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REVIEW

Circulating Fibroblast Growth Factor-21 in Patients with Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis

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Abstract

Background The pathogenesis of nonalcoholic fatty liver disease (NAFLD) is multifactorial. Fibroblast growth factor-21 (FGF-21) has been proposed to be associated with NAFLD, but data on its circulating levels in patients with NAFLD are to date conflicting.

Aims The synthesis and comparison of data on circulating FGF-21 between patients with NAFLD and controls without NAFLD.

Methods A comprehensive literature search was conducted in PubMed, Cochrane Library and Scopus, complemented by hand-searching. Forty-four observational studies with overall 15,563 participants (9548 controls and 6015 NAFLD patients) were included in the study.

Results Circulating FGF-21 was higher in patients with NAFLD compared to controls (standardized mean difference [SMD]: 0.61; 95% confidence interval [CI]: 0.44, 0.77; $p < 0.00001$). Subgroup analysis showed higher FGF-21 levels in patients with nonalcoholic steatohepatitis (NASH) compared to controls (SMD: 1.30; 95% CI: 0.35, 2.24; $p = 0.007$), but not between hepatic steatosis and controls, or hepatic steatosis and NASH. Furthermore, the findings were more robust in the subgroup of studies with NASH-related cirrhosis than those without them ($p = 0.0004$). Sensitivity analysis further supported the findings. Heterogeneity was high in all comparisons. Meta-regression analyses showed that FGF-21 SMD between NAFLD patients and controls was positively associated with the rate of patients with type 2 diabetes mellitus per study, and this could explain 49.2% of the heterogeneity among studies.

Conclusions Circulating FGF-21 levels were higher in NAFLD patients than controls, which may be possibly attributed to those with advanced disease (NASH and related cirrhosis).

Lay summary Circulating fibroblast growth factor-21 levels were higher in patients with nonalcoholic fatty liver disease compared to controls. This is primarily attributed to the higher levels observed in patients with advanced disease (steatohepatitis and related cirrhosis).

Keywords Fibroblast growth factor-21 · Metabolic dysfunction-associated steatohepatitis · Metabolic dysfunction-associated steatotic liver disease · Nonalcoholic fatty liver disease · Nonalcoholic steatohepatitis

Abbreviations

AASLD	American Association for the Study of Liver Diseases
ALT	Alanine Aminotransferase
APASL	Asian-Pacific Association for the Study of the Liver
AST	Aspartate Aminotransferase
BMI	Body Mass Index

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CI	Confidence Interval
CT	Computed Tomography
EASD	European Association for the Study of Diabetes
EASL	European Association for the Study of the Liver
EASO	European Association for the Study of Obesity
ELISA	Enzyme-Linked Immunosorbent Assay
FGF-21	Fibroblast growth factor-21
GGT	Gamma-Glutamyl Transferase
HOMA-IR	Homeostasis Model Assessment-Insulin Resistance
IR	Insulin Resistance
MAFLD	Metabolic (dysfunction-)Associated Fatty Liver Disease
MASLD	Metabolic dysfunction-Associated Steatotic Liver Disease
MASH	Metabolic dysfunction-Associated Steatohepatitis
MeSH	Medical Subject Headings
MOOSE	Meta-analysis of Observational Studies in Epidemiology
MRE	Magnetic Resonance Elastography
MRI	Magnetic Resonance Imaging
MRI-PDFF	Magnetic Resonance Imaging-proton density fat fraction
MRS	Magnetic Resonance Spectroscopy
NA	Not Available
NAFL	Nonalcoholic Fatty Liver
NAFLD	Nonalcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Nonalcoholic Steatohepatitis
NOS	Newcastle-Ottawa Scale
PECO	Population, Exposure, Comparison, Outcome
PEI	Proximity Extension Immunoassay
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized Controlled Trial
SD	Standard Deviation
SMD	Standardized Mean Difference
T2DM	Type 2 Diabetes Mellitus

Introduction

Nonalcoholic fatty liver disease (NAFLD) is a highly prevalent chronic liver disease affecting at least one third of the global population [1]. It was first described in 1980 and now is the second major cause leading to hepatic transplantation in the US, due to the end-stage liver disease and hepatocellular carcinoma [2]. The frequency of NAFLD increases in parallel with obesity, type 2 diabetes mellitus (T2DM) and other components of the metabolic syndrome. Histologically, NAFLD ranges from hepatic steatosis that may progress to nonalcoholic steatohepatitis (NASH), hepatic fibrosis and liver cirrhosis [3]. Regarding its pathogenesis, NAFLD is a multifactorial disease. Various genetic variants, environmental factors and epigenetic modifications contribute to its pathogenesis [4]. Dysregulation in metabolic homeostasis, insulin resistance (IR) and imbalance of various cytokines, adipokines, hepatokines and other mediators also play central role to the pathogenesis of NAFLD [5].

In 2019, a new nomenclature, metabolic (dysfunction)-associated fatty liver disease (MAFLD), was proposed in order to emphasize metabolic dysregulation in NAFLD [6]. In 2023, a multi-society Delphi consensus proposed the term metabolic dysfunction-associated steatotic liver disease (MASLD) in order to replace the stigmatizing term "fatty" [7]. According to the latter consensus, NASH was recommended to be renamed as metabolic dysfunction-associated steatohepatitis (MASH) [7]. Apart from changes in the nomenclature, MAFLD and MASLD emerged with new diagnostic criteria, which may reflect better the pathophysiology of the disease [6, 7].

Fibroblast growth factor-21 (FGF-21) is an hepatokine with pleiotropic effects on lipid and carbohydrates metabolism [8]. FGF-21 was shown to decrease IR by augmenting the uptake of glucose in muscle; in the liver, FGF-21 decreases intrahepatic lipid accumulation by increasing fatty acid β-oxidation and decreasing de novo lipogenesis [8]. The potentially pleiotropic effects of FGF-21 on various organs, based mainly on data from experimental studies, are depicted in Fig. 1 [8–11]. In clinical terms, higher FGF-21 levels were shown in patients with NAFLD than in individuals without NAFLD in most, but not all studies; similarly, data on circulating FGF-21 levels between patients with simple hepatic steatosis and steatohepatitis are inconclusive. The clarification of FGF-21 levels when the disease advances seems to be important in the light of FGF-21 analogs that are currently under investigation in patients with NASH; higher FGF-21 levels may target to limit the progression of the disease, but may also indicate a state of FGF-21 resistance or insensitivity, which may complicate the effect of FGF-21 analogs [8].

Taking all the above into account, the main aim of this systematic review and meta-analysis was to quantitatively synthesize and compare existing data regarding circulating FGF-21 levels in patients with NAFLD and controls, i.e., individuals without NAFLD or other liver diseases. A secondary aim was to compare FGF-21 levels between patients with hepatic steatosis and NASH. In this systematic review and meta-analysis, we kept the terminology of NAFLD rather than the newer ones of MAFLD or MASLD, because

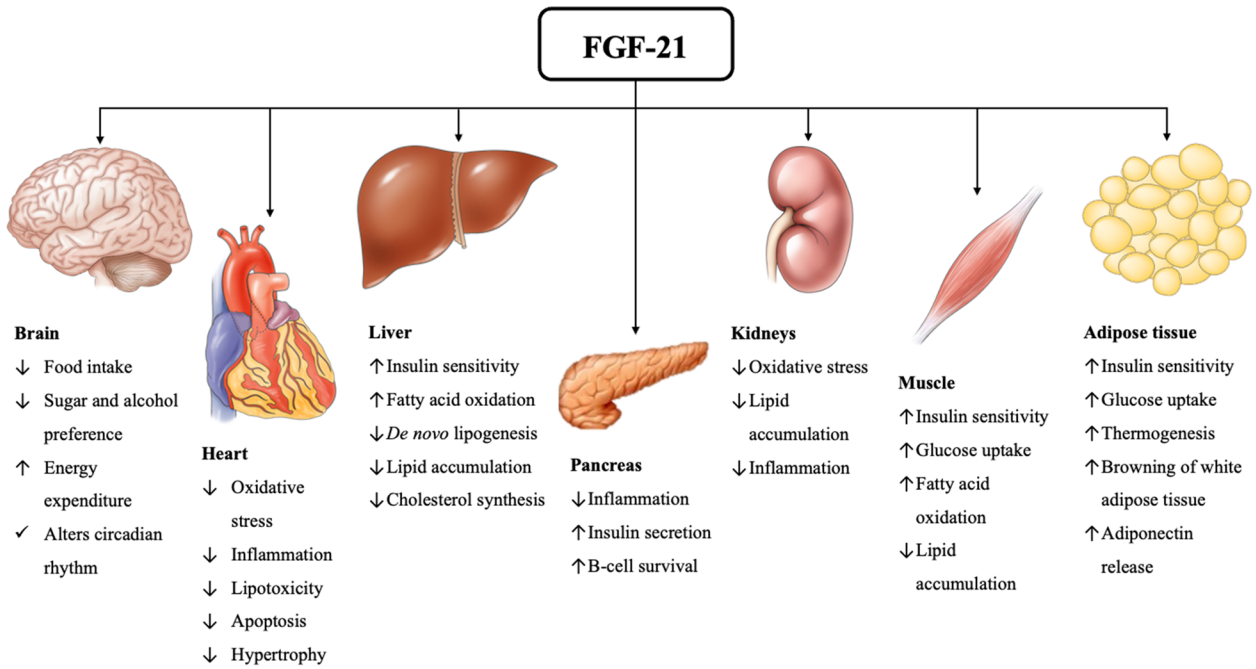

Fig. 1 Potential metabolic effects of FGF-21 based mainly on experimental data [8–11]. FGF-21 is a hepatokine suggesting exerting pleiotropic effects on systemic metabolism by targeting a variety of organs through multiple endocrine pathways. These actions position FGF-21 as a potentially key metabolic regulator and a promising therapeutic target in obesity, T2DM and other metabolic diseases, including NAFLD. Abbreviations: FGF-21, fibroblast growth factor-21; NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus

the diagnosis of the diseases was based on the diagnostic criteria of NAFLD in the majority of the included studies.

