristic analysis. A p value <0.05 was deemed significant for two-tailed tests. The analyses were performed in SPSS version 29.0 and R version 4.3.2.

Results

Of 104 simulated records screened, eight were excluded because of incomplete tissue quality or missing metabolic data, leaving 96 observations for analysis. The groups were similar in age and sex distribution. BMI, waist circumference, HOMA-IR, triglycerides, ALT, and NAS increased progressively across the control, simple steatosis, and NASH groups. Diabetes was present in 5.6% of participants with simple steatosis and 30.6% with NASH (p<0.001).

Table 1: Clinical, biochemical, and histological characteristics

Variable	Control (n=24)	Simple steatosis (n=36)	NASH (n=36)	p-value
Age (years)	43.2 ± 10.1	45.8 ± 9.6	47.3 ± 8.9	0.18
Male sex, n (%)	13 (54.2)	20 (55.6)	21 (58.3)	0.94
BMI (kg/m2)	24.1 ± 2.4	29.0 ± 3.1	31.2 ± 3.6	<0.001
Waist circumference (cm)	84.6 ± 8.7	98.1 ± 9.5	104.2 ± 10.8	<0.001
Type 2 diabetes, n (%)	0 (0.0)	2 (5.6)	11 (30.6)	<0.001
HOMA-IR	1.6 ± 0.7	3.2 ± 1.3	4.5 ± 1.8	<0.001
Triglycerides (mg/dL)	118.5 ± 32.8	168.4 ± 44.7	201.6 ± 56.3	<0.001
HDL cholesterol (mg/dL)	51.6 ± 10.2	43.8 ± 9.1	39.5 ± 8.7	<0.001
ALT (U/L)	24.7 ± 8.1	46.8 ± 19.4	72.5 ± 31.6	<0.001
AST (U/L)	23.8 ± 6.9	34.9 ± 13.2	51.6 ± 22.4	<0.001
Hepatic steatosis (%)	2.1 ± 1.4	28.6 ± 12.3	46.8 ± 16.7	<0.001
NAFLD activity score	0.8 ± 0.6	3.1 ± 0.8	5.4 ± 0.7	<0.001

Values are mean ± SD unless otherwise stated. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; NASH, non-alcoholic steatohepatitis. Hepatic miR-34a-5p and miR-33a-5p showed stepwise increases with histological severity, while miR-122-5p declined. Oxidative regulators SIRT1, PPARA, and CPT1A were significantly lower in simple steatosis and further reduced in NASH. Conversely, FASN, SCD1, and ACACA were increased, indicating a shift toward de novo lipogenesis. Protein abundance followed the corresponding messenger RNA patterns: SIRT1 and CPT1A were 53% and 49% lower in NASH than in controls, whereas FASN was 2.21-fold higher (all false-discovery-rate-adjusted p<0.001).

Table 2: Hepatic miRNA, target-gene, and protein expression

Marker (relative expression)	Control	Simple steatosis	NASH	FDR p-value
miR-34a-5p	1.00 ± 0.29	2.05 ± 0.72	3.54 ± 1.21	<0.001
miR-33a-5p	1.00 ± 0.25	1.62 ± 0.51	2.41 ± 0.83	<0.001
miR-122-5p	1.00 ± 0.31	0.74 ± 0.22	0.49 ± 0.18	<0.001
SIRT1 mRNA	1.00 ± 0.21	0.71 ± 0.20	0.47 ± 0.16	<0.001
PPARA mRNA	1.00 ± 0.24	0.69 ± 0.21	0.43 ± 0.17	<0.001
CPT1A mRNA	1.00 ± 0.23	0.68 ± 0.19	0.44 ± 0.15	<0.001
FASN mRNA	1.00 ± 0.27	1.61 ± 0.49	2.48 ± 0.81	<0.001
SCD1 mRNA	1.00 ± 0.30	1.43 ± 0.46	2.05 ± 0.67	<0.001
ACACA mRNA	1.00 ± 0.26	1.38 ± 0.41	1.91 ± 0.59	<0.001
SIRT1 protein	1.00 ± 0.19	0.73 ± 0.18	0.47 ± 0.14	<0.001
CPT1A protein	1.00 ± 0.22	0.75 ± 0.20	0.51 ± 0.17	<0.001
FASN protein	1.00 ± 0.25	1.52 ± 0.43	2.21 ± 0.66	<0.001

Expression is normalized to the control mean (1.00). ACACA, acetyl-CoA carboxylase alpha; CPT1A, carnitine palmitoyl transferase 1A; FASN, fatty-acid synthase; FDR, false-discovery rate; PPARA, peroxisome proliferator-activated receptor-alpha; SCD1, stearoyl-CoA desaturase 1; SIRT1, sirtuin 1. miR-34a-5p correlated inversely with SIRT1 and PPARA and positively with NAS. miR-33a-5p correlated inversely with CPT1A and positively with HOMA-IR. miR-122-5p correlated inversely with FASN and NAS. In adjusted logistic regression, higher miR-34a-5p and miR-33a-5p remained independently associated with NASH, whereas higher miR-122-5p was protective. A clinical model containing BMI, HOMA-IR, and ALT yielded an area under the receiver operating characteristic curve (AUC) of 0.82. Addition of the three hepatic miRNAs increased the AUC to 0.91 (95% confidence interval 0.84-0.97; p=0.018 for AUC comparison).

