Evaluation of dietary patterns influence on hepatic epigenetic gene modulation and dietary recommendations for the prevention of NAFLD to the general population

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Resumen

Introducción: NAFLD se ha convertido en un importante problema de salud pública y su prevalencia está aumentando a nivel mundial. Hacer hincapié en la compleja relación entre los patrones dietéticos y las modificaciones epigenéticas, como la metilación del ADN o la expresión de miARNs, puede ejercer un impacto positivo en la prevención y el manejo de los trastornos metabólicos, incluida la NAFLD dentro de los ODS 2030. Esta revisión tiene como objetivo evaluar la influencia de los patrones dietéticos en la modulación epigenética de genes hepáticos y proporcionar recomendaciones dietéticas para la prevención y el tratamiento de la NAFLD en la población general.

Métodos: Se aplicaron criterios integrales de selección y elegibilidad para identificar artículos relevantes que se centren en la interacción entre los regímenes dietéticos y los mecanismos epigenéticos, específicamente en relación con la función hepática en respuesta a la ingesta dietética o la suplementación. En el análisis final se incluyeron un total de 11 artículos, que abarcan estudios que evalúan los cambios epigenéticos en pacientes con NAFLD mediante modificaciones en los patrones dietéticos o suplementación de nutrientes.

Resultados: Los datos recopilados se organizaron según el tipo de estudios, categorizándolos en evaluaciones de cambios epigenéticos en pacientes con NAFLD mediante la modificación de patrones dietéticos o mediante la modificación/suplementación de diferentes tipos de nutrientes.

Conclusiones: El estudio subrayó la importancia de considerar intervenciones dietéticas en el tratamiento y la prevención de NAFLD, arrojando luz sobre el potencial de los patrones dietéticos para influir en la modulación epigenética del hígado. Esta revisión proporciona información valiosa sobre el papel de las intervenciones dietéticas en la NAFLD y ofrece recomendaciones para que la población general mitigue el riesgo de desarrollar este trastorno metabólico.

Palabras clave: patrón dietético, nutrición, epigenética, metilación del ADN, miARN, NAFLD.

Abstract

Introduction: NAFLD has emerged as a significant public health concern, with its prevalence increasing globally. Emphasizing the complex relationship between dietary patterns and epigenetic modifications such as DNA-methylation or miRNA expression can exert positive impact on preventing and managing metabolic disorders, including NAFLD within the 2030 SDG. This review aims to evaluate the influence of dietary

patterns on hepatic epigenetic gene modulation and provide dietary recommendations for the prevention and management of NAFLD in the general population.

Methods: A comprehensive screening and eligibility criteria were applied to identify relevant articles focusing on the interplay between dietary regimens and epigenetic mechanisms, specifically concerning hepatic function in response to dietary intake or supplementation. A total of 11 articles were included in the ultimate analysis, encompassing studies evaluating epigenetic changes in NAFLD patients through modifications in dietary patterns or nutrient supplementation.

Results: Collected data was organized based on the type of studies, categorizing them into evaluations of epigenetic changes in NAFLD patients by modifying dietary patterns or by the modification/supplementation of different types of nutrients.

Conclusions: The study underscored the importance of considering dietary interventions in the management and prevention of NAFLD, shedding light on the potential of dietary patterns to influence hepatic epigenetic gene modulation. This review provides valuable insights into the role of dietary interventions in NAFLD and offers recommendations for the general population to mitigate the risk of developing this metabolic disorder.

Key words: dietary pattern, nutrition, epigenetic, DNA-methylation, miRNA, NAFLD.

1. Introduction

1.1. Characteristics and prevalence of NAFLD

Nonalcoholic fatty liver disease (NAFLD) is a condition characterized by the accumulation of extra fat in the form of triglycerides (TG) in liver cells without excessive alcohol intake (1). It is considered a chronic liver disease that is becoming increasingly common and is associated with metabolic disorders such as insulin resistance (IR), obesity, type 2 diabetes mellitus (T2DM), and metabolic syndrome (MetS). NAFLD represents a range of conditions, starting with basic fat accumulation in the liver, progressing to non-alcoholic steatohepatitis (NASH), characterized by the presence of hepatocellular damage, inflammation and fibrosis. Liver fibrosis can potentially advance to liver, cirrhosis, and the development of hepatocellular carcinoma (HCC) (2) **(Figure1)**.

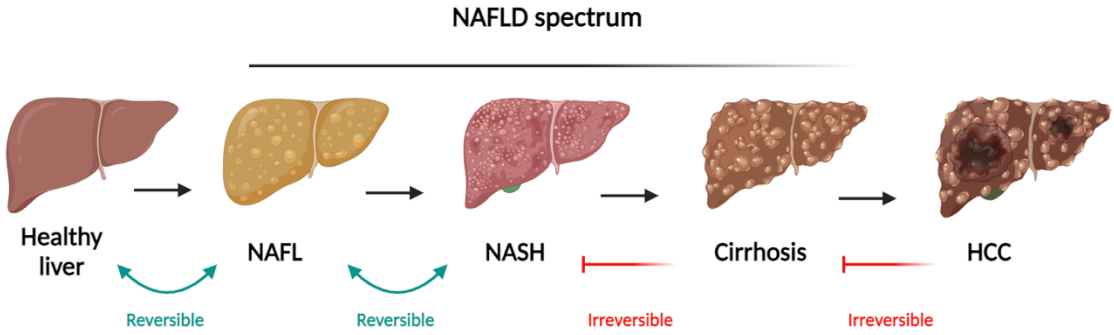

Figure 1: Schematic representation of NAFLD spectrum and the different stages evolving from a simple steatosis (NAFL), progressing to non-alcoholic steatohepatitis (NASH), both reversible, and cirrhosis and HCC as irreversible stages. Source: own elaboration adapted from Mungamuri SK et al. 2023 (79).

NAFLD is the most common cause of chronic disease around the world, both in adults and children. In recent years, the global prevalence of NAFLD has been continuing to increase at an alarming rate, much higher than previously anticipated, with an estimated prevalence of 25% worldwide, 26.9% in Europe (3) and 25,8% in Spain in people between 15- and 85-years age (4), which is expected to increase in the coming years. Current data from a recent meta-analysis has estimated that as many as 32% of the adult population is now affected by NAFLD, with the incidence and prevalence being higher among men compared to women. There is an even higher occurrence of 70% to 90% among individuals who have metabolic comorbidities such as obesity, T2DM, or MetS (5)(6). In fact, it has been described that NAFLD and MetS often coexist and

share common underlying factors such as obesity, IR and an unhealthy lifestyle, which includes a diet high in saturated fats, trans fats, and refined sugars (7). This diet, commonly consumed in western countries, is known as the western diet. As both MetS and NAFLD involve interactions of adipokines, cytokines, inflammatory factors and insulin resistance, some researchers have proposed that NAFLD can be regarded as a hepatic manifestation of MetS (8). Moreover, NAFLD is associated with an elevated risk of cardiovascular disease (CVD). Studies have demonstrated that individuals with NAFLD face substantial risks of developing conditions like hypertension, coronary heart disease, cardiomyopathy and cardiac arrhythmias, leading to increased cardiovascular morbidity and mortality (9, 10).

1.2. Pathogenesis and progression of NAFLD and environmental epigenetics

As numerous studies have elucidated, the underlying mechanisms for the development and progression of NAFLD are intricate and multifaceted. In light of this complexity, several contributing factors have been proposed, encompassing gut microbiota, metabolic disorders, genetic predisposition, epigenetic factors and lifestyle. Nowadays this is known as the “multiple-hit theory”, in which all the mentioned factors act together on genetically predisposed individuals to induce NAFLD. However, insulin resistance (IR) and liver fat accumulation seems to be the initial hits to the development of the disease (11) (**Figure 2**). How it has been described, the onset of fat accumulation begins as cytoplasmic lipid droplets within hepatocytes due to increased fatty acid uptake by the liver. Simultaneously, a reduction in fat transport via very-low-density lipoprotein (VLDL) is produced, along with mitochondrial dysfunction leading to an oxidative stress and an increase in de novo lipogenesis (DNL) (12). Collectively, these processes give rise to the reversible stage of simple steatosis. However, during the metabolism of these lipid droplets (LDs), specific metabolites and intermediate substances are generated, thereby influencing cellular equilibrium by creating a lipotoxic environment. This altered cellular environment induces stress within the cell, triggering inflammatory reactions. Consequently, the inflammatory response further accelerates fibrosis in the liver tissue, contributing for the advancement of the disease towards more severe stages such as NASH and HCC (13).