Methods

Literature Search

This systematic review and meta-analysis was conducted based on a pre-registered protocol in the international prospective register of systematic reviews (PROSPERO) registry (CRD42024537642). The reporting guidelines of the meta-analysis of observational studies in epidemiology (MOOSE) was also followed for the preparation of this manuscript [12].

First, the following research question was developed based on the Population, Exposure, Comparison, Outcome PECO model; “Are FGF-21 levels higher in patients with NAFLD compared with individuals without NAFLD?”. Three databases, i.e., PubMed, the Cochrane Library and Scopus, were searched using a comprehensive search query. In order to build the search query, we combined Medical Subject Headings (MeSH) terms with non-MeSH terms and connected them with Boolean operators. The following query was used for the searching in the PubMed: ((“Non-alcoholic Fatty Liver Disease”[Mesh]) OR NAFLD OR NAFL OR NASH OR (non-alcoholic fatty liver disease) OR (nonalcoholic fatty liver disease) OR (non alcoholic fatty liver disease) OR (non-alcoholic steatohepatitis) OR (non-alcoholic steatohepatitis) OR (non alcoholic steatohepatitis) OR (non-alcoholic fatty liver) OR (nonalcoholic fatty liver) OR (non alcoholic fatty liver) OR MAFLD OR (metabolic dysfunction-associated fatty liver disease) OR (metabolic dysfunction associated fatty liver disease) OR (metabolic associated fatty liver disease) OR MASLD OR (metabolic dysfunction-associated steatotic liver disease) OR MASH OR (metabolic dysfunction associated steatohepatitis)) AND ((“fibroblast growth factor 21”[Supplementary Concept]) OR FGF-21 OR FGF21 OR (FGF 21) OR (Fibroblast Growth Factor 21) OR (Fibroblast Growth Factor-21)). There were no language or publication date restrictions. Small adjustments in the search string were made based on the specific requirements of each database. The search was performed independently by two investigators (IF and MO), starting in May 10, 2024 up to January 10, 2025.

The literature search was further expanded by manually search in reference lists of all articles included in the meta-analysis, as well as the abstract books of three major gastroenterology and hepatology conferences (the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver and the Asian-Pacific Association for the Study of the Liver) between 2014 and

2024. Furthermore, automatic alerts were set up in the PubMed ("My NCBI"), the Cochrane Library ("Saved Search Alert") and Scopus ("Alerts"), to retrieve any relevant articles published after the initial search until the submission of this manuscript.

Inclusion and Exclusion Criteria

This is a meta-analysis of observational studies. Therefore, cross-sectional, case–control and cohort studies providing data on circulating FGF-21 levels for individuals with and without NAFLD were eligible. Inclusion criteria were: (i) studies including patients diagnosed with NAFLD using: hepatic histology after liver biopsy, abdominal ultrasonography, transient elastography, computed tomography (CT), magnetic resonance imaging (MRI), MRI-proton density fat fraction (MRI-PDFF), magnetic resonance spectroscopy (MRS), magnetic resonance elastography (MRE), other relevant imaging techniques, or noninvasive markers of hepatic steatosis and/or fibrosis; studies following the nomenclature of MAFLD or MASLD were also eligible; (ii) studies reporting quantitative measurement of circulating FGF-21 levels in the plasma or serum.

Exclusion criteria were: (i) studies with patients with other liver disease (e.g., alcoholic fatty liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver injury) or with mixed liver diseases (patients with NAFLD with other concomitant liver diseases); (ii) overlap of patients in different studies; (iii) studies with patients with NAFLD-associated hepatocellular carcinoma; (iv) studies for which additional data (e.g., FGF-21 levels per group) were absolutely necessary, but the corresponding author(s) did not provide them; (v) other types of studies, including experimental studies, reviews, opinions, editorials, commentaries, guidelines, hypotheses, book chapters, case reports or letters-to-the-editor; though, research letters-to-the-editor, i.e., containing original data, were considered.

When a study included more than one control group, the control group with the greatest similarity to NAFLD group was selected for the statistical analysis; in this regard, priority was given to body mass index (BMI), i.e., when a lean and an obese control group were included, we selected the obese group, which is usually more similar to the BMI of NAFLD patients.

Data Extraction

Two reviewers (IF and MO) independently performed the data extraction. Excel (Microsoft, Redmond, WA, USA) and EndNote (Clarivate Analytics, Philadelphia, PA, USA) were implemented in this procedure. The duplicates were removed and, subsequently, the two reviewers screened the titles and abstracts of all identified articles (stage of screening), excluding those that did not meet the prespecified inclusion and exclusion criteria. Next, the two reviewers independently evaluated the full-text articles, to select the appropriate ones for inclusion in the systematic review (stage of eligibility). For the automatic translation of articles published in non-English languages, Google Translate (https://translate.google.gr) was used as a supplementary tool; communication with the corresponding authors was also conducted to confirm the validity of the translation of essential data of their articles. Any disagreements between the reviewers were discussed with the involvement of the supervisor (SAP), until agreement was reached. The supervisor (SAP) guided the reviewers during the whole process and resolved any conflicts.

The next step was the extraction of relevant parameters from the included studies: (i) general features of the study (first author's surname, country of origin, publication year, study design); (ii) specific populations (e.g., children/adolescent population, morbidly obese population subjected to bariatric surgery, inclusion of patients with liver cirrhosis); (iii) main characteristics per group (number of patients or controls, age, sex, BMI, waist circumference, rate of T2DM); (iv) method of diagnosis of liver disease (NAFLD/MAFLD/MASLD) and method for the measurement of FGF-21 levels; (v) histological system used for the grading and staging of the liver disease; (vi) IR calculated with homeostasis model assessment-IR (HOMA-IR) in mean ± standard deviation; (vii) FGF-21 and liver function tests [alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT)] in mean ± SD.

In case essential data were missing (e.g., FGF-21 levels, number of patients per group), they were asked from the respective corresponding authors. In studies that essential data were available only in graphs, we used the online tool Graphreader (https://graphreader.com) to retrieve their numerical values. When needed, standard formulas were used through the online tool Meta-Converter (https://meta-converter.com) to merge study groups and to transform numerical data expressed in other forms to mean and SD. When essential data of importance were not available and the corresponding author(s) did not provide them, the study was excluded.

Quality Assessment

Newcastle–Ottawa scale (NOS; Ottawa Hospital Research Institute, Ottawa, ON, Canada) was the tool that was independently used by the two reviewers (IF and MO) for the quality assessment of included studies. The validity of each study was assessed within three domains: (i) the selection of the groups; (ii) the comparability of the groups; (iii) the assessment of the outcome. The scale of NOS ranges from 0 (very poor quality) to 9 (the highest quality). Any disagreement

between the reviewers was resolved after discussion with the involvement of the supervisor (SAP).

Outcomes

The standardized mean difference (SMD) with 95% Confidence Interval (95% CI) of circulating FGF-21 between patients with NAFLD and controls was the main outcome of this meta-analysis. Additionally, based on data retrieved from studies with histological grading and staging of NAFLD, secondary outcomes were the SMD of circulating FGF-21 between: (i) patients with simple hepatic steatosis (nonalcoholic fatty liver; NAFL) and controls; (ii) patients with NASH and controls, (iii) patients with NAFL and NASH.

Statistical Analysis

The softwares Revman (Review Manager, Version 5.4, Cochrane Collaboration, London, UK) and R (R Studio, the R Foundation for statistical computing, Vienna, Austria) were use for the statistical analysis. The level of statistical significance was set at $P < 0.05$ in all tests (two-sided). I^2 test was used for the evaluation of heterogeneity among studies. Given the expected heterogeneity among studies, the analysis was based on a random-effects inverse-variance model. Egger's test and visual assessment of the funnel plot asymmetry were used for the evaluation of the probability of publication. Subgroup analyses were conducted to compare circulating FGF-21 in: (i) patients with NAFL vs. controls; (ii) patients with NASH vs. controls; (iii) patients with NAFL vs. patients with NASH; (iv) studies with vs. without biopsy-proven NAFLD; (v) studies with NASH-related cirrhosis vs. studies without NASH-related cirrhosis (fibrosis stage F4). Furthermore, sensitivity analyses were conducted after excluding studies with: (i) children/adolescent populations; (ii) morbidly obese populations undergoing bariatric surgery; (iii) NOS score < 7; (iv) outliers of FGF-21 SMD; (v) the use of the definition of MAFLD for the diagnosis of the disease. Finally, meta-regression analysis with a random-effect model was conducted, to regress FGF-21 SMD between patients with NAFLD and controls for the following potential confounders: (i) age; (ii) sex; (iii) T2DM; (iv) BMI; (v) waist circumference; (vi) HOMA-IR.

Results

Literature Search

The initial search led to the retrieval of 1544 articles: 935 from Scopus, 484 from Pubmed and 125 from Cochrane library. In addition, 150 articles were retrieved via automatic alerts set in the above databases and 310 articles through handsearching in the abstract books of the three international conferences mentioned above. The process of identification, screening, eligibility and of the final selection of studies to be included in the systematic review and meta-analysis is fully presented in Fig. 2, which follows the reporting guidelines of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA), and is briefly reported hereby. After the removal of 830 duplicates, 1086 articles were excluded at the stage of screening. Therefore, 88 articles were included in the stage of eligibility. Communication with the corresponding author(s) was required for 35 of these articles. Seventeen authors of 11 studies responded and provided us the required data; their fine contribution is recognized in the acknowledgement section of this article. On the contrary, the corresponding authors of 21 studies did not reply or were unwilling to provide critical information (e.g., FGF-21 levels) for their studies, which, therefore, were excluded. The authors of other three studies did not respond to less crucial queries (e.g., the method of FGF-21 measurement), so their studies were included in the meta-analysis. Finaly, 44 studies were included in this systematic review and meta-analysis [13–56].