Table 3: Correlation and multivariable analyses

Analysis	Variable	Effect estimate	95% CI	p-value
Correlation	miR-34a-5p vs SIRT1	r = -0.72	-0.81 to -0.60	<0.001
Correlation	miR-34a-5p vs PPARA	r = -0.66	-0.76 to -0.52	<0.001
Correlation	miR-34a-5p vs NAS	r = 0.71	0.59 to 0.80	<0.001
Correlation	miR-33a-5p vs CPT1A	r = -0.68	-0.78 to -0.55	<0.001
Correlation	miR-33a-5p vs HOMA-IR	r = 0.49	0.32 to 0.63	<0.001
Correlation	miR-122-5p vs FASN	r = -0.47	-0.62 to -0.29	<0.001
Correlation	miR-122-5p vs NAS	r = -0.54	-0.67 to -0.38	<0.001
Adjusted NASH model	miR-34a-5p (per fold)	OR = 2.35	1.42 to 3.88	0.001
Adjusted NASH model	miR-33a-5p (per fold)	OR = 1.91	1.13 to 3.22	0.016
Adjusted NASH model	miR-122-5p (per fold)	OR = 0.38	0.17 to 0.83	0.015

The logistic model was adjusted for age, sex, BMI, HOMA-IR, and ALT. CI, confidence interval; NAS, NAFLD activity score; OR, odds ratio. In HepG2 cells, fatty-acid loading increased intracellular triglycerides 2.74-fold and increased miR-34a-5p and miR-33a-5p by 2.81-fold and 2.06-fold, respectively, while reducing miR-122-5p to 0.58-fold. Anti-miR-34a restored SIRT1 protein by 46.3% and reduced triglycerides by 28.4% relative to fatty-acid-loaded negative controls. Anti-miR-33a restored CPT1A by 39.8% and reduced triglycerides by 24.7%. miR-122 replacement reduced FASN by 31.5% and triglycerides by 22.9%. The combined intervention produced the greatest effect, reducing triglycerides by 41.6%, Oil Red O-positive area by 44.2%, and FASN protein by 38.7%, while increasing SIRT1 and CPT1A by 58.1% and 51.4%, respectively (all p<0.001).

Discussion

The most important result of this simulated translational analysis was a coordinated miRNA pattern that was linked to the deterioration of NAFLD histology: miR-34a-5p and miR-33a-5p were up-regulated, miR-122-5p was down-regulated, oxidative regulators were down-regulated and lipogenic enzymes were up-regulated in the liver. These changes were not restricted to statistical relationships. Responses that were similar in direction in fatty-acid-loaded hepatocytes indicated that the combined modulation of the three miRNAs resulted in a greater reduction in lipid accumulation than modulation of any single miRNA. The findings thus indicate that multiple post-transcriptional regulators are involved in the maintenance of impaired beta-oxidation and enhanced lipogenesis in the network model.

The results of the miR-34a are biologically plausible. miR-34a is able to directly inhibit SIRT1 and reduce PPARalpha-mediated fatty-acid oxidation. The strong inverse correlation between miR-34a and SIRT1, and the restoration of SIRT1 expression after miR-34a inhibition, in the present dataset, implies that this pathway could link histological progression with decreased metabolic flexibility. Experimental studies on NAFLD in iron overload also demonstrated that miR-34a overexpression led to triglyceride accumulation, mitochondrial dysfunction, and that the silencing of miR-34a reversed SIRT1 and decreased steatosis [13]. The progressive increase of miR-34a from simple steatosis to NASH also suggests that the function of miR-34a may not be limited to lipid storage, but may also involve oxidative stress, inflammation, and hepatocyte injury.

miR-33a showed a similar correlation with the severity of the disease and was most correlated with CPT1A. CPT1A is a rate limiting enzyme for entry of long chain fatty acids into the mitochondria and thus regulates beta-oxidation. The inverse miR-33a-CPT1A correlation and partial recovery of CPT1A upon anti-miR-33a treatment is consistent with known miR-33 biology. More recent hepatocyte-specific deletion experiments showed that miR-33 deficiency decreased lipid production, increased mitochondrial fatty-acid oxidation, increased insulin sensitivity, and prevented the progression from steatosis to steatohepatitis, fibrosis and HCC [14]. The present model is expanded to human histological categories and proposes that miR-33a may be more informative if used in conjunction with expression of oxidative enzymes, rather than as a single biomarker.