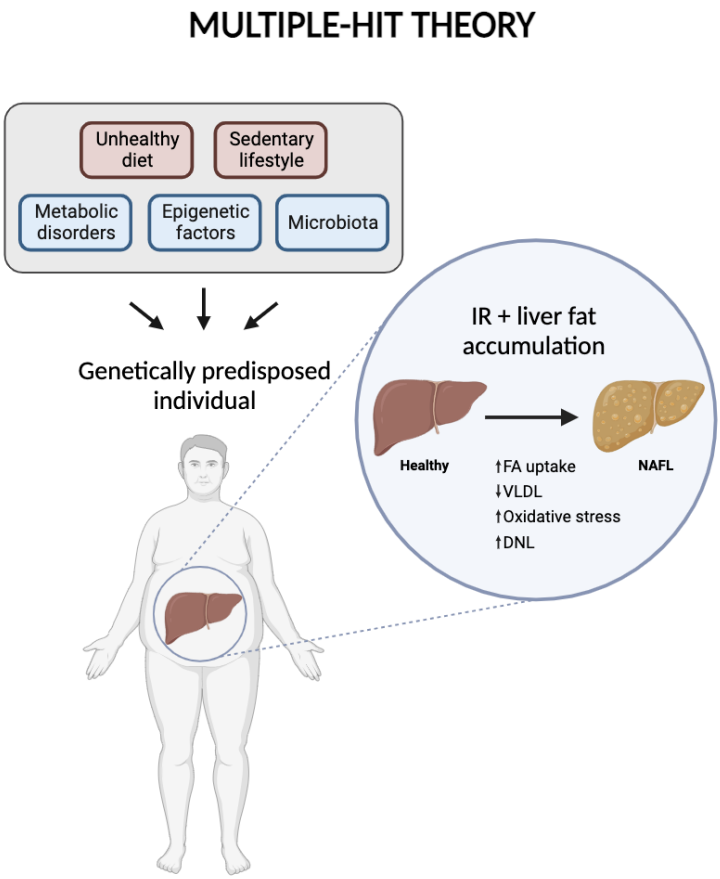

Figure 2: Diagram representing multiple-hit theory including the factors involved in the development and progression of NAFLD. Source: Own elaboration.

Although genetic factors are known to play a pivotal role in an individual's susceptibility to NAFLD, with gene variants including patatin like phospholipase domain containing 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), glucokinase regulator (GCKR), membrane-bound O-acyltransferase domain-containing 7 (MBOAT7), and hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13) (14); epigenetic modifications such as DNA methylation, histone alterations or miRNAs expression, can be influenced by lifestyle choices, such as dietary patterns. Epigenetics, in essence, refers to the reversible and heritable changes in gene expression without any alteration in the underlying nucleotide sequence, effectively serving as a mechanistic intermediary between genes and environmental factors (15). In this context, emerging research points to environmental factors, specifically dietary choices, as having a substantial impact on the epigenetic landscape of the liver. Notably, it is well-documented that the diet is responsible for approximately 15% of liver triglyceride (TG) accumulation

in NAFLD (16, 17). Thus, dietary components can lead to epigenetic modifications that can either exacerbate or mitigate the risk of NAFLD. These biochemical alterations involve changes to DNA and specific histones, mediated by various enzymes with critical roles in epigenetic gene regulation. The activity of these enzymes is known to be sensitive to dietary factors and cofactors generated by cellular intermediary metabolism. Consequently, these enzymes enable cells to adapt to changing conditions by selectively regulating the expression of specific genes, thereby establishing a direct link between diet, metabolism, gene expression and, ultimately, the overall impact on health, either beneficial or detrimental (18, 19).

1.2.1. DNA methylation

DNA methylation is the most studied epigenetic mechanism in NAFLD wherein a methyl (CH_3) group is covalently added to the DNA molecule from an S-adenosylmethionine (SAM), generated by one-carbon metabolism, to the fifth carbon of a cytosine residue. This modification results in the formation of 5-methylcytosine (5mC), typically occurring within cytosine-guanine dinucleotides-rich regions known as CpG islands, and it is catalyzed by the DNA-methyltransferases (DNMTs) family.
This methylation frequently alters gene functionality by affecting gene expression levels. In general, hypermethylation of CpG islands is associated with gene repression, whereas hypomethylation may activate gene transcription (18).

Different studies have linked differential DNA methylation to the pathogenesis of non-alcoholic fatty liver disease (NAFLD) in human liver biopsies (20-24). This aberrant DNA methylation has been shown to lead to inappropriate gene expression, contributing to the development and progression of the disease. For example, a human liver methylation study identified NAFLD-specific expression and DNA-methylation changes in key enzymes-encoding genes associated with lipid and glucose metabolism such as Igf1 (insulin-like growth factor 1), Igfbp2 (IGF binding protein 2), Acly (ATP citrate lyase), and PC (pyruvate carboxylase) (23).

Moreover, it has been shown how diet can be one of the main factors affecting DNA methylation. As mentioned before, S-adenosylmethionine availability is

crucial for the methylation process and it is synthesized from dietary methyl groups (i.e. methionine, choline, betaine, folate). In this regard, diets deficient in methyl donors can lead to either global hypomethylated DNA and changes in the methylation of specific genes (25).

1.2.2. Histone modifications

Histones are small globular DNA-binding proteins that play a pivotal role in the formation of chromatin. Within the nucleus, the DNA is wrapped around a histone octamer forming the nucleosome, the basic unit of chromatin. These proteins contain an N-terminal tail rich in positively charged amino acids that can undergo a large number of post-translational modifications, including acetylation and methylation. Such modifications play a crucial role in chromatin structure and gene expression by regulating effector molecule binding. For instance, histone acetyltransferases (HAT) catalyze the acetylation of the lysine (K) residues within histone tails resulting in chromatin opening, thereby permitting gene transcription machinery access. Conversely, histone deacetylases (HDAC) remove acetyl groups, leading to chromatin closure and gene repression restoration. On the other hand, histone methylation can act as a repressive or activating mark depending on the methyl group position on the K of histone 3 (H3). In this regard, a methylation in the K4 is an activating mark whereas methylation at K9 or K27 is repressive (26).

Histone modifications have been implicated in the development and progression of NAFLD by affecting the transcriptional activity of genes involved in lipid metabolism, inflammation and fibrosis. As previously mentioned, such modifications can be dinamically modulated by environmental factors without altering the underlying DNA sequence. For instance, Sirtuins (SIRTs), a subgroup of HDACs crucial in regulating cellular energy metabolism, have been described to be involved in NAFLD progression. These proteins can interact with histones and other proteins and remove acetyl groups thereby repressing gene expression. Moreover, it has been shown that the activation of SIRT1 results in a decrease of lipids and triglycerides accumulation in the liver and thus ameliorating NAFLD (27). In this sense, environmental factors such as caloric restriction (CR)

or the consumption of resveratrol, a natural polyphenol found in many plants, have been linked to the activation of SIRT1 (28).

1.2.3. microRNAs

MicroRNAs (miRNAs) are single-stranded, short RNA transcripts of 18-25 nucleotides long that can inhibit translation or induce degradation of target mRNAs through complementary base pairing, thus regulating gene expression at the post-transcriptional level (29). Despite accounting for the 1-5% of the human genome, these non-coding transcripts exhibit a key role in regulating mechanisms associated with metabolic homeostasis. Notably, miRNAs are implicated as significant mediators in metabolic disorders such as obesity, MetS, T2D and NAFLD (30).