Descriptives of the Included Studies

The main descriptives of the 44 included studies are presented in Table 1. They were conducted between 2010 and 2024 and they totally contain data from 15,563 individuals, 9548 controls and 6015 patients. Eighteen studies were conducted in Europe, 19 in Asia, six in America and one in Africa. There were 36 cross-sectional studies, three case–control studies and five cohort studies, from which only the baseline data were used and analyzed in the meta-analysis. NAFLD was diagnosed with liver biopsy in 15 studies, abdominal ultrasonography in 14 studies, transient elastography in three studies, MRI-PDFF in three, MRI in two and MRS in two studies, CT in one, non-invasive indices in one and both ultrasonography and non-invasive indices in one study; the criteria of MAFLD were implemented in two studies, whereas those of MASLD in none. Furthermore, nine studies referred to pediatric/adolescent populations, four studies to patients with morbid obesity subjected to bariatric surgery (Table 1) and four studies included patients with NASH-related cirrhosis (Table S1). Circulating levels of FGF-21 were measured by enzyme-linked immunosorbent assay (ELISA) in 42 studies, except one that were measured with proximity extension immunoassay (PEI) and one that the method of FGF-21 measurement was not available (Table 1).

Demographic and laboratory characteristics extracted from the included studies are presented in the Table S1. For each study, data for the NAFLD and control groups were

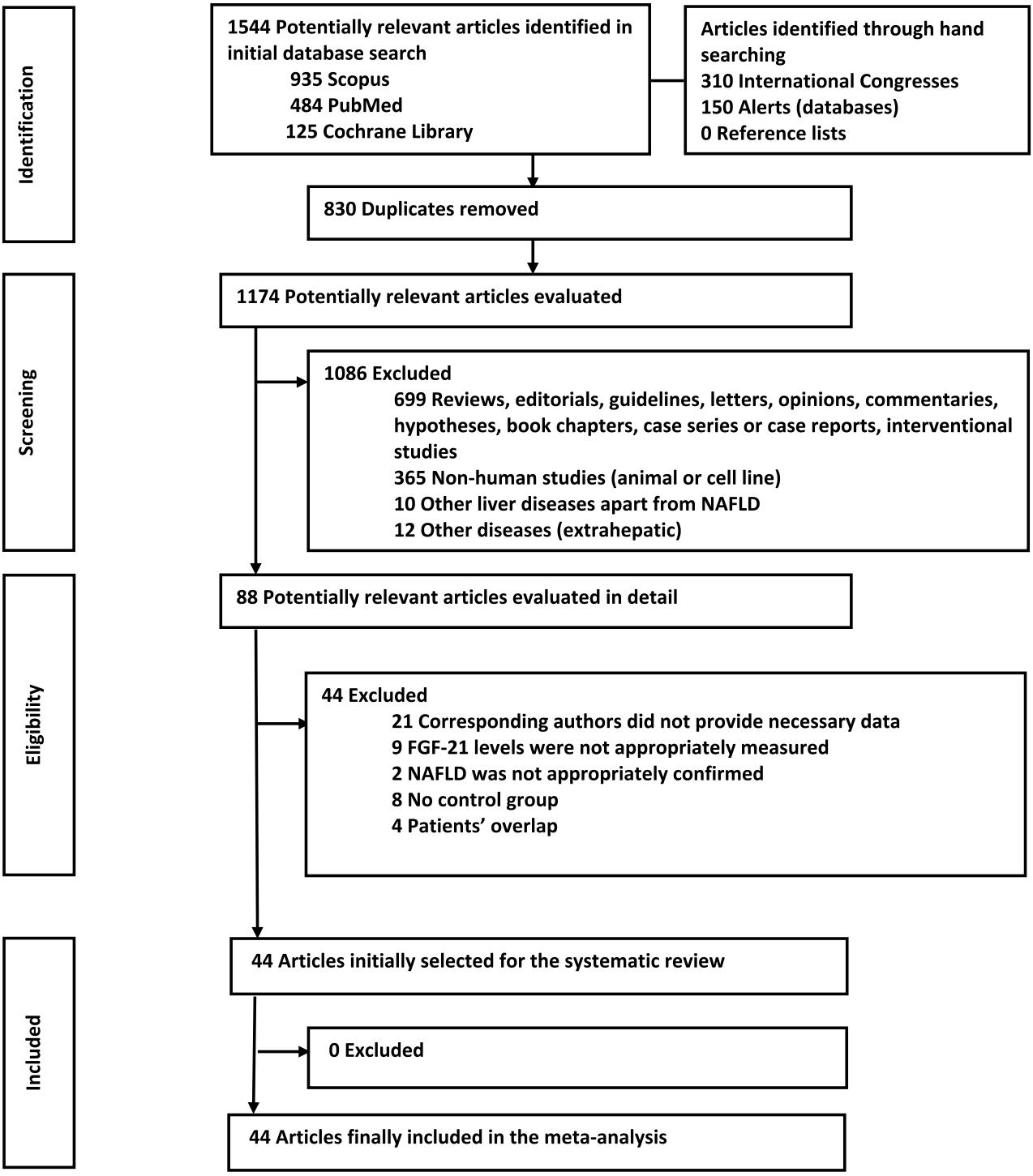

Fig. 2 Flowchart depicting the process of the literature search, according to the PRISMA statement. Abbreviations: FGF-21, fibroblast growth factor-21; NAFLD, nonalcoholic fatty liver disease; PRISMA, preferred reporting items for systematic reviews and meta-analyses

separately presented, including the number of patients (and men), age, BMI (kg/m^2), waist circumference (cm), the number of patients with T2DM, FGF-21 levels (ng/ml), AST (IU/L), ALT (IU/L), GGT (IU/L), HOMA-IR, and the number of patients with NASH-related cirrhosis.

Quality of the Included Studies

The NOS score was used for the evaluation of all studies (Table 1). There were 19 studies (43.2%) with NOS score <7. The mean ($\pm$ SD) NOS score was 6.68 ($\pm$ 1.30).

Table 1 Main descriptives of the studies included in the systematic review and meta-analysis

First author, Year, Origin[†]	Study design	Method of NAFLD diagnosis	Method of FGF-21 measurement	NOS score	Additional information
Abozaid, 2023, Netherlands [13]	Cohort study	Abdominal ultrasonography	PEI	8	
Ajaz, 2021, United Kingdom [14]	Cross-sectional	Liver biopsy	ELISA	4	All patients, but not controls, with NASH
Alisi, 2013, Italy [25]	Cross sectional	Liver biopsy	ELISA	8	Pediatric population
Babak, 2017, Ukraine [36]	Cross-sectional	Abdominal ultrasonography	ELISA	5	
Bahijri, 2023, Saudi Arabia [47]	Cross-sectional	Abdominal ultrasonography	ELISA	9	All patients and controls with T2DM
Barb, 2019, USA [52]	Cross-sectional	Liver biopsy	ELISA	8	
Chang, 2022, South Korea [53]	Cross-sectional	MRI -PDFF	ELISA	8	
Dushay, 2010, USA [54]	Cross-sectional	Liver biopsy	ELISA	7	
Elshinshawy, 2023, Egypt [55]	Cross-sectional	Transient elastography	ELISA	6	All patients, but not controls, with hypothyroidism; all patients and controls without T2DM
Flisiak-Jackiewicz, 2019, Poland [56]	Cross-sectional	Abdominal ultrasonography	ELISA	7	Pediatric population; all patients and controls without T2DM
Franck, 2023, Germany [15]	Cross sectional	Liver biopsy	ELISA	5	
Gallego-Duran, 2024, Spain [16]	Cross-sectional	Liver biopsy	ELISA	5	
Giannouli, 2023, Greece [17]	Case–control	Abdominal ultrasonography	ELISA	7	Adolescent population; all patients and controls with polycystic ovary syndrome
Goralska, 2023, Poland [18]	Cross-sectional	Non-invasive index of steatosis: FLI	ELISA	5	
Hua, 2019, Taiwan [19]	Cross-sectional	Abdominal ultrasonography	ELISA	7	Pediatric population
Ji, 2019, China [20]	Cross-sectional	Abdominal ultrasonography	ELISA	9	
Jiang, 2014, China [21]	Cross-sectional	Abdominal ultrasonography	ELISA	6	Patients and controls without T2DM
Ko, 2023, South Korea [22]	Cross-sectional	MRI-PDFF	ELISA	7	Pediatric population
Koliaki, 2015, Germany [23]	Cross-sectional	Liver biopsy	NA	6	Patients and controls with morbid obesity subjected to bariatric surgery
Koot, 2013, Netherlands [24]	Cross-sectional	MRS	ELISA	6	Pediatric and adolescent population
Li H, 2013, China [26]	Cross-sectional	Abdominal ultrasonography	ELISA	6	
Li X, 2011, China [27]	Cross-sectional	Abdominal ultrasonography	ELISA	7	Patients and controls without T2DM
Li X, 2024, China [28]	Cross-sectional	MAFLD criteria	ELISA	8	Patients and controls without T2DM
Lin D, 2023, China [29]	Cross sectional	Liver biopsy	ELISA	5	
Lin H, 2022, USA [30]	Cohort study	MRI	ELISA	9	Adolescent population; all patients and controls without T2DM
Liu, 2020, China [31]	Cross-sectional	Abdominal ultrasonography	ELISA	6	
Małecki, 2017, Poland [32]	Cross-sectional	Abdominal ultrasonography	ELISA	5	Pediatric population
Monserrat-Mesquida, 2020, Spain [33]	Cross-sectional	MRI-PDFF	ELISA	8	
Pafili, 2022, Germany [34]	Cross-sectional	Liver biopsy	ELISA	9	Patients and controls with morbid obesity subjected to bariatric surgery