In contrast to the previously reported increase in circulating miR-122 in liver injury, hepatic miR-122 was found to be decreased as NAS increased. This paradox is anticipated as active secretion and passive leakage of miR-122 from damaged hepatocytes contribute to the extracellular pool, while the intrahepatic pool is the remaining regulatory pool.

The free fatty acids may inhibit the expression of miR-122 in the liver and activate a lipogenic pathway to promote triglyceride accumulation in the liver and muscle [15]. In our cell model, replacement of miR-122 reduced the expression of FASN and intracellular triglycerides, suggesting a homeostatic, rather than merely pathogenic, intracellular function of miR-122. The results highlight the importance of biological compartment when proposing miR-122 as a diagnostic or therapeutic marker. The observed rise in FASN, SCD1 and ACACA suggests an improvement in de novo lipogenesis. These enzymes are regulated by insulin, SREBP1c, and carbohydrate-responsive element-binding protein, as well as epigenetic mechanisms, but miRNA can either upregulate or downregulate their activity. The decrease in FASN following replacement of miR-122 and the enhanced effect following combined treatment indicate that there may be cross-talk between miRNA pathways that regulates lipogenesis. Clinically relevant interaction because circulating miRNA panels have typically discriminated NASH better than single miRNA measurements. A study that used a combination of miR-122, miR-192, miR-21 and cytokeratin-18 had better classification of NASH than the conventional markers alone [16]. The AUC improvement (0.82 to 0.91) for the present model also supports multi-marker integration.

From a therapeutic perspective, miRNAs are very appealing because they can have multiple targets within a metabolic pathway. The same property, however, poses issues of safety such as unintended gene suppression, activation of the immune system, tissue distribution outside of the liver, and loss of normal physiological functions. Experimental miRNA therapies were the subject of a systematic review, revealing that the most commonly studied approach was the antagonism of miR-34a, which has been linked to the improvement of hepatic triglycerides and aminotransferases, though most studies were preclinical [17]. Thus, delivery systems need to be hepatocyte selective, have the correct dose, be reversible and have minimal off-target activity. Theoretically, combination therapy allows for lower doses of each agent, but this needs to be formally evaluated for toxicity.

There are also some significant limitations to the study. First, all numerical data is simulated and not used to make conclusions about clinical efficacy or causal inference. Secondly, a Cross-Sectional design cannot establish whether changes in miRNA occur before or after metabolic deterioration. Third, there is a difference between HepG2 and primary human hepatocytes in terms of lipid handling and insulin responsiveness. Fourth, miRNA measurements are still technically challenging due to the quality of the RNA, the choice of reference genes, and the heterogeneity of tissues. Lastly, the model was limited to three miRNAs and six metabolic targets, while NAFLD has a broader network of hepatocytes, Kupffer cells, stellate cells, endothelial cells, adipose tissue and the gut. Recent case-control studies have also demonstrated that miRNA expression is disease state dependent, and that panels containing miR-122, miR-192 and miR-33 family members may be more effective than single miRNAs [18]. Multicentre prospective cohorts, paired serum and liver measurements, single cell or spatial transcriptomics, and target-specific intervention studies in primary human systems should be used in future research.

The model highlights the potential integration of tissue miRNA profiling, enzyme-level measurements, histology, and functional perturbation in a single study, despite these limitations.

The most significant translational implication is not that a single miRNA is responsible for NAFLD, but that the suppression of SIRT1/PPARalpha by miR-34a, repression of CPT1A by miR-33a, and lack of miR-122-mediated inhibition of lipogenesis could all contribute to maintaining hepatic TG levels and driving progression to NASH.

Conclusion

In this simulated study, progressive NAFLD was defined as higher levels of miR-34a-5p and miR-33a-5p, lower levels of miR-122-5p, lower levels of SIRT1/PPARA/CPT1A and higher levels of FASN/SCD1/ACACA. A coordinated regulatory network, not a single miRNA mechanism, was supported by the combined miRNA correction that led to the greatest reduction in hepatocyte accumulation of triglycerides. These miRNAs are yet to be validated in prospective human cohorts and primary liver models before they can be used as diagnostic or therapeutic agents.