Numerous miRNAs have been described as important gene regulators controlling fatty acid metabolism and cholesterol homeostasis in the liver. These include miR-27b, miR-33, miR-34a, miR-122 and miR-223 (31). Specifically, miR-122 is the most abundant miRNA in the liver and plays a key role in liver homeostasis hepatocyte differentiation and lipid metabolism. Functionally, miR-122 has been demonstrated to promote excess lipid production and TG secretion by suppressing SIRT1 expression (32). For instance, Carlos J Pirola, et al., found that circulating levels of miR-122, miR-192 and miR-375 were upregulated and were positively correlated with disease severity using a screening strategy of global serum miRNA profiling in 47 NAFLD patients (33). In this context, many studies have also reported how diet can influence the expression of miRNAs. The quality of food intake and several dietary components, such as fatty acids, vitamin D and E, dietary fiber or selenium (FA) can affect miRNA expression profile or function (34, 35), thus impacting health status. In addition, increased circulating miR-122 expression has been associated with the severity of NAFLD and stands out as a potential non-invasive biomarker and therapeutic target of this liver disease (36).

1.3. Influence of dietary patterns in metabolic diseases

Nutrition stands as one of the key factors contributing to metabolic disease progression. It is well established that an unhealthy dietary pattern, typically characterized by excessive caloric intake, an abundance of sugars and saturated

fats and a deficiency in fiber, polyunsaturated fatty acids and specific micronutrients, significantly influences the onset and advancement of NAFLD and other associated metabolic conditions (37). On the other hand, having better diet quality such as Mediterranean diet (MedDiet), vegetarian or DASH (Dietary Approaches to Stop Hypertension), may exert a positive impact in preventing and managing metabolic disorders (38). For example, in a cross-sectional study comprising 328 participants aged between 55 and 75 years, diagnosed with MetS and enrolled in the PREDIMED-Plus trial, adherence to the Mediterranean diet exhibited an inverse correlation with NAFLD. This adherence was linked to notable enhancements in serum lipid profiles, insulin resistance (IR), and liver enzymes (39).

Regarding specific nutrients, other findings have suggested that vitamin E or resveratrol may improve liver function among NAFLD patients by reducing oxidative stress due to its antioxidative properties (40, 41).

In line with all of these observations, diverse dietary interventions and nutrients evaluation, have been demonstrated to exert epigenetic modifications by modifying food intake and composition, thus significantly impacting the epigenome. For instance, caloric restriction (CR) and dietary restriction (DR) have been found to induce alterations in DNA methylation and histone modifications, thereby influencing gene expression and contributing to metabolic well-being. Likewise, intermittent fasting (IF) and periodic fasting (PF) have the capacity to modulate epigenetic regulation in various tissues, including adipose tissues, liver, and pancreas. Additionally, other dietary approaches such as the ketogenic diet (KD) or Mediterranean diet have been shown to elicit epigenetic effects (15, 42). Conversely, a Western dietary pattern, typified by containing high levels of processed food, red meat, high-fat dairy and refined grains, has the potential to impact gene expression at the transcriptional level, predisposing individuals to NAFLD (15).

In the context of the increasing prevalence it is important to address the prevention and management of NAFLD. By comprehending the complex relationship between dietary patterns and the epigenetic modifications driven in NAFLD pathogenesis and using dietary interventions to modify the hepatic epigenetic environment, it will open an opportunity to significantly impact on the

incidence and progression of NAFLD. Moreover, the efforts in this regard are closely linked to the global health agenda enclosed within the framework of the 2030 Sustainable Development Goals (SDG). Achieving these goals requires a concerted effort to reduce the burden of non-communicable diseases, including NAFLD, and to improve overall health and well-being on a global scale. By aligning the research and recommendations with the 2030 SDG, this review aims to contribute to the global mission of fostering healthier populations and reducing the prevalence of NAFLD. In this regard, here will be explored the intricate interplay between dietary patterns, epigenetics, and NAFLD, with a focus on formulating evidence-based dietary recommendations for both prevention and management.

2. Objectives

General objective:

The general aim of this literature review is to comprehensively examine existing studies investigating the impact of dietary patterns on the epigenetic regulation of the liver function and to formulate precise dietary recommendations for the prevention of Non-Alcoholic Fatty Liver Disease (NAFLD) in the general population, within the framework of the 2030 Sustainable Development Goals (SDG) of mitigating the burden of non-communicable diseases to ensure healthy lives and promote well-being for all.

Specific objectives:

1. To identify the dietary patterns that have been studied in relation to liver epigenetic regulation and their effects on the development or prevention of NAFLD.
2. To analyze the epigenetic mechanisms involved in the pathogenesis of NAFLD and how dietary patterns can influence them.
3. To provide specific dietary recommendations for the prevention of NAFLD in the general population based on the available evidence, exploring the health implications of dietary interventions, including their potential to reduce the burden of other non-communicable diseases, thus aligning with the 2030 Sustainable Development Goals.

Research question

The research question has been formulated using the PICO format identifying the following elements:

- P (Population/Patient/Problem): population with NAFLD
- I (Intervention): dietary patterns (i.e.: western diet)
- C (Comparison): Mediterranean diet or another dietary pattern (i.e.: low-fat diet, caloric restriction, etc.)
- O (Outcome): Hepatic epigenetic modulation and dietary recommendations for the prevention of NAFLD.

Therefore, the PICO in the research question would be: In the population with NAFLD (P), how does a dietary pattern, as western diet (I), compared to another dietary pattern or Mediterranean diet (C) affect hepatic epigenetic modulation and what are the dietary recommendations for the prevention of NAFLD (O)?

3. Methodology

3.1. Data sources and search strategy

For the purpose of this comprehensive review, a systematic literature review was conducted in accordance with PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocol) guidelines. The primary aim was to synthesize available evidence in a rigorous and replicable manner, addressing the specific research question: "How does diet influence hepatic epigenetic modulation when comparing different dietary patterns?"

The search process encompassed two electronic databases: PubMed and Web of Science. Relevant articles were initially assessed based on their titles and/or abstracts. The search strategy employed a combination of Medical Subject Heading (MeSH) terms and non-Medical Subject Heading terms that were pertinent to the subject matter. Key terms included: "epigen*," "hepatic epigen*," "DNA methylation," "histone modification," "epigenetic changes," "diet," "dietary pattern," "food pattern," "eating pattern," "dietary habit," "eating habit," "dietary behavior, “nutritional programming”, “epigenetic diet”, “nutritional epigenetics”, "fatty liver," "NAFLD," "Non-alcoholic Fatty Liver Disease," "Steatosis of Liver," "steatohepatitis," "steatosis", “NASH” “metS” Additionally, Boolean operators

such as "AND" and "OR" were employed to interrelate terms and refine the search results.

3.2. Study selection, inclusion and exclusion criteria

The **inclusion criteria** for this review were defined as follows:

- Published full-text articles from the preceding ten years, specifically from 2013 to 2023, both included, to ensure the incorporation of the most current research findings.
- Studies conducted in human subjects, thereby focusing on research that directly pertains to the human context.
- Articles published in either the English or Spanish language, facilitating access to a broader spectrum of research literature.
- Studies evaluating the epigenetics changes influenced by certain diets and its relation with NAFLD.

The **exclusion criteria** were established as follows:

- Studies published prior 2013
- Studies conducted on animals, in vitro models or other non-human models
- Articles published in languages other than English or Spanish
- Publications that were not full-text accessible form or having insufficient information
- Review articles
- Articles that did not directly address the research question related to dietary patterns and hepatic epigenetic modulation in the context of NAFLD.

In October and November of 2023, an initial research was undertaken to identify potentially relevant studies within the PubMed and Web of Science databases. Following the application of the aforementioned selection criteria, 38 articles were retrieved from PubMed, while 147 were obtained from Web of Science. Consequently, a total of 172 articles were identified for screening, since 13 were duplicated, then were excluded.

