Safety and efficacy of FGF21 analogs in nonalcoholic steatohepatitis: a meta-analysis of randomized clinical trials

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Abstract

Introduction: The efficacy and safety of fibroblast growth factor 21 (FGF21) analogs in patients with nonalcoholic steatohepatitis (NASH) remains unclear. Therefore, we aimed to compare the use of FGF21 analogs with placebo in patients with NASH. Methods: We searched Medline, Embase, and Cochrane databases from inception to December 2023. The primary outcomes were: triglycerides levels, high-density lipoprotein (HDL) levels, low-density lipoprotein (LDL) levels, NASH resolution, N-terminal type III collagen propeptide (PRO-C3) levels, and adverse events. Subgroup analyses were performed according to drug dosages. We used Review Manager 5.4 to pool the data, assessing heterogeneity with I2. Results: Eight studies reporting data of 967 patients were included in this review. Follow-up ranged from 16 to 121 weeks and dosage ranged from 3 to 70mg a day. When compared to placebo, FGF21 analogs were significantly associated with a reduction in triglycerides (MD -22.07; 95% CI -36.67 to -7.47) and PRO-C3 (MD -6.12; 95% CI -8.05 to -4.20). There were no significant differences in the resolution of NASH (RR 3.57; 95% CI 0.91 to 13.94). Higher rates of adverse events (RR 1.08; 95% CI 1.01 to 1.14) were observed in patients who received the intervention. Conclusion: Despite a higher incidence of adverse events for FGF21 analogs, they showed positive effects on the lipid profile and biomarker for the formation of fibrotic tissue. However, no improvement in NASH resolution was observed. Keywords: FGF21 analog; pegozafermin; efruxifermin; pegbelfermin; fibrosis; NASH.

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Abstract

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Methods: We searched Medline, Embase, and Cochrane databases from inception to December 2023. The primary outcomes were: triglycerides levels, high-density lipoprotein (HDL) levels, low-density lipoprotein (LDL) levels, NASH resolution, N-terminal type III collagen propeptide (PRO-C3) levels, and adverse events. Subgroup analyses were performed according to drug dosages. We used Review Manager 5.4 to pool the data, assessing heterogeneity with I^2.

Results: Eight studies reporting data of 967 patients were included in this review. Follow-up ranged from 16 to 121 weeks and dosage ranged from 3 to 70mg a day. When compared to placebo, FGF21 analogs were significantly associated with a reduction in triglycerides (MD -22.07; 95% CI -36.67 to -7.47) and PRO-C3 (MD -6.12; 95% CI -8.05 to -4.20). There were no significant differences in the resolution of NASH (RR 3.57; 95% CI 0.91 to 13.94). Higher rates of adverse events (RR 1.08; 95% CI 1.01 to 1.14) were observed in patients who received the intervention.

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Keywords : FGF21 analog; pegozafermin; efruxifermin; pegbelfermin; fibrosis; NASH.

ABBREVIATIONS

FDA – food and drug administration

FGF21 – fibroblast growth factor 21

HbA1c – glycated hemoglobin

HDL – high-density lipoprotein

LDL – low-density lipoprotein

MD – mean difference

NAFLD – non-alcoholic fatty liver disease

NASH – nonalcoholic steatohepatitis

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-Analysis

PRO-C3 - N-terminal type III collagen propeptide

RCT – randomized controlled trial

RR – risk ratio

1. Introduction

Non-alcoholic steatohepatitis (NASH) represents an advanced form of non-alcoholic fatty liver disease (NAFLD), characterized by inflammation and fibrosis in the liver.[1] Currently, it affects approximately 20 to 30% of the global population, emerging as the leading hepatic cause of morbidity and mortality.[2] The significant increase in the incidence of this condition and its potentially lethal complications, such as cirrhosis or hepatocellular carcinoma, highlight it as a significant challenge for public health.[3,4] It is important to emphasize that, to date, there are no drugs approved by the Food and Drug Administration specifically intended for the treatment of NASH, which emphasizes the urgency of implementing effective strategies to address this condition.[5]

Fibroblast growth factor 21 (FGF21), a hormone that regulates lipid and glucose metabolism, has shown hepatoprotective effects in preclinical studies. These findings suggest a potential therapeutic role for FGF21 in NAFLD, paving the way for the development of more effective therapeutic strategies in addressing this condition.[6,7] Current literature and clinical practice offer promising perspectives on the therapeutic potential of FGF21 analogs in NAFLD, highlighting their role as mediators of homeostasis and inflammatory process in the disease metabolism. These analogs demonstrate the ability to reduce liver fat, prevent hepatocyte damage and suppress inflammation.[8,9] However, the variability in individual responses and the lack of consensus on assessment criteria pose significant challenges to the clinical implementation of these therapies.