Table 1 (continued)

First author, Year, Origin[†]	Study design	Method of NAFLD diagnosis	Method of FGF-21 measurement	NOS score	Additional information
Praktiknjo, 2019, Germany [35]	Cross-sectional	Transient elastography	ELISA	7	All patients and controls with HIV infection, without obesity
Qian, 2019, China [37]	Cross-sectional	MRS	ELISA	7	
Shen J, 2012, China [38]	Case–control	Liver biopsy	ELISA	8	
Shen Y, 2023, China [39]	Cohort study	Abdominal ultrasonography	ELISA	7	
Shen Y, 2013, China [40]	Cross-sectional	Abdominal ultrasonography	ELISA	6	
Singh, 2024, USA [41]	Cross-sectional	Liver Biopsy	ELISA	5	Patients, but not controls, with morbid obesity subjected to bariatric surgery
Sydor, 2022, Germany [42]	Cross-sectional	Transient elastography	ELISA	6	
Tanaka, 2022, Japan [43]	Cross-sectional	MAFLD criteria	ELISA	7	
Tucker, 2020, USA [44]	Cohort study	CT	ELISA	7	
Van Hove, 2024, USA [45]	Cross-sectional	Liver biopsy	ELISA	5	Pediatric patients but not controls diagnosed with NASH
Waluga, 2017, Poland [46]	Cross-sectional	Liver biopsy	ELISA	7	Patients and controls with morbid obesity subjected to bariatric surgery
Wargny, 2018, France [48]	Cohort study	MRI	ELISA	6	
Xu, 2024, China [49]	Cross-sectional	Abdominal ultrasonography and non-invasive indices	ELISA	5	
Yang, 2015, China [50]	Cross sectional	Liver biopsy	ELISA	8	
Yilmaz, 2010, Turkey [51]	Case–control	Liver biopsy	ELISA	7	

[†]: Studies are sorted alphabetically according to the surname of the first author. *CT* computed tomography, *ELISA* enzyme-linked immunosorbent assay, *FGF-21* fibroblast growth factor-21, *FLI* fatty liver index, *HIV* human immunodeficiency virus, *MAFLD* metabolic dysfunction-associated fatty liver disease, *MRI* magnetic resonance imaging, *MRI-PDFF* magnetic resonance imaging-proton density fat fraction, *MRS* magnetic resonance spectroscopy, *NA* not available, *NAFLD* nonalcoholic fatty liver disease, *NASH* nonalcoholic steatohepatitis, *NOS* Newcastle–Ottawa Scale, *PEI* Proximity extension immunoassay, *T2DM* type 2 diabetes mellitus

Main and Secondary Outcomes

Circulating FGF-21 was higher in patients with NAFLD compared to controls (SMD: 0.61; 95% CI: 0.44, 0.77; P < 0.00001) (Table 2; Fig. 3). Heterogeneity among studies was high (I^2 = 94%). Egger's test suggested statistically significant publication bias (p = 0.030) and visual asymmetry was observed in the relevant funnel plot (Fig. S1). Subgroup comparisons according to the disease severity in studies with histological confirmation showed: (i) no statistical difference in circulating FGF-21 between patients with NAFL and controls (n = 9; SMD: 0.22; 95% CI: −0.49, 0.93; p = 0.540; Table 3; Fig. S2a-b); (iii) higher FGF-21 levels in patients with NASH compared

Table 2 FGF-21 SMD and related statistics between patients with NAFLD and controls in the sum of studies and in sensitivity analyses

Comparison NAFLD versus Controls	All studies (n = 44)	After excluding studies with pediatric/adolescent populations (n = 35)	After excluding studies with populations undergoing bariatric surgery (n = 40)	After excluding studies with NOS < 7 (n = 25)	After excluding studies with outliers of FGF-21 SMD (N = 41)	After excluding studies with the use of the definition of MAFLD (n = 42)
SMD (95% CI); p-value	0.61 (0.44, 0.77); < 0.00001	0.70 (0.55, 0.85); < 0.00001	0.59 (0.41, 0.76); < 0.00001	0.37 (0.17, 0.58); 0.0004	0.58 (0.44, 0.72); < 0.00001	0.62 (0.44, 0.79); < 0.00001
I^2; p-value	94%; < 0.00001	92%; < 0.00001	95%; < 0.00001	95%; < 0.00001	91%; < 0.00001	94%; < 0.00001
Egger's test p-value	0.030	0.0004	0.049	0.422	0.012	0.031

CI confidence interval, *FGF-21* fibroblast growth factor-21, *MAFLD* metabolic dysfunction-associated fatty liver disease, *NAFLD* nonalcoholic fatty liver disease, *NOS* Newcastle–Ottawa Scale, *n* number of studies included in the analysis, *SMD* standardized mean difference

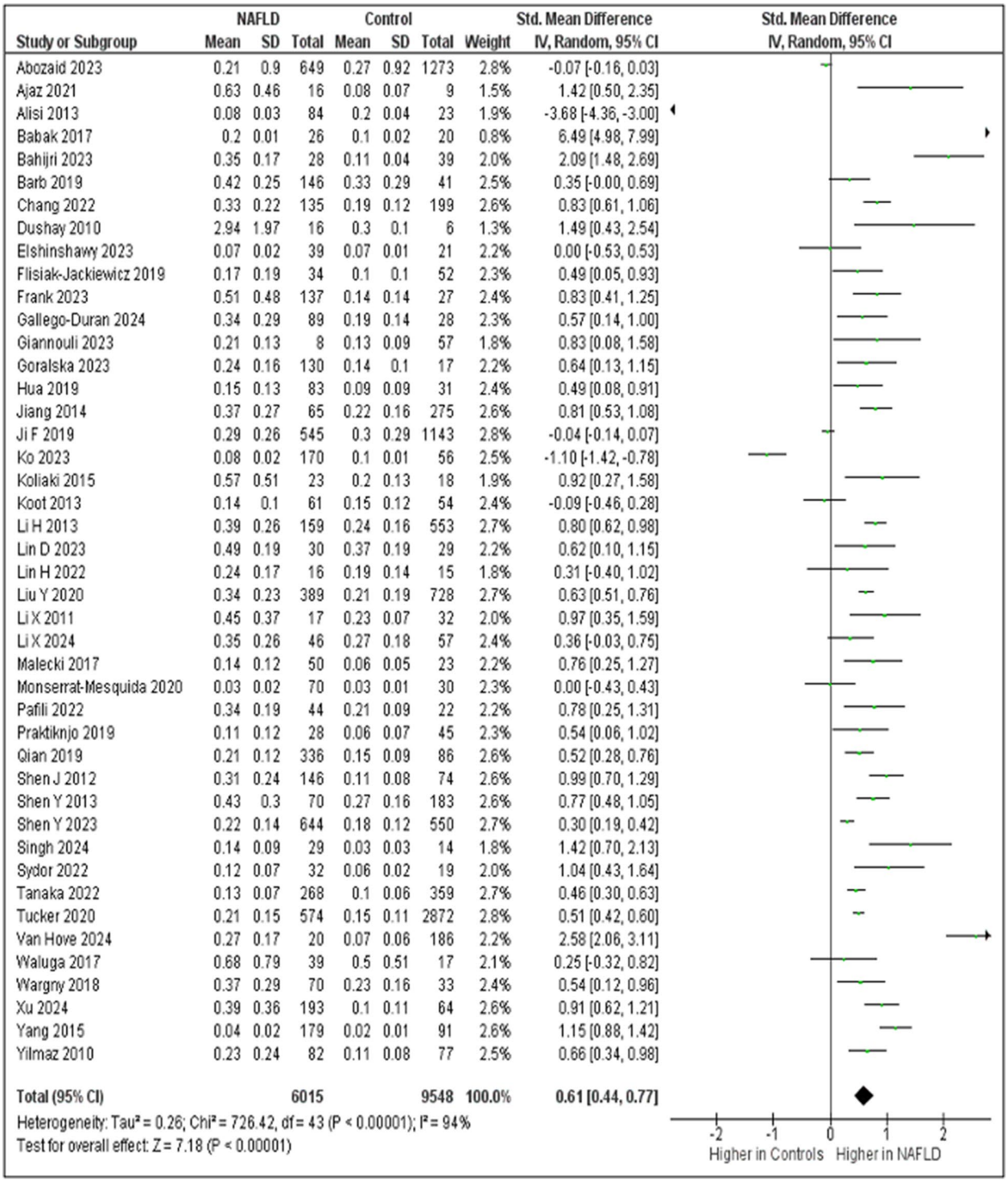

Fig. 3 Forest plot for the comparison of circulating FGF-21 between patients with NAFLD and controls in all studies (n = 44) included in the meta-analysis. Abbreviations: CI, confidence intervals; FGF-21, fibroblast growth factor-21; IV, inverse variance; NAFLD, nonalcoholic fatty liver disease; SD, standard deviation

to controls (n = 12; SMD: 1.30; 95% CI: 0.35, 2.24; P = 0.007; Table 3; Fig. S2c-d); no statistical difference in circulating FGF-21 between patients with NAFL and patients with NASH (n = 9; SMD: 1.17; 95% CI: −0.06, 2.39; p = 0.060; Table 3; Fig. S2e-f). Heterogeneity in these three subgroup comparisons remained high, whereas Egger's test showed not significant publication bias, which was confirmed by the visualization of funnel plots.