3.3. Screening and eligibility criteria

In adherence to the established inclusion criteria, the initial set of articles sought to evaluate the impacts of dietary regimens on epigenetic mechanisms. Within this context, a number of articles were excluded due to their lack of consideration for the interplay between epigenetics and dietary patterns (61 articles excluded). Subsequently, articles that failed to center their focus on the influence of hepatic epigenetic mechanisms concerning liver function in response to dietary intake or supplementation were also excluded (n= 79). Finally, in a subsequent phase, articles that yielded inconclusive results or those that adopted distinct mechanistic approaches unrelated to epigenetics in the context of NAFLD, were subject to exclusion (n= 24). As a result, the ultimate analysis encompassed a total of 11 articles.

A flow diagram showing the selection process is depicted in **Figure 3**.

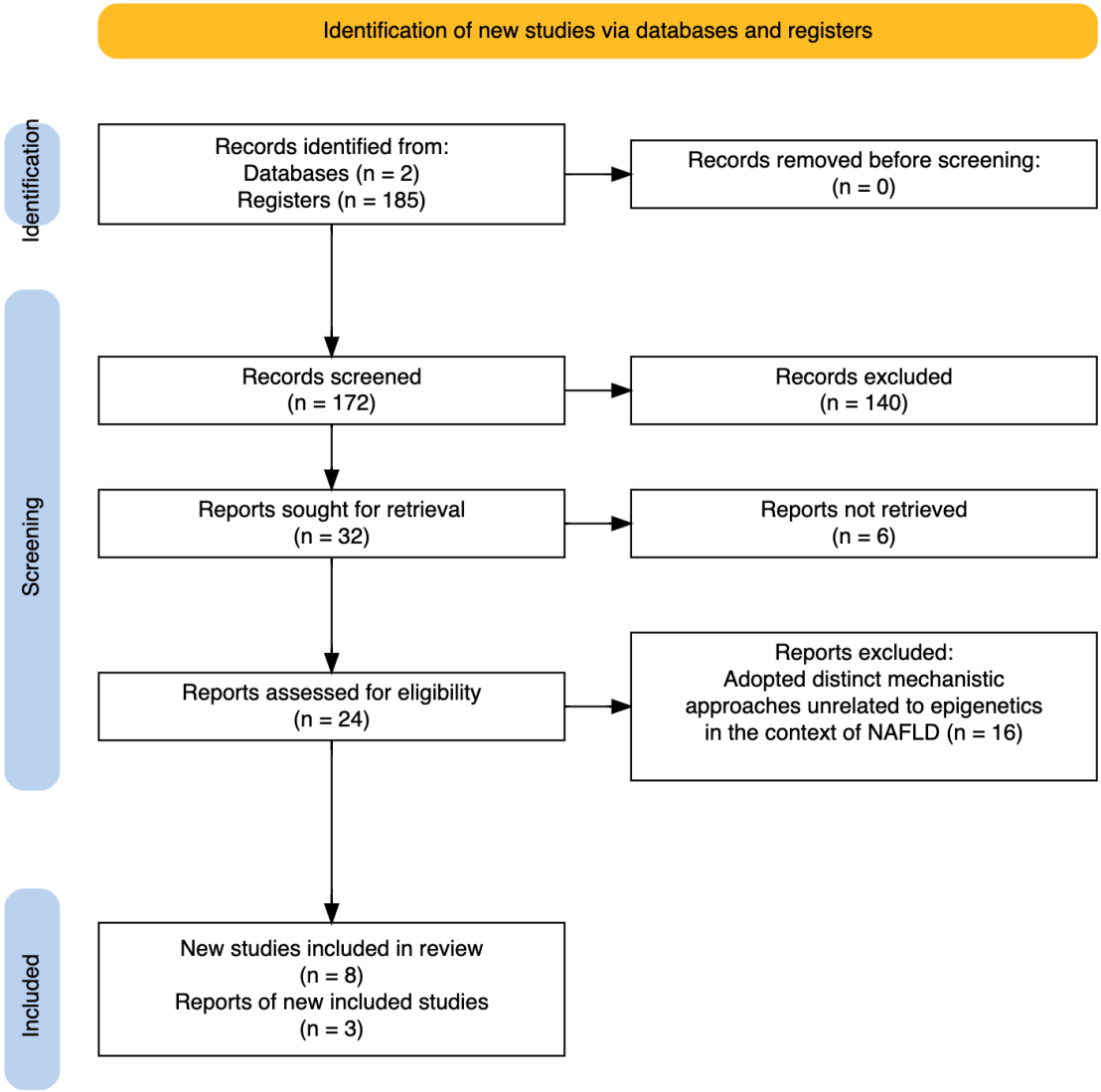

Figure 3: PRISMA flow diagram. Summarizing the selection of papers included in this review.

4. Results

The acquired information was organized based on the type of studies evaluating epigenetic changes in NAFLD patients by modifying dietary patterns **(Table 1)** or by the modification/supplementation of different type of nutrients **(Table 2)**.

Table 1: Epigenetic effect of diet intervention in NAFL/MetS subjects

	Diet intervention						
	Study reference	**Type of study**	**Intervention**	**Source of biological sample**	**Epigenetic mechanism**	**Epigenetic signature**	**Outcomes**
Low fat / low carb - MED diets	43	Sub-study of the CENTRAL randomized controlled trial	120 participants from CENTRAL trial were conducted to an 18-month regimen of either LF or MED/LC diets with 28 g/day of provided walnuts, with or without PA (PA+/PA-)	Blood cells	DNA-methylation	2095 different CpGs located among 41 genes were analyzed. Significant correlations for 5 CpG methylation in steatosis related genes predicted NAFLD: - AC074286.1 (cg15996499) - CRACR2A (cg04614981) - A2MP1 (cg14335324) - FARP1 (cg21126338) - FARP1 (cg00071727)	After 18-months significant reductions of IHF%, weight and WC were observed, with no differences between diet groups LF and MED/LC
LF: low-fat; MED/LC: Mediterranean-low carbohydrate diet; PA: physical activity; IHF: Intrahepatic fat; WC: waist circumference							
MED diet	44	Clinical trial	18 participants with medium-to-high-grade steatosis trained to follow the Mediterranean diet including fiber supplements	Blood cells	DNA-methylation and Histone modification	Histone modifications: Reduced levels of H3 acetylation in monocytes and lymphocytes. DNA-methylation:	Improvement of the anthropometric, biochemical and liver steatosis status. Genome-wide methylation patterns changed towards

						11485 CpG sites hypermethylated; 142 hypomethylated	the pattern for healthy blood. Methylation changes in blood separated liver biopsies from NAFLD patients according to the fibrosis grade.
H3: Histone 3							
Low-carb diet	45	Randomized controlled trial	50-65 years old participants with NAFLD and NASH were randomly assigned to four groups: Ex, LCD, exercise, ELCD, and No groups. 6-month intervention.	Blood cells	DNA-methylation	Differentially methylated CpGs before and after intervention: 100118 (Ex), 268582 (LCD), 270663 (ELCD) and 259249 (No) CpG After exclusion of No group: 430 (Ex), 2807 (LCD) and 1648 (ELCD) CpGs; 404 (Ex), 2661 (LCD) and 1575 (ELCD) genes.	Lower methylation levels pre-intervention than post-intervention. LCD and ELCD intervention on human NAFLD can induce DNA methylation alterations at critical genes in blood, e.g., GAB2 (validated in liver and adipose of NASH mice model)
Ex: exercise; LCD: low carbohydrate diet; ELCD: exercise plus low carbohydrate diet; No: No intervention							
RESMENA hypocaloric -MED diet	46	Sub-study of the Randomized-prospective RESMENA study	40 participants with MetS from the RESMENA study were evaluated before and after 8-wk hypocaloric-MED diet	Blood cells	miRNA	Expression of miR-155-3p was decreased in WBC; Let-7b was upregulated after treatment.	RESMENA diet improved most anthropometric and biochemical features. Low consumption of lipids and saturated fat were associated with higher expression of let-7b after the nutritional intervention

MetS: metabolic syndrome; WBC: white blood cells

RESMENA hypocaloric -MED diet and AHA diet	47	Sub-study of the Randomized-prospective RESMENA study	24 patients with MetS features from the RESMENA study were selected from two dietary groups: RESMENA or AHA diets.	Blood cells	miRNA	49 miRNAs differentially expressed (35 from AHA and 14 from MD diet). miR-410, miR637, miR-214 and miR-190 with the most significant expression changes.	After 8w intervention: Significant changes in anthropometric parameters (BW, BMI, WS and waist/hip ratio). Improvement of metabolic profile.