Recently, two large, planned trials have investigated the role of FG21 analogs as a therapy of choice for clinical and laboratory benefits in patients with NASH (Non-Alcoholic Steatohepatitis). These studies have substantially increased the population of randomized patients receiving efruxifermin, pegbelfermin, or pegozafermin as a strategy for resolving NASH and improving metabolic profiles.[10,11] Therefore, we aimed to perform a meta-analysis of Randomized Controlled Trials (RCTs) to assess the safety and efficacy of FGF21 analogs in the treatment of NASH.

2. Material and Methods

2.1 Eligibility criteria

Inclusion in this meta-analysis was restricted to studies that met all the following eligibility criteria: (1) randomized controlled trials (RCTs); (2) comparing FGF21 analogs to placebo; (3) enrolling patients with NASH; and (4) reporting at least one of the outcomes of interest. There was no restriction on follow-up, as the preliminary literature review did not find substantial heterogeneity in follow-up between different

studies. We excluded studies with (1) no control group; (2) population overlap; or (3) in patients without NASH.

2.2 Search strategy and data extraction

We systematically searched Medline, Embase, and Cochrane Central Register of Controlled Trials from inception to December 2023 with the following search terms: ("FGF21 analogue" OR pegozafermin OR pegbelfermin OR efruxifermin) AND ("non-alcoholic hepatic steatosis" OR steatohepatitis OR NASH OR "fatty liver disease"). The references from all included studies were also searched manually for any additional studies. Two authors (M.S. and L.B.) independently extracted the data following predefined search criteria and quality assessment. The prospective meta-analysis protocol was registered on PROSPERO on January 15, 2024, under protocol CRD42024497414.

2.3 Endpoints and subanalyses

Efficacy outcomes included NASH resolution, glycated hemoglobin (HbA1c), serum triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), PRO-C3, and body weight. Adverse events were the safety outcome of interest. Prespecified subanalysis included FGF21 analog dosing data: (1) low dose: 3 mg to 18 mg; (2) medium dose: 20 mg to 36 mg; and (3) high dose: higher than 40 mg.

2.4 Quality assessment

Quality assessment of RCTs was performed using the Cochrane Collaboration's tool for assessing risk of bias in randomized trials (RoB-2), in which studies are scored as high, low, or unclear risk of bias in 5 domains: selection, performance, detection, attrition, and reporting biases. [12] Publication bias was investigated, for outcomes that included all studies in this meta-analysis, by funnel-plot analysis of point estimates according to study weights.

2.5 Statistical analysis

We performed this systematic review and meta-analysis following the Cochrane Collaboration and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement guidelines.[13,14] Treatment effects were compared using mean differences (MD) and 95% confidence intervals (CI) for continuous outcomes, and risk ratio (RR) with 95% CI for binary endpoints using the DerSimonian and Laird random-effects model. We used the Cochran Q test and I^2 statistics to assess for heterogeneity; P values inferior to 0.01 were considered significant for heterogeneity. We considered $I^2 < 25\%$; $25\% < I^2 < 75\%$, and $I^2 > 75\%$ as low, medium and high heterogeneity, respectively. Review Manager 5.1.7 (Cochrane Centre, The Cochrane Collaboration, Denmark) was used for statistical analysis.

Sensitivity analysis

To explore the robustness of the results and identify possible outliers, we performed a leave-one-out sensitivity analysis for outcomes with high heterogeneity, by systematically removing each study from the pooled result. Additionally, another sensitivity analysis was performed by removing studies with a high risk of bias.

3. Results

3.1 Study selection and characteristics

As detailed in Figure 1, the initial search yielded 317 results. After the removal of duplicate records and ineligible studies, 28 remained and were fully reviewed based on inclusion criteria. Of these, a total of 8 RCTs were included, comprising 967 patients.[10,11,15-20] A total of 689 (71.2%) patients received FGF21 analogs and 278 patients (28.8%) received placebo. The follow-up ranged between 16 to 121 weeks. The mean patient age was 55.5 years and 37% patients in the FGF21 analogs group were males. Study characteristics are reported in Table 1. Three studies used efruxifermin as a treatment regimen;[15-17] whereas two studies used pegozafermin.[18,19] The remaining studies used pegbelfermin.[10,11,20]