Table 3 FGF-21 SMD and related statistics in subgroup analysis according to NAFLD severity

	NAFL vs. Controls (n = 9)	NASH vs. Controls (n = 12)	NASH vs. NAFL (n = 9)
SMD (95% CI); p-value	0.22 (−0.49, 0.93); p = 0.540	1.30 (0.35, 2.24); p = 0.007	1.17 (-.0.06, 2.39); p = 0.060
I^2; p-value	95%; p < 0.00001	97%; p < 0.00001	98% p < 0.00001
Egger's test p-value	0.795	0.999	0.579

CI confidence interval, *FGF-21* fibroblast growth factor-21, *NAFLD* nonalcoholic fatty liver disease, *n* number of studies, *SMD* standardized mean difference

In the subgroup analysis for the comparison between patients with NAFLD and controls among studies with and without histological confirmation of the disease, there was no difference between subgroups (p = 0.590); circulating FGF-21 was higher in patients with NAFLD than controls within both subgroups (Table 4; Fig. S2 g-i). Heterogeneity remained high in both subgroup comparisons. In the subgroup analysis for the comparison between patients with NAFLD and controls within studies with and without the inclusion of patients with NASH-related liver cirrhosis, there was statistically significant difference between subgroups (p = 0.0004), with FGF-21 SMD being higher in studies with the inclusion of patients with NASH-related cirrhosis (SMD: 1.01, 95% CI: 0.84, 1.18, p < 0.00001; Table 4; Fig. S2j-l). Notably, heterogeneity radically decreased in the subgroup of studies including patients with NASH-related cirrhosis (I^2 = 0%; p = 0.480), whereas it remained high in the absence of patients with NASH-related cirrhosis (Table 4; Fig. S2j). No significant publication bias was suggested by Egger's test and funnel plots (Table 4; Fig. S2 h-i, S2 k-l).

In the sensitivity analyses for the comparison between patients with NAFLD and controls, after excluding studies with: (i) pediatric/adolescent populations (n = 9; Fig. S3a); (ii) morbidly obese populations undergoing bariatric surgery (n = 4; Fig. S3b); (iii) NOS score < 7 (n = 19; Fig. S3c); (iv) outliers of FGF-21 SMD (n = 3; Fig. S3 d); (v) the use of the definition of MAFLD for the diagnosis of the disease (n = 2; Fig. S3e), FGF-21 levels remained higher in patients with NAFLD compared to controls in all the analyses (Table 2). Heterogeneity remained essentially unchanged in all the sensitivity analyses. Egger's test p-value remained statistically significant in all comparisons, except for that following the exclusion of studies with NOS score < 7 (p = 0.422). Visual inspection of funnel plots indicated a degree of asymmetry (Fig. S4a-e).

In the univariate meta-regression analysis, the percentage of patients with T2DM was positively associated with FGF-21 SMD between patients with NAFLD and controls and could explain 49.2% of the heterogeneity among studies (Table 5; Fig. S5). The rest of the selected potential confounders, i.e., age, sex, BMI, waist circumference and HOMA-IR were not significantly associated with FGF-21 SMD between patients with NAFLD and controls (Table 5). Thus, these parameters could not explain a part of the heterogeneity among studies.

Discussion

This systematic review and meta-analysis demonstrated higher circulating FGF-21 in patients with NAFLD compared to controls (Table 2; Fig. 3). Within the subgroup of studies with histological confirmation of the disease, FGF-21 levels were higher in patients with NASH than controls, but not in patients with NAFL compared to controls or patients with NASH [Table 3; Fig. S2a, S2c, S2e]. Notably, in another subgroup analysis, higher FGF-21 between patients with NAFLD and controls was observed within studies that included patients with NASH-related cirrhosis than those that did not include them (Table 4; Fig. S2j). Altogether these findings indicate

Table 4 FGF-21 SMD and related statistics between patients with NAFLD and controls in subgroup analysis within studies: (i) with vs. without histological confirmation of NAFLD with liver biopsy and (ii) with vs. without the inclusion of patients with NASH-related cirrhosis

	Liver Biopsy (n = 15)	Without Liver Biopsy (n = 29)	NASH related cirrhosis (n = 4)	Without NASH related cirrhosis (n = 40)
SMD (95% CI); p-value	0.69 (0.20, 1.17); 0.006	0.54 (0.37, 0.72); < 0.00001	1.01 (0.84, 1.18); < 0.00001	0.57 (0.40,0.74); < 0.00001
I^2; p-value	94%; < 0.00001	94%; < 0.00001	0%; 0.48	94%; < 0.00001
Egger's test p-value	0.599	0.053	0.103	0.062
p-value for difference (between subgroups)	0.590		0.0004	

CI confidence interval, *FGF-21* fibroblast growth factor-21, *NAFLD* nonalcoholic fatty liver disease, *n* number of studies, *SMD* standardized mean difference

Table 5 Univariate meta-regression analysis of FGF-21 SMD between NAFLD patients and controls with potential confounders

Confounder	Age (years)	Sex (men%)	T2DM (%)	BMI (kg/m^2)	Waist Circumference (cm)	HOMA-IR
N	32	31	14	32	16	14
Beta (95%CI)	0.013 (−0.005, 0.031)	−1.414 (−3.685, 0.856)	1.043 (0.400, 1.686)	0.022 (−0.023, 0.066)	0.012 (−0.012, 0.036)	0.038 (−0.125, 0.202)
p-value	0.158	0.222	0.0015	0.339	0.328	0.647
Adjusted R square	2.74%	1.52%	49.18%	0.00%	0.00%	0.00%

BMI body mass index, *CI* confidence interval, *HOMA-IR* homeostasis model assessment-insulin resistance, *FGF-21* fibroblast growth factor-21, *NAFLD* nonalcoholic fatty liver disease, *N* number of studies with available data on each potential confounder, *SMD* standardized mean difference, *T2DM* type 2 diabetes mellitus

that higher circulating FGF-21 levels observed in patients with NAFLD than controls may be primarily attributed to more severe disease; however, the interpretation of this result should be approached with caution, because of the relatively small number of studies with histological confirmation or with the inclusion of patients with NASH-related cirrhosis.

Trying to investigate the high heterogeneity among studies, subgroup, sensitivity and meta-regression analyses were performed. The inclusion of patients with NASH-related cirrhosis, i.e., the whole histological spectrum of NAFLD minimized the heterogeneity (Table 4; Fig. S2j). This implies that not inclusion of the full spectrum of NALFD in the relevant studies is partly accounted for the observed heterogeneity; however, the interpretation of this result should also be approached with caution, due to the small number of studies having included patients with NASH-related cirrhosis. Beyond this finding, the other subgroup and sensitivity analyses did not essentially reduce the heterogeneity among studies (Tables 2–4). The meta-regression analysis showed that the percentage of patients with T2DM may be accounted for about 49% of the heterogeneity among studies (Table 5; Fig. S5). This seems rational since higher rates of T2DM were observed in individuals with than without NAFLD [57], and FGF-21 was shown higher in patients with than without T2DM [58]. Nonetheless, this finding should be cautiously interpreted, because of the small number of studies with data on T2DM in this meta-analysis (n = 14). The other potential confounders, i.e., age, sex, BMI, waist circumference and HOMA-IR could not explain a part of heterogeneity among studies. Based on the above findings, the inclusion of patients across the full spectrum of NAFLD and the number of patients with T2DM are highly recommended in the future relevant studies.

Egger's test showed an overall publication bias in the comparison between patients with NAFLD and controls and most funnel plots also showed a degree of asymmetry (Fig. S2b, S2 d, S2f, S2 h-i, S2k-l, S4a-e). However, Egger's test was not significant and the funnel plot showed lower degree of asymmetry in all subgroup analyses (Tables 3–4), as well as in the sensitivity analysis after the exclusion of studies with NOS < 7, i.e., those with estimated lower quality. The latter finding may imply that most relevant studies of higher quality, i.e., those providing the most reliable findings, were included in this systematic review and meta-analysis.

From a pathophysiologic point of view, the production of FGF-21 may increase when NAFLD progresses to more advanced disease, as a counterbalancing mechanism against the disease progression [8]. Data from experimental studies have shown that FGF-21, acting through on its receptor (FGFR1) on the cell membrane with β-Klotho as co-receptor, increases fatty acid β-oxidation and decreases de novo lipogenesis, thereby attenuating hepatic steatosis. Additionally, FGF-21 may attenuate hepatic inflammation by inhibiting the nuclear factor kappa B pathway, decreasing pro-inflammatory cytokines (e.g., interleukin-1β) and increasing anti-inflammatory cytokines (e.g., interleukin-10), decreasing intrahepatic oxidative stress and endoplasmic reticulum stress. FGF-21 may also possibly decrease hepatic fibrosis, by attenuating the hepatic expression of transforming growth factor-β [8]. Notably, FGF-21 seems to increase insulin sensitivity in the liver [59].

However, higher FGF-21 concentrations in advanced disease may also imply a state of FGF-21 resistance or insensitivity [8]. In this case, the administration of FGF-21 analogs, in order to achieve supraphysiological concentrations of FGF-21, thus surpassing the barrier of FGF-21 resistance or insensitivity, may be beneficial, thus limiting the progression of the disease, or may fail to limit the progression of the disease. Indeed, FGF-21 analogs (e.g., pegbelfermin, efruxifermin) have been investigated in clinical trials of NASH. In two phase 2b RCTs, pegbelfermin was administered in patients with NASH and advanced hepatic fibrosis (FALCON 1 trial) [60] or NASH-associated compensated cirrhosis (FALCON 2 trial) [61], without, however meeting its primary endpoints. On the contrary, in a phase 2b RCT, efruxifermin administration in patients with NASH and moderate or advanced hepatic fibrosis (HARMONY) provided more favorable results; more specifically, efruxifermin improved hepatic fibrosis and resolved NASH in higher

rates than placebo [62]. The above considering, the main clinical implication of our meta-analysis is the consolidation of higher circulating FGF-21 concentrations, when the disease progresses to NASH or NASH-related cirrhosis. Whether the administration of FGF-21 analogs to achieve supraphysiological FGF-21 concentrations are definitely beneficial or not for NASH may hopefully be shown by the ongoing clinical trials.