BW: body weight; BMI: body mass index; WS: waist circumference

Table 2: Epigenetic effect of nutrient modification/supplementation in NAFL/MetS

Nutrient evaluation							
Study reference	**Type of study**	**Participants**	**Nutrient evaluation**	**Source of biological sample**	**Epigenetic mechanism**	**Epigenetic signature**	**Outcomes**
48	Case-control	18 control participants and 47 patients with NAFLD	Methyl-donor nutrients	Liver biopsies	DNA-methylation	Global DNA-hypomethylation in patients with NAFLD	Significantly lower levels of global DNA methylation in patients with NAFLD than control participants; Global DNA methylation level decreased with the aggravation of hepatic inflammation grade and disease progression. Severity of NAFLD correlated positively with the serum homocysteine level
49	Case-control	35 diabetic and 60 nondiabetic obese subjects	Vitamin 12 and folate levels	Liver biopsies with or without signs of NAFLD	DNA-methylation	236 CpG sites (94%) hypomethylated and 15 sites (6%) hypermethylated in subjects with T2D	Significant sites in diabetic subjects were hypomethylated. Significantly reduced circulating folate levels in the T2D compared with the nondiabetic subjects were observed

T2D: type 2 diabetes

50	Double Blind Randomized Placebo Controlled Clinical Trial (pilot)	24 patients with NAFLD	n-3 PUFA capsules contained fish oil (503 mg of DHA + 102 mg of EPA) or placebo capsules (750 mg of oleic acid)	Blood	miR-122	No changes in miR-122 circulating levels	n-3 PUFA were incorporated in erythrocytes after six months of fish oil supplementary intake. n-3 PUFA were effective in reducing ALP and liver fibrosis without altering the expression of circulating miR-122 in individuals with NAFLD
PUFA: polyunsaturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid: ALP: alkaline phosphatase							
51	Randomized, double-blind, placebo-controlled clinical trial	52 patients with NAFLD	4 g/day supplementation of fish oil (2100 mg EPA and 924 mg DHA) or placebo (oleic acid) over a 6-month period	Blood	miRNA-122	Ongoing research	
52	Controlled intervention trial	12 healthy subjects and 12 patients with MetS	Acute high- and low-polyphenols EVOO intake (55 ml after 12h of fasting) - single dose	Blood	miRNAs	Supressed miRNAs: miR-146b-5p; miR-19a-3p; miR-181b-5p; miR-107; miR-769-5p; miR-192-5p Upregulated miRNAs: miR-23b-3p; miR-519b-3p	Acute EVOO intake led to significant changes in gene and miRNA expression in PBMCs of both healthy subjects and patients with MetS (more significant in healthy) High-polyphenols EVOO led to more significant changes in gene and

							miRNA expression compared to low-polyphenols EVOO
EVOO: extra virgin olive oil; PBMCs: peripheral blood mononuclear cells							
53	Randomized placebo-controlled trial	82 patients with MetS	TRM group received 400 mg capsules (δ-tocotrienol 250 mg; Resveratrol 150 mg) and placebo received (cellulose 400 mg capsule) twice daily for 24 weeks.	Blood	miRNAs	TRM supplementation resulted in a significant upregulation of miR-130b and miR-221, as well as downregulation of miR-122	TRM supplementation improved MetS parameters, including central obesity, impaired fasting glucose, dyslipidemia, and hypertension.
TRM: δ-tocotrienol and resveratrol mixture							

4.1. Epigenetic evaluation of Low fat, Mediterranean and Low carb diet interventions in NAFL subjects

Several studies in the last few years have highlighted the relationship between diet modifications and its effect on the metabolic rewiring towards a healthier state. Nevertheless, only a few studies investigated its association with epigenetic changes due to biological sample limitations. In this regard, some investigations determined how both Mediterranean and low-fat diets may have a positive impact in NAFLD bearing patients by modifying the global DNA-methylation and improving the anthropometric, biochemical and liver steatosis status. Thus, Yaskolka Meir A. and collaborators investigated the effects of lifestyle interventions on epigenetic signatures of liver fat in a randomized controlled trial where 120 participants from the CENTRAL trial were conducted to an 18-month regimen of either Low-fat (LF) or Mediterranean-Low carb (MED/LC) diets with 28 g/day of provided walnuts, with or without physical activity (PA). At baseline, an association between liver fat deposition levels and higher triglycerides levels, with the methylation of FARP1 gene (cg0007172 CpG site), was found. Using a NAFLD prediction model by specific CpG methylation within the AC074286.1, CRACR2A, A2MP1, and FARP1 genes, methylation of these CpG predicted lower fat depositions in the liver. After the 18-month diet and physical activity intervention, significant reductions of intrahepatic fat (IHF), weight and waist circumference (WC) were observed. In this sense, different lifestyle interventions resulted in significant differential methylation effects in some specific CpG sites. Whereas the low-fat diet was associated with an increased methylation in the A2MP1 gene (cg14335324 CpG site), the MED/LC diet correlated with lower methylation at the same (43).

An additional interventional study was conducted to investigate the influence of epigenetic mechanisms on the regulation of gene expression during the treatment of NAFLD. The study recruited 18 participants with medium-to-high grade of steatosis and were subsequently instructed to follow the Mediterranean diet along with fiber supplements. The adherence to the diet was evaluated and physiological parameters were measured at three different timepoints: baseline (prior dietary intervention) and after 30 and 60 days of diet. Analysis of the blood cells methylomes indicated an unhealthy dietary pattern of the participants before

the intervention. Notably, after diet change, a decrease in liver steatosis was observed along with statistically significant changes in the methylome of blood cells, where 11.627 CpG sites displayed significant alterations in the methylation levels. Specifically, the majority of the methylation changes observed were gains with 11.485 hypermethylated CpG sites and 142 CpG hypomethylated sites. Of particular interest was the comparison of median global methylation levels across the three timepoints with those found in healthy blood samples. Remarkably, the methylation levels at the second and third timepoints closely resembled those observed in healthy blood samples. Furthermore, the study also compared the relative acetylation levels of histone H3 in lymphocytes and monocytes between the different timepoints before and after the intervention where a decrease in the relative acetylation was observed. According to the literature, deacetylation of H3 on lysines 9 and 14 has been inversely correlated with DNA methylation and associated with actively transcribed loci signifying a notable shift towards healthier methylation/histone modification profiles following the dietary intervention. Moreover, the study found that methylation levels at CpG sites undergoing diet-related methylation changes in blood cells stratified liver biopsies from NAFLD patients according to fibrosis grade (44).

On the other hand, Na Wu and collaborators conducted a randomized controlled trial in order to investigate the effect of the exercise and Low-carb diet intervention in human NAFLD and non-alcoholic steatohepatitis (NASH) by exploring the genome-wide methylation changes of specific candidate genes (e.g. GAB2) in blood after 6-month intervention. The participants were individuals of 50-65 years old with NAFLD and NASH randomly assigned to four groups: exercise (Ex), Low-carbohydrate diet (LCD), exercise and Low-carb diet (ELCD) and a group with No intervention (No). After comparing the DNA-methylation levels in each group, differentially methylated CpGs were identified between baseline and follow-up time points after 6 months of intervention. Specifically, 100118 (Ex), 268582 (LCD), 270663 (ELCD) and 259249 (No) CpGs. After exclusion of No group: 430 (Ex), 2807 (LCD), 1648 (ELCD) CpGs and 404 (Ex), 2661 (LCD) and 1575 (ELCD) genes were found to be differentially methylated. Notably, the study identified GAB2 gene, which is involved in inflammatory and fibrotic pathways in the liver, as a candidate gene affected by exercise and diet interventions, and

associated with improvements in NAFLD and NASH conditions when silenced. The methylated changes of GAB2 were also validated in liver and adipose tissue of NASH bearing mice after diet and exercise interventions (45).