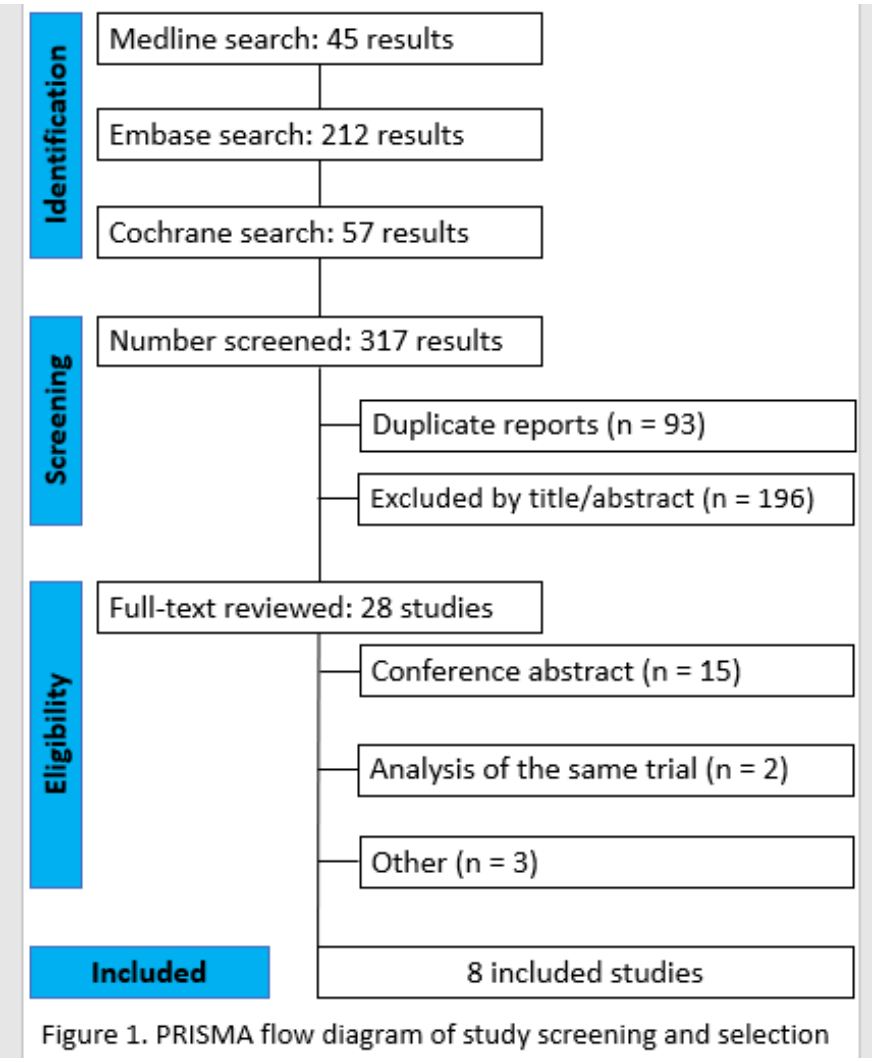

Figure 1. PRISMA flow diagram of study screening and selection

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Table 1. Baseline characteristics of included studies

Study	FGF	Follow-up^{+}, weeks	Patients FGF/PB	Male, % FGF/PB	Age^{+}, y FGF/PB	BMI ++, FGF/PB	Fibrosis stage III/IV, % FGF/PB	ALT, U/liter FGF/PB	TG, mg/ml FGF/PB
BALANCED 2021	Efruxifermin	30	59/21	47.5/28.5	52.0/52.4	37.5/37.6	28.8/38	57.4/50.7	177.7/208.3
HARMONY 2023	Efruxifermin	96	85/43	38.8/37.2	54.4/ 55	37.7/38.7	63.5/69.7	56.5/62.2	156.1/ 169.7
ENLIVEN 2023	Pegozafermin	46	151/71	36.4/32	55.2/56.3	35.8/38.1	64.9/69.1	58.7/49.6	172.6/170.3
FALCON 1 2024	Pegbelfermin	48	148/49	41,2/40,8	56.6/57.5	35.6/35.2	100	53.4/53.4	163.8/ 200.4