There are some meta-analyses of clinical trials investigating the effects of FGF-21 analogs on NAFLD. In a meta-analysis of 8 RCTs, FGF-21 analogs were shown to reduce NAFLD activity score (NAS) (without worsening of fibrosis) and fibrosis stage (without worsening of MASH) [63]. Similar results were provided by other relevant meta-analysis of 7 [64] or 6 [65] clinical trials. The results of the existing meta-analyses should cautiously be interpreted, because of the small number of included studies, which, importantly, were mostly sponsored. Furthermore, different FGF-21 analogs (e.g., efruxifermin, pegbelfermin, and pegozafermin) were synthesized together, which renders difficult the interpretation of the results of the relevant meta-analyses. There is also a meta-analysis of diagnostic accuracy, which supported that FGF-21 provided a pooled sensitivity of 0.62 (95% CI 0.50–0.73) and specificity of 0.78 (0.70–0.84) for diagnosing NASH, based on the results of four studies [66]. However, we could not recommend the use of FGF-21 as a non-invasive index of NASH, based on the results of this [66] or our meta-analysis; of course, our results warrant more diagnostic accuracy studies to clarify whether FGF-21 may add value in the non-invasive diagnosis of NASH or related fibrosis, alone or, more possibly, in combination with other parameters.

There is also a recent bi-directional Mendelian randomization study supporting that FGF-21 was negatively regulated by NAFLD, whereas positively regulated by obesity and T2DM [67]. However, FGF-21 did not improve NAFLD, obesity or T2DM, possibly owing to FGF-21 resistance [67]. This study contradicts the main result of our meta-analysis, i.e., higher FGF-21 in patients with NAFLD than controls; however, the reasons why FGF-21 was differently regulated by NAFLD and obesity or T2DM were not clear, since their possible effects on FGF-21 are expected to be towards the same direction. The authors of this study also supported that FGF-21 resistance may inhibit any counterbalancing effects of FGF-21 changes not only in NAFLD, but also in obesity and T2DM, which is in accordance with our speculation above.

This systematic review and meta-analysis investigating circulating FGF-21 levels in patients with NAFLD carries a degree of originality. Furthermore, our findings are highly relevant with the above-mentioned clinical trials investigating FGF-21 analogs for the treatment of NASH, as mentioned above, especially in the light of potential FGF-21 resistance or insensitivity observed in advanced disease. However, this systematic review and meta-analysis has certain limitations. First, the inclusion of observational studies cannot show a cause-effect association between FGF-21 and NAFLD. Second, the comparison of circulating FGF-21 levels between different grades of hepatic steatosis, different stages of hepatic fibrosis and different degree of hepatic inflammation was not feasible, because of the very small number of studies providing the specific histological information; even when FGF-21 levels were reported, the groups were differently defined in different studies. For example, when reported, fibrosis stages were grouped as F0 vs. F1-F4, or F0-1 vs. F2-F3, or F0-2 vs. F3-4 etc. in different studies, which rendered the synthesis of grouping for fibrosis stages insecure; however, FGF-21 levels were higher in NAFLD patients than controls among studies having included patients with NASH-related cirrhosis, which equals with fibrosis stage F4 (Table 4; Fig. S2j), thus providing an indirect indication of higher FGF-21 levels in the end stage of fibrosis. Third, the pre-planned subgroup analysis based on different methods of FGF-21 measurement was not feasible, because all but two studies utilized ELISA for FGF-21 quantification; however, different ELISA kits from different manufacturers might have affected the heterogeneity among studies. Fourth, heterogeneity among studies was high and could be explained only partly by the sensitivity analyses (Table 4; Fig. S2j) and meta-regression analyses (Table 5; Fig. S5). Furthermore, Egger's test showed an overall publication bias, despite the extensive manual searching we performed. Last, we adopted the nomenclature of NAFLD and not that of MASLD that has been more recently suggested, because, as mentioned above, most studies included in the systematic review and meta-analysis were based on the nomenclature and definition of NAFLD (Table 1). Although there is significant overlap between NAFLD, MAFLD and MASLD, the proposed shift from NAFLD to MAFLD or MASLD should cautiously be performed because of the different definitions among them [68]. In this regard, it has been recommended that all different names of the disease may be used during this period of transition with the necessary flexibility [69].

Conclusions

Higher circulating FGF-21 levels were shown in patients with NAFLD, which may be possibly attributed to those with advanced disease (NASH and or NASH-related cirrhosis). These results may imply a counterbalancing increase of FGF-21, when NAFLD progresses to advanced disease, thus possibly supporting the ongoing clinical trials of FGF-21 analogs in patients with NASH. However, FGF-21 resistance or insensitivity, when the disease progresses may also be considered, a condition which, if validated, may interfere with the results of the relevant clinical trials.

Key References

1 • Fouad Y, Alboraie M, Shiha G. Epidemiology and diagnosis of metabolic dysfunction-associated fatty liver disease. Hepatol Int. 2024;18:827–33. https://doi.org/10.1007/s12072-024-10704-3.

This review provides a contemporary summary of the epidemiology and the diagnosis of the disease.

6 •• Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease : An international expert consensus statement. J Hepatol. 2020;73:202–9. https://doi.org/10.1016/j.jhep.2020.03.039.

This consensus introduced the nomenclature and the definition of metabolic dysfynction-associated fatty liver disease.

7 •• Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023;79:1542–56. https://doi.org/10.1016/j.jhep.2023.06.003.

This Delphi consensus introduced the nomenclature and the definition of metabolic dysfunction-associated steatotic liver disease.

60 • Loomba R, Sanyal AJ, Nakajima A, Neuschwander-Tetri BA, Goodman ZD, Harrison SA, et al. Pegbelfermin in Patients With Nonalcoholic Steatohepatitis and Stage 3 Fibrosis (FALCON 1): A Randomized Phase 2b Study. Clin Gastroenterol Hepatol. 2024;22:102–12. https://doi.org/10.1016/j.cgh.2023.04.011.

This is a phase 2b clinical trial investigating the effect of pegbelfermin, a FGF-21 analog in patient with steatohepatitis and advanced fibrosis.

61 • Abdelmalek MF, Sanyal AJ, Nakajima A, Neuschwander-Tetri BA, Goodman ZD, Lawitz EJ, et al. Pegbelfermin in Patients With Nonalcoholic Steatohepatitis and Compensated Cirrhosis (FALCON 2): A Randomized Phase 2b Study. Clin Gastroenterol Hepatol. 2024;22:113–23. https://doi.org/10.1016/j.cgh.2023.04.012.

This is a phase 2b clinical trial investigating the effect of pegbelfermin, an FGF-21 analog in patient with steatohepatitis and compansated cirrhosis.

62 • Harrison SA, Frias JP, Neff G, Abrams GA, Lucas KJ, Sanchez W, et al. Safety and efficacy of once-weekly efruxifermin versus placebo in non-alcoholic steatohepatitis (HARMONY): a multicentre, randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Gastroenterol Hepatol. 2023;8:1080–93. https://doi.org/10.1016/S2468-1253(23)00272-8.

This is a phase 2b clinical trial investigating the effect of efruxifermin, a FGF-21 analog in patient with steatohepatitis.

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Authors' Contribution IF: Conceptualization; Methodology; Investigation; Data curation; Formal analysis; Visualization; Writing-original draft; Writing-review & editing. MO: Methodology; Investigation; Data curation; Formal analysis; Visualization; Writing-original draft; Writing-review & editing. AG: Validation; Writing-review & editing. OG: Validation; Writing-review & editing SAP: Conceptualization: Methodology, Investigation; Data curation; Validation; Writing-original draft; Writing-review & editing; Supervision. All authors approved the final version to be submitted and agreed to be accountable for all aspects of the work to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Declarations