Additionally, two correlated studies were conducted to assess the impact of a dietary strategy for weight loss on the expression of specific microRNAs (miRNAs) in white blood cells (WBC) among participants diagnosed with Metabolic Syndrome (MetS). Notably, NAFLD is considered the hepatic manifestation of MetS. In this context, J. L. Marques-Rocha et al. investigated the effects of a Mediterranean-based nutritional intervention (RESMENA dietary pattern) on anthropometric measurements, biochemical markers, and the expression of selected inflammation-related genes and miRNAs in WBC. The study enrolled 40 participants diagnosed with MetS from the RESMENA-S study, a controlled prospective intervention lasting 8 weeks. The RESMENA-S study aimed to reduce MetS features through a 30% energy restriction over 6 months, incorporating components of the Mediterranean diet. The results indicated improvements in most anthropometric and biochemical features post-intervention, with significant reductions in body weight (BW), waist circumference (WC), fat mass, total cholesterol (TC), triacylglycerols, glucose, and insulin levels. Notably, miR-155-3p decreased significantly, while the tumor suppressor miRNA let-7b exhibited substantial upregulation. Although no correlation was found between these miRNAs and proinflammatory gene expression, they were statistically associated with changes in diet quality, as assessed by the Healthy Eating Index (HEI). Low consumption of lipids and saturated fats correlated with higher let-7b expression following the nutritional intervention (46).

Conversely, P.S. Marsetti et al., part of the same research group, investigated the miRNA expression pattern in WBC with two weight-loss dietary strategies in a subset of 24 MetS patients from the RESMENA study. The 8-week intervention included two energy-restricted diets: the RESMENA diet, characterized by moderately high protein, and the AHA diet based on American Heart Association recommendations. Both diets imposed a 30% caloric restriction based on total energy needs. Both dietary strategies in the RESMENA study improved the metabolic profile and anthropometric parameters, such as BW, BMI, WC, and waist/hip ratio. No significant differences were observed in anthropometric and

biochemical parameters when comparing the two dietary strategies. A total of 49 miRNAs (35 from AHA and 14 from MD diet) exhibited differential expression after the 8-week weight-loss intervention. Notably, miR-410 and miR-637 were upregulated, while miR-214 and miR-190 were downregulated, with the most significant expression changes observed with the AHA dietary pattern intervention. The expression of several miRNAs was significantly associated with anthropometric and biochemical parameters (47).

4.2. Evaluation of specific nutrients levels/interventions and its epigenetic effect in NAFL subjects

The liver is a major site of both lipid and one-carbon metabolism and these two pathways interact by contributing to tissue homeostasis. Alterations in one-carbon metabolism are strongly associated with hepatic lipid metabolism. In this regard, investigations have highlighted the importance of these nutrients in liver function revealing that a reduction of dietary one-carbon sources can induce NAFLD or NASH. A case-control study conducted by Lai et al., aimed to assess hepatic global DNA methylation and serum one-carbon metabolite concentrations in patients with NAFLD examining the potential associations between these parameters with liver histology. The study involved the evaluation of liver biopsies from 18 control participants and 47 patients with NAFLD. The findings revealed significantly lower levels of global DNA methylation in livers from individuals with NAFLD compared to those from control participants. Moreover, the decrease in global methylation levels correlated with the aggravation of hepatic inflammation grade and disease progression, being participants with borderline NASH the most remarkably affected group with the lowest levels compared to control individuals. Additionally, the authors identified a positive correlation of global DNA methylation level with serum homocysteine concentrations and a lower betaine/choline ratio. An increasing trend in the homocysteine level was observed with steatosis. Participants with borderline NASH had a significantly higher homocysteine level and a lower betaine/choline ratio than controls (48).

In another case-control study, liver biopsies obtained from 35 individuals diagnosed with type 2 diabetes (T2D) and 60 biopsies from non-diabetic control subjects were subjected to analysis to explore the genome-wide pattern of DNA

methylation in the liver and its correlation with circulating folate levels. All participants were enrolled in the Kuopio Obesity Surgery Study, and histological assessments of all biopsies confirmed normal liver status as well as varying degrees of steatosis and non-alcoholic steatohepatitis (NASH) within both groups. The genome-wide analysis uncovered 251 distinct CpG sites exhibiting differential methylation in the livers of individuals with T2D. The majority of these significant CpG sites (236; 94%) demonstrated hypomethylation, while a smaller fraction (15; 6%) exhibited hypermethylation. These findings were correlated with vitamin B12 and folate levels revealing non-significant differences in vitamin B12 levels between T2D and non-diabetic subjects. However, subjects with T2D displayed significantly reduced erythrocyte folate levels compared with non-diabetic subjects. Despite the study did not focus specifically on fatty liver, but in the epigenetic alterations of subjects with T2D, the study showed that a significant proportion of both diabetic (77%) and non-diabetic (55%) subjects had simple steatosis or NASH (49).

Considering other nutrients, a preliminary investigation was conducted in NAFLD patients to evaluate the impact of fish-oil omega-3 polyunsaturated fatty acids (PUFA) supplementation. This randomized, double-blind, placebo-controlled clinical study aimed to investigate whether supplementation could effectively reduce miR-122 circulating levels in NAFLD patients. Over a six-months period, 13 patients were administered capsules containing n-3 PUFA derived from fish oil plus vitamin E, gelatin, purified water and glycerin as a humectant. Each capsule contained approximately 503 mg of docosahexaenoic acid (DHA) and 102 mg of eicosapentaenoic acid (EPA). Conversely, the placebo control group consisted of 11 patients who received olive oil containing capsules with about 750 mg of oleic acid, in addition to smaller amounts of palmitic acid and linoleic acid. Throughout the trial, each individual consumed three capsules per day. However, there was no observed alteration in the expression of circulating miR-122 in both groups, the results showed that omega-3 PUFA were incorporated in erythrocytes after six months of fish oil supplementary intake, and that n-3 PUFA were effective in reducing alkaline phosphatase (ALP) and liver fibrosis in individuals with NAFLD (50).

Building up on this, the same research group recently published a study protocol for a new randomized, double-blind and placebo-controlled clinical trial to assess the effect of fish oil supplementation on the concentration of miRNA-122, FGF-21 and liver fibrosis in NAFLD patients. This trial mirrors the previous study but increasing patients and adjusting dosage. Specifically, 52 patients will be recruited, with 26 allocated in each group during a 6-month period. The n-3 PUFA group will receive 4 g/day supplementation of fish oil (containing 2100 mg EPA and 924 mg DHA) while the placebo group will be administered refined olive oil containing 750 mg of oleic acid. The primary outcome of the study will be the ability of EPA-rich fish oil, compared to placebo, to reduce miR-122 concentration in NAFLD patients after 6 months. Secondary, will be the ability of EPA-rich fish oil, compared with placebo, to reduce the concentration of FGF-21 and improve the degrees of hepatic fibrosis and steatosis estimated and liver biomarkers (51).

Continuing with miRNAs regulated by nutrient intake, Simona D'Amore et al. employed a controlled trial intervention to evaluate the effects of acute extra virgin olive oil (EVOO) intake on gene and miRNA expression in peripheral blood mononuclear cells (PBMCs). The study included 12 healthy subjects and 12 patients with MetS who were divided in two groups: high-polyphenols and low-polyphenols EVOO intake. Independently of the group, subjects consumed 50 ml (44g) of EVOO in a single dose, at 8 am and after 12 h fasting. During the following 4 h after EVOO administration, the participants abstained from food, drinks and exercise. After overnight fasting and 4 h after EVOO administration, samples for serum biochemistry and whole blood for PBMC isolation were collected. The study identified specific changes in miRNA expression following the acute intake of EVOO. Specifically, EVOO rich in polyphenols, led to significant changes in the gene expression profiling of key genes related to lipid metabolism, inflammation, proliferation, and cancer; and the expression of specific miRNAs in PBMCs in both healthy and MS patients, but particularly in healthy subjects. The specific changes found in miRNA expression were 6 suppressed: 1) miR-146b-5p; 2) the oncogenic miR-19a-3p; 3) miR-181b-5p; 4) miR-107; 5) miR-769-5p; 6) miR-192-5p and 2 upregulated: 1) the anti-inflammatory miR-23b-3p and 2) the tumor-suppressor miR-519b-3p. Intriguingly

most of these miRNAs (with the exception of miR-19a-3p) were not significantly modulated in the PBMCs of patients with MetS (52).