References

1. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohep atitis (NASH): a systematic review. Hepato logy. 2023;77(4):1335-1347. doi: 10.1097/HE P.0000000000000004. PMID: 36626630.
2. European Association for the Study of the Liver; European Association for the Study of Diabetes; European Association for the Study of Obesity. EASL-EASD-EASO Clinical Practice Guidelines on the management of metabolic dysfunction-associated steatotic liver disease (MASLD). J Hepatol. 2024;81(3):492-542. doi: 10.1016/j.jhep.2024.04.031. PMID: 38851997.
3. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. Nat Med. 2018;24(7):908-922. doi: 10.1038/s415 91-018-0104-9.
4. Agbu P, Carthew RW. MicroRNA-mediated regulation of glucose and lipid metabolism. Nat Rev Mol Cell Biol. 2021;22(6):425-438. doi: 10.1038/s41580-021-00354-w. PMID: 337 72227.
5. Hochreuter MY, Dall M, Treebak JT, Barres R. MicroRNAs in non-alcoholic fatty liver disease: progress and perspectives. Mol Metab. 2022;65:101581. doi: 10.1016/j.molmet.2022. 101581. PMID: 36028120.
6. Pirola CJ, Fernandez Gianotti T, Castano GO, Mallardi P, San Martino J, Mora Gonzalez Lopez Ledesma M, et al. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. Gut. 2015;64(5):800-812. doi: 10.1136/gutjnl-2014-306996. PMID: 24973316.
7. Miyaaki H, Ichikawa T, Kamo Y, Taura N, Honda T, Shibata H, et al. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. Liver Int. 2014;34(7):e302-e307. doi: 10.1111/liv.12429. PMID: 24313922.
8. Ding J, Li M, Wan X, Jin X, Chen S, Yu C, Li Y. Effect of miR-34a in regulating steatosis by targeting PPARalpha expression in nonalcoho lic fatty liver disease. Sci Rep. 2015;5:13729. doi: 10.1038/srep13729. PMID: 26330104.
9. Davalos A, Goedeke L, Smibert P, Ramirez CM, Warrier NP, Andreo U, et al. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. Proc Natl Acad Sci U S A. 2011;108(22):9232-9237. doi: 10.1073/pnas.1102281108. PMID: 21576 456.
10. Gerin I, Clerbaux LA, Haumont O, Lanthier N, Das AK, Burant CF, et al. Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. J Biol Chem. 2010;285(44):33652-33661. doi: 10.1074/jbc.M110.152090. PMID: 20732877.
11. Zhang M, Sun W, Zhou M, Tang Y. MicroRNA-27a regulates hepatic lipid metabolism and alleviates NAFLD via repressing FAS and SCD1. Sci Rep. 2017;7:14493. doi: 10.1038/s41598-017-15141-x.
12. Sun C, Huang F, Liu X, Xiao X, Yang M, Hu G, et al. miR-21 regulates triglyceride and cholesterol metabolism in non-alcoholic fatty liver disease by targeting HMGCR. Int J Mol Med. 2015;35(3):847-853. doi: 10.3892/ijm m.2015.2076. PMID: 25605429.
13. Wang L, Sun M, Cao Y, Ma L, Shen Y, Velikanova AA, et al. miR-34a regulates lipid metabolism by targeting SIRT1 in non-alcoholic fatty liver disease with iron overload. Arch Biochem Biophys. 2020;695:108642. doi: 10.1016/j.abb.2020.108642. PMID: 33098 868.
14. Fernandez-Tussy P, Cardelo MP, Zhang H, Sun J, Price NL, Boutagy NE, et al. miR-33 deletion in hepatocytes attenuates MASLD-MASH-HCC progression. JCI Insight. 2024;9(19):e168476. doi: 10.1172/jci.insigh t.168476. PMID: 39190492.
15. Chai C, Rivkin M, Berkovits L, Simerzin A, Zorde-Khvalevsky E, Rosenberg N, et al. Metabolic circuit involving free fatty acids,

microRNA 122, and triglyceride synthesis in liver and muscle tissues. Gastroenterology. 2017;153(5):1404-1415. doi: 10.1053/j.gastro. 2017.08.013. PMID: 28802563.
16. Becker PP, Rau M, Schmitt J, Malsch C, Hammer C, Bantel H, et al. Performance of serum microRNAs-122, -192 and -21 as biomarkers in patients with non-alcoholic steatohepatitis. PLoS One. 2015;10(11): e0142661. doi: 10.1371/journal.pone.0142661. PMID: 26565986.
17. Zhu Y, Tan JK, Wong SK, Goon JA. Therapeutic effects of microRNAs on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: a systematic review and meta-analysis. Int J Mol Sci. 2023;24(11):9168. doi: 10.3390/ijms2411916 8. PMID: 37298120.
18. Stoica VC, Apostol D, Diculescu MM, Gardan IP, Gardan DA, Maruntelu I, et al. Time for micro-RNAs in steatotic liver disease: a case-control study. Front Endocrinol (Lausanne). 2024;15:1349524. doi: 10.3389/ fendo.2024.1349524. PMID: 38549763.