Competing interests The authors declare no competing interests

References

1. Fouad Y, Alboraie M, Shiha G. Epidemiology and diagnosis of metabolic dysfunction-associated fatty liver disease. Hepatol Int. 2024;18:827–33. https://doi.org/10.1007/s12072-024-10704-3
2. Pais R, Barritt AS, Calmus Y, Scatton O, Runge T, Lebray P, et al. NAFLD and liver transplantation : Current burden and expected challenges. J Hepatol. 2016;65:1245–57. https://doi.org/10.1016/j.jhep.2016.07.033.
3. Polyzos SA, Mantzoros CS. Nonalcoholic fatty future disease. Metabolism. 2015;65:1007–16. https://doi.org/10.1016/j.metabol.2015.12.009.
4. Parola M, Pinzani M. Molecular Aspects of Medicine Liver fibrosis in NAFLD / NASH : from pathophysiology towards diagnostic and therapeutic strategies. Mol Aspects Med. 2024;95: 101231. https://doi.org/10.1016/j.mam.2023.101231.
5. Makri E, Goulas A, Polyzos SA. Epidemiology, Pathogenesis, Diagnosis and Emerging Treatment of Nonalcoholic Fatty Liver Disease. Arch Med Res. 2021;52:25–37. https://doi.org/10.1016/j.arcmed.2020.11.010.
6. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease : An international expert consensus statement. J Hepatol. 2020;73:202–9. https://doi.org/10.1016/j.jhep.2020.03.039.
7. Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023;79:1542–56. https://doi.org/10.1016/j.jhep.2023.06.003.
8. Raptis DD, Mantzoros CS, Polyzos SA. Fibroblast Growth Factor-21 as a Potential Therapeutic Target of Nonalcoholic Fatty Liver Disease. Ther Clin Risk Manag. 2023;19:77–96. https://doi.org/10.2147/TCRM.S352008.
9. Chen Z, Yang L, Liu Y, Huang P, Song H, Zheng P. The potential function and clinical application of FGF21 in metabolic diseases. Front Pharmacol. 2022;13:1089214. https://doi.org/10.3389/fphar.2022.1089214.
10. Lin X, Liu YB, Hu H. Metabolic role of fibroblast growth factor 21 in liver, adipose and nervous system tissues. Biomed reports. 2017;6:495–502. https://doi.org/10.3892/br.2017.890.
11. Staiger H, Keuper M, Berti L, de Angelis MH, Häring HU. Fibroblast Growth Factor 21-Metabolic Role in Mice and Men. Endocr Rev. 2017;38:468–88. https://doi.org/10.1210/er.2017-00016.
12. Brooke BS, Schwartz TA, Pawlik TM. MOOSE Reporting Guidelines for Meta-analyses of Observational Studies. JAMA Surg. 2021;156:787–8. https://doi.org/10.1001/jamasurg.2021.0522.
13. Abozaid YJ, Ayada I, Van Kleef LA, Vallerga CL, Pan Q, Brouwer WP, et al. Plasma proteomic signature of fatty liver disease: The Rotterdam Study. Hepatology. 2023;78:284–94. https://doi.org/10.1097/HEP.0000000000000300.
14. Ajaz S, McPhail MJ, Gnudi L, Trovato FM, Mujib S, Napoli S, et al. Mitochondrial dysfunction as a mechanistic biomarker in patients with non-alcoholic fatty liver disease (NAFLD). Mitochondrion. 2021;57:119–30. https://doi.org/10.1016/j.mito.2020.12.010.
15. Franck M, John K, Al Aoua S, Rau M, Geier A, Schattenberg JM, et al. Hepatokine-based identification of fibrotic NASH and improved risk stratification in a multicentre cohort of NAFLD patients. Liver Int. 2023;43:2668–79. https://doi.org/10.1111/liv.15686.
16. Gallego-Durán R, Ampuero J, Maya-Miles D, Pastor-Ramírez H, Montero-Vallejo R, Rivera-Esteban J, et al. Fibroblast growth factor 21 is a hepatokine involved in MASLD progression. United Eur Gastroenterol J. 2024;1–13. https://doi.org/10.1002/ueg2.12534.
17. Giannouli A, Stefanaki C, Kouskoutis C, Konidari M, Mani I, Konidari K, et al. Hepatokine Profile in Adolescents with Polycystic Ovary Syndrome: A Case-Control Study. J Clin Med. 2023;12:5744. https://doi.org/10.3390/jcm12175744.
18. Goralska J, Razny U, Gruca A, Zdzienicka A, Micek A, Dembinska-Kiec A, et al. Plasma Cytokeratin-18 Fragment Level Reflects the Metabolic Phenotype in Obesity. Biomolecules. 2023;12:476. https://doi.org/10.3390/biom13040675.
19. Hua MC, Huang JL, Hu CC, Yao TC, Lai MW. Including Fibroblast Growth Factor-21 in Combined Biomarker Panels Improves Predictions of Liver Steatosis Severity in Children. Front Pediatr. 2019;7:420. https://doi.org/10.3389/fped.2019.00420.
20. Ji F, Liu Y, Hao JG, Wang LP, Dai MJ, Shen GF, et al. KLB gene polymorphism is associated with obesity and non-alcoholic fatty liver disease in the Han Chinese. Aging (Albany NY). 2019;11:7847–58. https://doi.org/10.18632/aging.102293.
21. Jiang S, Zhang R, Li H, Fang Q, Jiang F, Hou X, et al. The single nucleotide polymorphism rs499765 is associated with fibroblast growth factor 21 and nonalcoholic fatty liver disease in a Chinese population with normal glucose tolerance. J Nutrigenet Nutrigenomics. 2014;7:121–9. https://doi.org/10.1159/000367943.
22. Ko HJ, Woo S, Han J, Kim YM, Lim HJ, Kim MJ, et al. Which obesity index is the most useful marker for predicting hepatic steatosis in children and adolescents with obesity? A cross-sectional study using quantitative magnetic resonance imaging. Obes Res Clin Pract. 2023;17:335–42. https://doi.org/10.1016/j.orcp.2023.05.013.
23. Koliaki C, Szendroedi J, Kaul K, Jelenik T, Nowotny P, Jankowiak F, et al. Adaptation of Hepatic Mitochondrial Function in Humans with Non-Alcoholic Fatty Liver Is Lost in Steatohepatitis. Cell Metab. 2015;21:739–46. https://doi.org/10.1016/j.cmet.2015.04.004.
24. Koot BGP, van der Baan-Slootweg OH, Bohte AE, Nederveen AJ, van Werven JR, Tamminga-Smeulders CLJ, et al. Accuracy of prediction scores and novel biomarkers for predicting nonalcoholic fatty liver disease in obese children. Obesity (Silver Spring). 2013;21:583–90. https://doi.org/10.1002/oby.20173.
25. Alisi A, Ceccarelli S, Panera N, Prono F, Petrini S, De Stefanis C, et al. Association between Serum Atypical Fibroblast Growth Factors 21 and 19 and Pediatric Nonalcoholic Fatty Liver Disease. PLoS ONE. 2013;8: e67160. https://doi.org/10.1371/journal.pone.0067160.
26. Li H, Dong K, Fang Q, Hou X, Zhou M, Bao Y, et al. High serum level of fibroblast growth factor 21 is an independent predictor of non-alcoholic fatty liver disease: a 3-year prospective study in China. J Hepatol. 2013;58:557–63. https://doi.org/10.1016/j.jhep.2012.10.029.
27. Li X, Fan X, Ren F, Zhang Y, Shen C, Ren G, et al. Serum FGF21 levels are increased in newly diagnosed type 2 diabetes with non-alcoholic fatty liver disease and associated with hsCRP levels independently. Diabetes Res Clin Pract. 2011;93:10–6. https://doi.org/10.1016/j.diabres.2011.02.034.
28. Li X, Zheng K, Liu L, Zhang T, Gu W, Hou X, et al. Relationship of postprandial fibroblast growth factor 21 with lipids, inflammation and metabolic dysfunction-associated fatty liver disease during oral fat tolerance test. Front Endocrinol (Lausanne). 2024;15:1–11. https://doi.org/10.3389/fendo.2024.1343853.
29. Lin D, Sun Q, Liu Z, Pan J, Zhu J, Wang S, et al. Gut microbiota and bile acids partially mediate the improvement of fibroblast