To assess the impact of a 24-week supplementation with a mixture of δ-tocotrienol and resveratrol (TRM) on the relative expression of microRNAs (miRNAs), Fatima et al. conducted a randomized, placebo-controlled trial involving 82 patients diagnosed with Metabolic Syndrome (MetS) within the age range of 18 to 80 years. The participants were equally and randomly assigned to two groups: the TRM group and the placebo group. The TRM group received 400 mg capsules containing δ-tocotrienol (250 mg) and resveratrol (150 mg), while the placebo group received capsules containing 400 mg of cellulose. Both groups administered their respective supplements twice daily for the duration of 24 weeks. Following the intervention, the TRM supplementation demonstrated a significant upregulation of two specific miRNAs, miRNA-130b-5p and miRNA-221-5p. These miRNAs are associated with central obesity, inflammation, and insulin resistance, respectively. Furthermore, the results indicated a downregulation of miRNA-122, suggesting a correlation with the improvement of dyslipidemia. Conversely, no significant differences in miRNA expression were observed in the placebo group. Upon completion of the intervention, daily TRM supplementation exhibited improvements in biochemical parameters linked to MetS, including central obesity, impaired fasting glucose, dyslipidemia, and hypertension, within the treatment group. Additionally, a reduction in inflammatory, oxidative stress, and insulin resistance biomarkers was noted, attributed to the modulatory effect of MetS-related miRNAs (53).

5. Discussion

The present study aimed to comprehensively examine the current available evidence on the impact of dietary patterns on the epigenetic regulation of liver function. The majority of the studies included, focused on the changes of the DNA methylation patterns of both liver biopsies or peripheral mononuclear blood cells (PMBCs) and the expression of specific miRNAs of patients with NAFLD or diagnosed with MetS, after diet interventions or specific nutrients supplementation. The findings point out that specific dietary interventions by modifying food intake and composition, have the potential to influence the

epigenetic regulation of liver function in the context of NAFLD. As it has been described in the literature, excessive caloric intake and a deficiency in certain nutrients such as fiber, polyunsaturated fatty acids and specific micronutrients including vitamins and antioxidants, impact the gene expression favoring the progression of many metabolic diseases including NAFLD. Conversely better diet quality such as Mediterranean, vegetarian or DASH diets may exert positive changes in the metabolic rewiring being suitable for the prevention or managing of the metabolic disorders (11, 70). The interventions included in this review were all mainly based in the Mediterranean diet with caloric restriction or low carbohydrate dietary pattern. Regarding the epigenetic effect, lower levels of global DNA methylation were observed in livers from individuals with NAFLD compared to control participants and the decrease in global methylation levels correlated with the aggravation of hepatic inflammation grade and disease progression. After diet change, higher methylation levels were observed indicating an effect in the regulation of gene expression. In fact, all diet interventions improved most anthropometric and biochemical features measured compared to baseline highlighting the impact of dietary one-carbon sources including choline, folate, methionine and betaine, in DNA-methylation and the importance of these nutrients on liver homeostasis and its implications in the development of NAFL or NASH. Regarding this, one-carbon metabolism may be linked with NAFLD by the mechanism of VLDL synthesis and the export of TG from the liver. Choline plays a key role in packaging and transportation of TG into VLDL, thus aberrant levels of one-carbon metabolites may impact in reducing VLDL secretion and a subsequent accumulation of fat in the liver (54). In line with this, one of the two case-control studies evaluating methyl-donor metabolites levels in patients with NALFD found a positive correlation between severity of NAFLD and serum homocysteine level and the other one pointed a significantly reduced circulating folate levels in the T2D participants compared with the nondiabetic subjects. Folate and homocysteine are interconnected through one-carbon metabolism pathway where a deficiency of folate can lead to an accumulation of homocysteine which cannot be remethylated to methionine to be used as the final methyl donor in the majority of methyltransferase reactions thereby, reducing the methylation potential and potentially impacting DNA methylation (55).

This study also recapitulated other dietary interventional approaches that were followed to understand the potential of different miRNAs to modulate gene expression/metabolic pathways in a context of metabolic disorder NAFLD-related. In the last decade, miRNAs have emerged as key regulators of physiological processes, including inflammation, proliferation, glucose and lipid metabolism (56) and the analysis of its expression in the study of human health and disease is rapidly evolving. In line with this, the two studies evaluating weight-loss dietary interventions (RESMENA and AHA) found both dietary patterns to be useful for losing weight, improving biochemical features of MetS and to induce significant miRNAs expression changes in WBC. Specifically, the AHA dietary pattern intervention showed specific weight-loss miRNA expression related to the diet (miR-190, miR-214, miR-410, mirR-637) while others correlated with biochemical and anthropometric features (miR-587, miR-2115, miR-410, miR-96). Of note, the AHA is a healthy eating plan framed within the American Heart Association guidelines designed to reduce the risk of heart disease and improve health by changing dietary habits. The recommendations include specific guidelines for intake of fruits, vegetables, whole grains, fish, saturated fat, dietary cholesterol, added sugar and salt (57).

Additionally, specific nutrients and dietary supplements has been evaluated in this review as potential regulators of miRNAs in patients with NAFLD. Despite EVOO and fish oil Omega-3 PUFA-rich did not show significant miRNA changes in patients after intervention, interesting results regarding liver function improvement were collected. Fish-oil supplementation ameliorated liver damage after 6-month intervention and a single acute dose of 55 ml EVOO rich in polyphenols was able to transiently ameliorate insulin sensitivity in healthy subjects but not in MetS-bearing subjects. Nevertheless, the latter can be attribute to the short-term 24-hours intervention and a longer period administration could potentially reveal significant results in MetS patients. In line with that, both fatty acids omega-3 (EPA and DHA) and EVOO have been associated before with the reduction of liver fat, inflammation and improvement of insulin resistance and different supplementation with polyunsaturated fatty acids omega-3 may contribute to an improvement of several biochemical features (60) as well as the modulation of the expression of specific miRNAs (61). Indeed,

since Omega-3 fatty acids are precursors of eicosanoids, signaling molecules playing key roles in the regulation of immunity and inflammation, these fatty acids have anti-inflammatory effects and can regulate hepatic lipid composition and improve IR (62). Similar effects are linked to the phytochemicals present in EVOO whose properties may have potential benefits to treat against steatosis (59, 63). Thus, considering these beneficial traits and the scarce evidence in the epigenetical approach, further research addressing n-3 PUFA, EVOO and miRNAs relationship may be necessary to provide additional insights.

Conversely, supplementation with a mixture of δ-tocotrienol and resveratrol (TRM) significantly regulated miRNAs associated with central obesity, inflammation and insulin resistance (miRNA-130b, miRNA-221) and improved dyslipidemia by downregulating miRNA-122, overall resulting in an improvement of MetS features. Moreover, these miRNAs were linked with a reduction in adipocytokines and oxidative stress biomarkers, making them good candidates to be tracked in response to treatment of MetS. Both, delta-tocotrienol (a subtype of vitamin E) and resveratrol has been studied for its antioxidant properties which are beneficial for liver function, nevertheless the mechanisms underlying their beneficial effects have not been fully elucidated yet and further research is still needed for their clinical validation (64, 65).

In summary, the scientific evidence available to date suggests that dietary patterns and specific dietary compounds can modulate epigenetic mechanisms capable of regulating liver function in health and disease. However, these studies have some limitations due to several reasons: (i) the epigenetic research applied to metabolic diseases is an emerging field that still have some limitations in the understanding of the molecular mechanisms taking place in the interplay between diet and epigenetic changes. These modifications are complex and depend on a variety of interindividual parameters, such as sex, gender, ethnicity, genetic variants or metabolic tissue. (ii) Other limitations rely on the heterogeneity in the study design. The variability in the methodology, the analysis technique, the target population and the time of intervention introduce some challenges in the interpretation of the results. In this regard, there is a specific need for more longitudinal studies in different racial cohorts. Tracking individuals over longer-periods time could yield valuable insights into diet-induced epigenetic

modifications. (iii) Finally, human samples to assess DNA-methylation or histone modifications, represents a technical limitation due to the invasive procedure. Liver biopsies have been established as gold standard samples to study liver disease, nevertheless, as shown here, recent advancements have introduced new methods for quantifying DNA-methylation from peripheral blood, however, how diet affects the blood methylome is not fully understood yet. Overcoming these technical challenges is critical for advancing our understanding of the intricate relationship between diet, epigenetics, and liver function (66).

5.1. Evidence-based dietary recommendations for the prevention and management of NAFLD to the general population

To further complement the presented analysis, this review aimed to compilate evidence-based dietary recommendations for the prevention and management of NAFLD taking into consideration the epigenetic modulation of liver function influenced by the intake of various dietary components. As previously noted, dietary modifications have been shown to be effective in controlling metabolic diseases. Given the current lack of consensus on a specific pharmacological approach to treat and improve fatty liver diseases, lifestyle modifications focused on weight loss, diet and exercise, became the first line of therapy (67).

Insulin resistance, inflammation and oxidative stress represent the main underlying features in the development and progression of fatty liver disease. In light of this, dietary patterns capable of ameliorating these mechanistic aspects will be potentially good candidates to treat and prevent NAFLD (68).

Although further clinical trials are still needed to optimize the best dietary treatment approach, most current guidelines recommend low calorie diets as a weight loss strategy. Targeting a 7–10% of body weight loss is highlighted as crucial for the management of NAFLD (69). Alternatively, a plant-based Mediterranean-related diet is widely recommended as the most effective approach to treat and prevent NAFLD due to its high antioxidants and anti-inflammatory properties. The latter is characterized by high consumption of fruits, vegetables, whole grains, legumes, nuts and seeds, and low intake of sugar (70). Moreover, the Mediterranean diet can also include moderate consumption of fish, reduced intake of red meat and It is highly recommended the exclusion of

processed food, and food and beverages high in added fructose (71). It is worthy to note that MedDiet or its plant-based variant would also be an optimal dietary pattern to provide one-carbon metabolites including folate and related B vitamin as it has been described in this review. These compounds play a crucial role in supplying methyl groups for methylation reactions thereby contributing to the maintenance of DNA-methylation patterns to prevent or mitigate NAFLD development and progression (72).

Building upon the aforementioned findings, the following evidence-based key dietary recommendations for the prevention and management of NAFLD are summarized as follows:

1. **Adopt a diet mainly plant-based and minimize saturated fats to reduce inflammation:**
   Consume a diet that is mainly composed of plant-based food minimizing animal-derived nutrient sources rich in saturated fats, particularly red meat.
2. **High-fiber intake to stimulate gut microbiota and regulate fasting glucose:**
   Increase consumption of high-fiber meals by incorporating a variety of fruits, vegetables, legumes and whole grains into daily meals.
3. **Increase PUFAs and MUFAs fatty acids to optimize lipid profile:**
   Increase the consumption of polyunsaturated fatty acids (PUFA), specifically omega-3 fatty acids by including oily fish such as salmon, sardines or trout in the diet and add monounsaturated fatty acids (MUFAs) through the intake of extra-virgin olive oil, nuts and seeds.
4. **Limit consumption of highly processed food, soft drinks and added fructose and salt to avoid and mitigate fatty liver accumulation.**
5. **Avoid alcohol or keep it consumption below the risk threshold (30g men, 20g women).**

6. Applicability and new lines of research

Current findings addressing the influence of dietary patterns in the regulation of the epigenetic mechanisms in NAFLD, may have significant applications in diagnosis and treatment of metabolic diseases. A growing body of evidence is unveiling new perspectives in the implementation of different strategies to

address common non-communicable diseases through the understanding of the underlying molecular mechanisms and the development of population-appropriate interventions that may help to identify disease-associated environments and biomarkers suitable to prevent, detect and treat (73, 74).

Promoting healthy eating habits among the population is a key strategy for the prevention and management of several metabolic disorders including NAFLD. As pointed before, the epigenetic field faces the challenge of implementing new prospective and multi-ethnic interventional studies to unravel the intricate mechanisms bridging gene regulation and environmental factors such as diet, together with its metabolic outcomes over the time among diverse individuals. In line with this, emerging lines of research may have the scientific imperative to reflect the diversity of the population based on sex, gender and ethnicity in order to contribute to more effective and equitable health care practices.

Coming up with the purpose to promote new insights in the aforementioned approaches, here is proposed a diet-intervention study.

1. **Aim of the study:**

   Analyze the effect of a low-calorie plant-based Mediterranean diet in DNA-methylation pattern and lipid metabolism related miRNAs expression in PBMCs of a multi-ethnic cohort with NAFLD.

2. **Type of study and design:**

   The study will consist in a prospective cohort study targeting individuals with clinical NAFLD at baseline which will be treated with a single dietary intervention consisting in a plant-based Mediterranean diet over a 24-month period follow-up, in order to evaluate long-term impact of the dietary intervention.

   - Population: adult women and men, between 40-60 years age including equal representative groups of different ethnicities (multi-ethnic cohort).
   - Variables:
     - Independent: adoption of a low-calorie plant-based Mediterranean diet adapted to the target population and following the frequency of consumption established in the dietary guidelines of The Public Health Agency of Catalonia (75).

- Dependent: DNA-methylation pattern and lipid metabolism-related miRNAs of peripheral blood mononuclear cells before, meanwhile and after the intervention.

3. **Data collection:**

- Clinical assessment: anthropometric measures, biochemical markers, liver fat content and liver inflammation markers.
- Dietary surveys to evaluate the establishment and adherence to the diet
- Blood sample collection and DNA and RNA sampling extraction for epigenetic profiling and liver inflammation markers.

4. **Analysis**

Three follow-up timepoints will be set: baseline, at 12-month and after 24-month intervention. All data will be collected and compared between timepoints and between groups using specific statistical analysis based on the nature of the data

- Liver fat content will be analyzed as IHF% through magnetic resonance imaging (non-invasive).
- DNA-methylation changes (CpGs candidates) of the PBMCs will be analyzed based on GWAS catalog hits for NAFLD traits.
- Microarray analysis for miRNA profile expression.

Besides reducing health inequalities among diverse global communities by incorporating different ethnic groups and implementing a plant-based Mediterranean diet intervention, the present proposal has the potential to make a substantial contribution to ongoing global efforts in the field of sustainable development. Specifically, it addresses health, nutrition and environmental sustainability by addressing the promotion of health and well-being through a sustainable dietary pattern and a more equitable understanding of the epigenetic effects of dietary interventions in different population groups.

7. Conclusions

The present review aimed to analyze the influence of dietary patterns on hepatic gene modulation in individuals with NAFLD through different epigenetic modifications. Although environmental epigenetics is an emerging field whose evidence is scarce to fully understand and elucidate the mechanisms involved in gene-environment interaction, the existing studies to date are capable of

establishing at least a link between changes in dietary habits towards healthier patterns and modifications in DNA methylation patterns and differential expression of specific miRNAs associated with metabolic pathways involved in the development and progression of NAFLD. Specifically, dietary patterns based on the Mediterranean diet show relevant benefits that must be addressed in greater depth to understand the underlying mechanisms of action and their potential clinical applications.

However, there are several limitations to which these studies are exposed, thus difficult the interpretation of the data necessary to establish new preventive, diagnostic and therapeutic tools. In this regard, a greater number of studies are necessary that homogenize and reduce heterogeneity in the design of interventions, with long-term approaches and including diverse population groups that minimize the inequalities of scientific findings and allow a more precise and equitable approach in terms of prevention and treatment.

8. References

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