growth factor 21 on methionine-choline-deficient diet-induced non-alcoholic fatty liver disease mice. Free Radic Biol Med. 2023;195:199–218. https://doi.org/10.1016/j.freeradbiomed.2022.12.087.
30. Lin H, Mercer KE, Ou X, Mansfield K, Buchmann R, Børsheim E, et al. Circulating microRNAs Are Associated With Metabolic Markers in Adolescents With Hepatosteatosis. Front Endocrinol (Lausanne). 2022;13: 856973. https://doi.org/10.3389/fendo.2022.856973.
31. Liu Y, Ji F, Hao DA, Hao JG, Wang LP, Dai MJ, et al. A novel FGF21-based noninvasive scoring model to diagnose NAFLD in Chinese population. Acta Medica Mediterr. 2020;36:1727–33. https://doi.org/10.19193/0393-6384_2020_.3_271.
32. Małecki P, Mania A, Tracz J, Łuczak M, Mazur-Melewska K, Figlerowicz M. Adipocytokines as Risk Factors for Development of Nonalcoholic Fatty Liver Disease in Children. J Clin Exp Hepatol. 2021;11:646–53. https://doi.org/10.1016/j.jceh.2021.03.002.
33. Monserrat-Mesquida M, Quetglas-Llabrés M, Abbate M, Montemayor S, Mascaró CM, Casares M, et al. Oxidative Stress and Pro-Inflammatory Status in Patients with Non-Alcoholic Fatty Liver Disease. Antioxidants (Basel). 2020;9:759. https://doi.org/10.3390/antiox9080759.
34. Pafili K, Kahl S, Mastrototaro L, Strassburger K, Pesta D, Herder C, et al. Mitochondrial respiration is decreased in visceral but not subcutaneous adipose tissue in obese individuals with fatty liver disease. J Hepatol. 2022;77:1504–14. https://doi.org/10.1016/j.jhep.2022.08.010.
35. Praktiknjo M, Djayadi N, Mohr R, Schierwagen R, Bischoff J, Dold L, et al. Fibroblast growth factor 21 is independently associated with severe hepatic steatosis in non-obese HIV-infected patients. Liver Int. 2019;39:1514–20. https://doi.org/10.1111/liv.14107.
36. Babak OY, Molodan VI, Lapshyna KA, Prosolenko KO. Biomarkers usage in minimally invasive diagnosis of nonalcoholic steatohepatitis in nonalcoholic fatty liver disease patients. New Armen Med J. 2017;11:46 – 51. https://repo.knmu.edu.ua/handle/123456789/18470.
37. Qian LL, Wu L, Zhang L, Zhang J, Zhou J, Li YH, et al. Serum biomarkers combined with ultrasonography for early diagnosis of non-alcoholic fatty liver disease confirmed by magnetic resonance spectroscopy. Acta Pharmacol Sin. 2020;41:554–60. https://doi.org/10.1038/s41401-019-0321-x.
38. Shen J, Chan HLY, Wong GLH, Choi PCL, Chan AWH, Chan HY, et al. Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers. J Hepatol. 2012;56:1363–70. https://doi.org/10.1016/j.jhep.2011.12.025.
39. Shen Y, Hu T, Tan H, Xu Y, Wang Y, Ma X, et al. Insight to the association among fibroblast growth factor 21, non-alcoholic fatty liver disease and cardiovascular outcomes: A population-based study. Cytokine. 2023;170: 156318. https://doi.org/10.1016/j.cyto.2023.156318.
40. Shen Y, Ma X, Zhou J, Pan X, Hao Y, Zhou M, et al. Additive relationship between serum fibroblast growth factor 21 level and coronary artery disease. Cardiovasc Diabetol. 2013;12:124. https://doi.org/10.1186/1475-2840-12-124.
41. Singh C, Jin B, Shrestha N, Markhard AL, Panda A, Calvo SE, et al. ChREBP is activated by reductive stress and mediates GCKR-associated metabolic traits. Cell Metab. 2024;36:144–58. https://doi.org/10.1016/j.cmet.2023.11.010.
42. Sydor S, Dandyk C, Schwerdt J, Manka P, Benndorf D, Lehmann T, et al. Discovering Biomarkers for Non-Alcoholic Steatohepatitis Patients with and without Hepatocellular Carcinoma Using Fecal Metaproteomics. Int J Mol Sci. 2022;23:8841. https://doi.org/10.3390/ijms23168841.
43. Tanaka M, Takahashi S, Higashiura Y, Sakai A, Koyama M, Saitoh S, et al. Circulating level of fatty acid-binding protein 4 is an independent predictor of metabolic dysfunction-associated fatty liver disease in middle-aged and elderly individuals. J Diabetes Investig. 2022;13:878–88. https://doi.org/10.1111/jdi.13735.
44. Tucker B, McClelland RL, Allison MA, Budoff MJ, Wu BJ, Barter PJ, et al. Relationship of fibroblast growth factor 21 levels with inflammation, lipoproteins and non-alcoholic fatty liver disease. Atherosclerosis. 2020;299:38–44. https://doi.org/10.1016/j.atherosclerosis.2020.03.009.
45. Van Hove JLK, Friederich MW, Strode DK, Van Hove RA, Miller KR, Sharma R, et al. Protein biomarkers GDF15 and FGF21 to differentiate mitochondrial hepatopathies from other pediatric liver diseases. Hepatol Commun. 2024;8: e0361. https://doi.org/10.1097/HC9.0000000000000361.
46. Waluga M, Kukla M, Zorniak M, Kajor M, Liszka L, Dyaczynski M, et al. Fibroblast growth factor-21 and omentin-1 hepatic mRNA expression and serum levels in morbidly obese women with non-alcoholic fatty liver disease. J Physiol Pharmacol. 2017;68:363–74. https://pubmed.ncbi.nlm.nih.gov/28820393/.
47. Bahijri S, Eldakhakhny B, Enani S, Ajabnoor G, Al-Mowallad AS, Alsheikh L, et al. Fibroblast Growth Factor 21: A More Effective Biomarker Than Free Fatty Acids and Other Insulin Sensitivity Measures for Predicting Non-alcoholic Fatty Liver Disease in Saudi Arabian Type 2 Diabetes Patients. Cureus. 2023;15:1–10. https://doi.org/10.7759/cureus.50524.
48. Wargny M, Ducluzeau PH, Petit JM, Le May C, Smati S, Arnaud L, et al. Circulating PCSK9 levels are not associated with the severity of hepatic steatosis and NASH in a high-risk population. Atherosclerosis. 2018;278:82–90. https://doi.org/10.1016/j.atherosclerosis.2018.09.008.
49. Xu K, He BW, Yu JL, Kang HM, Zheng TT, Chen ZY, et al. Clinical significance of serum FGF21 levels in diagnosing nonalcoholic fatty liver disease early. Sci Rep. 2024;14:25191. https://doi.org/10.1038/s41598-024-76585-6.
50. Yang M, Xu D, Liu Y, Guo X, Li W, Guo C, et al. Combined Serum Biomarkers in Non-Invasive Diagnosis of Non-Alcoholic Steatohepatitis. PLoS ONE. 2015;10: e0131664. https://doi.org/10.1371/journal.pone.0131.
51. Yilmaz Y, Eren F, Yonal O, Kurt R, Aktas B, Celikel CA, et al. Increased serum FGF21 levels in patients with nonalcoholic fatty liver disease. Eur J Clin Invest. 2010;40:887–92. https://doi.org/10.1111/j.1365-2362.2010.02338.x.
52. Barb D, Bril F, Kalavalapalli S, Cusi K. Plasma Fibroblast Growth Factor 21 Is Associated With Severity of Nonalcoholic Steatohepatitis in Patients With Obesity and Type 2 Diabetes. J Clin Endocrinol Metab. 2019;104:3327–36. https://doi.org/10.1210/jc.2018-02414.
53. Chang JS, Ahn JH, Kang SH, Koh SB, Kim JY, Baik SK, et al. Metabolic Stress Index Including Mitochondrial Biomarker for Noninvasive Diagnosis of Hepatic Steatosis. Front Endocrinol (Lausanne). 2022;13: 896334. https://doi.org/10.3389/fendo.2022.896334.
54. Dushay J, Chui PC, Gopalakrishnan GS, Varela-Rey M, Crawley M, Fisher FM, et al. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. Gastroenterology. 2010;139:456–63. https://doi.org/10.1053/j.gastro.2010.04.054.
55. Elshinshawy S, Elhaddad H, Abdel Alem S, Shaker O, Salam R, Yosry A, et al. The Interrelation Between Hypothyroidism and Non-alcoholic Fatty Liver Disease, a Cross-sectional Study. J Clin Exp Hepatol. 2023;13:638–48. https://doi.org/10.1016/j.jceh.2023.03.004.
56. Flisiak-Jackiewicz M, Bobrus-Chociej A, Wasilewska N, Tarasow E, Wojtkowska M, Lebensztejn DM. Can hepatokines be regarded as novel non-invasive serum biomarkers of intrahepatic lipid content in obese children? Adv Med Sci. 2019;64:280–4. https://doi.org/10.1016/j.advms.2019.02.005.

57. Athyros VG, Polyzos SA, Kountouras J, Katsiki N, Anagnostis P, Doumas M, et al. Non-Alcoholic Fatty Liver Disease Treatment in Patients with Type 2 Diabetes Mellitus; New Kids on the Block. Curr Vasc Pharmacol. 2020;18:172–81. https://doi.org/10.2174/1570161117666190405164313.
58. Wang YS, Ye J, Cao YH, Zhang R, Liu Y, Zhang SW, et al. Increased serum / plasma fibroblast growth factor 21 in type 2 diabetes mellitus : a systematic review and meta-analysis. Postgrad Med J. 2019;1–6. https://doi.org/10.1136/postgradmedj-2018-136002.
59. Markan KR, Naber MC, Ameka MK, Anderegg MD. Circulating FGF21 Is Liver Derived and Enhances Glucose Uptake During Refeeding and Overfeeding. Diabetes. 2014;63:4057–63. https://doi.org/10.2337/db14-0595.
60. Loomba R, Sanyal AJ, Nakajima A, Neuschwander-Tetri BA, Goodman ZD, Harrison SA, et al. Pegbelfermin in Patients With Nonalcoholic Steatohepatitis and Stage 3 Fibrosis (FALCON 1): A Randomized Phase 2b Study. Clin Gastroenterol Hepatol. 2024;22:102–12. https://doi.org/10.1016/j.cgh.2023.04.011
61. Abdelmalek MF, Sanyal AJ, Nakajima A, Neuschwander-Tetri BA, Goodman ZD, Lawitz EJ, et al. Pegbelfermin in Patients With Nonalcoholic Steatohepatitis and Compensated Cirrhosis (FALCON 2): A Randomized Phase 2b Study. Clin Gastroenterol Hepatol. 2024;22:113–23. https://doi.org/10.1016/j.cgh.2023.04.012.
62. Harrison SA, Frias JP, Neff G, Abrams GA, Lucas KJ, Sanchez W, et al. Safety and efficacy of once-weekly efruxifermin versus placebo in non-alcoholic steatohepatitis (HARMONY): a multicentre, randomised, double-blind, placebo-controlled, phase 2b trial. Lancet Gastroenterol Hepatol. 2023;8:1080–93. https://doi.org/10.1016/S2468-1253(23)00272-8.
63. Jeong C, Han N, Jeon N, Rhee SJ, Staatz CE, Kim MS, et al. Efficacy and Safety of Fibroblast Growth 21 Analogs for the Treatment of Metabolic Dysfunction-Associated Steatohepatitis : A Systematic Review and Meta-Analysis. Clin Pharmacol Ther. 2024;116:72–81. https://doi.org/10.1002/cpt.3278.
64. de Oliveira FD, Khalil SM, de Santana Sato EDB, de Souza MHG, Meine GC. Efficacy and Safety of Fibroblast Growth Factor 21 Analogues for Metabolic Dysfunction-Associated Steatohepatitis: A Systematic Review and Meta-Analysis. Ann Nutr Metab. 2025;81:51–60. https://doi.org/10.1159/000541583.
65. Theofilis P, Oikonomou E, Karakasis P, Pamporis K, Dimitriadis K, Kokkou E, et al. FGF21 Analogues in Patients With Metabolic Diseases: Systematic Review and Meta-Analysis of Randomised Controlled Trials. Liver Int. 2025;45: e70016. https://doi.org/10.1111/liv.70016.
66. He L, Deng L, Zhang Q, Guo J, Zhou J, Song W, et al. Diagnostic Value of CK-18, FGF-21, and Related Biomarker Panel in Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. Biomed Res Int. 2017;2017:9729107. https://doi.org/10.1155/2017/9729107.
67. He Q, Li Y, Yu R, Lin M. Association of FGF21 with Metabolic and Cardiovascular Diseases: A Mendelian Randomization Analysis. Exp Clin Endocrinol Diabetes. 2025. https://doi.org/10.1055/a-2549-6889.
68. Polyzos SA, Mantzoros CS. Metabolic dysfunction-associated steatotic liver disease: Recent turning points for its diagnosis and management. Metabolism. 2024;157: 155936. https://doi.org/10.1016/j.metabol.2024.155936.
69. Lonardo A, Bril F, Caldwell SH, Eslam M, Fan JG, Gish RG, et al. Researchers call for more flexible editorial conduct rather than abruptly adopting only the new MASLD nomenclature. J Hepatol. 2024;80:e192–4. https://doi.org/10.1016/j.jhep.2024.01.012.