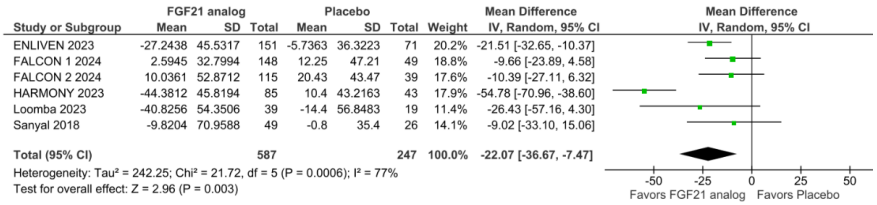
Study	FGF	Follow-up^{+}, weeks	Patients FGF/PB	Male, % FGF/PB	Age^{+}, y FGF/PB	BMI ++, FGF/PB	Fibrosis stage III/IV, % FGF/PB	ALT, U/liter FGF/PB	TG, mg/ml FGF/PB
FALCON 2 2024	Pegbelfermin	121	115/39	35.6/38.4	58.6/61.4	35.6/35.4	100	48.7/48.3	154.0/133.4
Sanyal 2018	Pegbelfermin	16	49/26	34.6/38.4	52/47	34.4/37	7/1	67.9/80	198.2/ 171
Loomba 2023	Pegozafermin	53	62/19	38.7/36.8	51.7/52.6	34.8/33.8	NA	42.2/38.8	NA
BALANCED Cohort C 2023	HEC88473	16	20/10	20/70	57.1/61.1	36.0/39.1	NA	31.7/32.7	134.6/121.7

BMI++ The body-mass index is the weight in kilograms divided by the square of the height in meters; Fibrosis stage: fibrosis stages according to the Clinical Research Network (CRN) in patients with nonalcoholic steatohepatitis (NASH); $^{+}$mean or median; ALT: alanine aminotransferase; TG: Triglycerides; HbA1c: Glycated hemoglobin; FGF: FGF21 analog; PB: placebo; U: units; L: liter; mg: milligram; mL: milliliter

3.2 Pooled analyses of all studies

Regarding the lipid panel, there was a statistically significant reduction in triglycerides (MD -22.07; 95% CI -36.67 to -7.47; p=0.0006; I^2=77%; Figure 2A) and LDL (MD -7.10; 95% CI -13.40 to -0.80; p=0.03; I^2=71%; Figure 2B) in the FGF21 analog group in comparison to placebo. Moreover, there was an increase in HDL levels in the intervention group (MD 6.28; 95% CI 3.34 to 9.22; p=0.0001; I^2=80%; Figure 3A) compared to placebo. The leave-one-out sensitivity analysis confirmed the result and reduced heterogeneity with the removal of HARMONY (MD 4.75; 95% CI 3.38 to 6.12; I^2=2%; Figure S13).

A



B

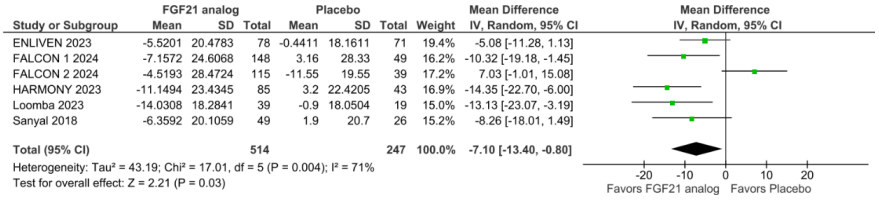


Figure 2 A. Triglyceride levels were significantly lower in the FGF21 analog group. **B** : LDL levels were

significantly lower in the FGF21 analog group. IV = Inverse Variance; CI = Confidence Interval; M-H: Mantel Haenszel; SD = Standard Deviation.

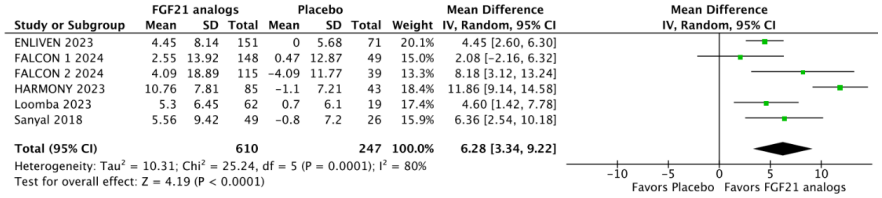


Figure 3 A: HDL levels were significantly higher in the FGF21 analog group. **B:** Pro C3 was significantly lower in the FGF21 analog group. IV: Inverse Variance; CI = Confidence Interval; M-H: Mantel Haenszel; SD = Standard Deviation.

In those who received the intervention, there was a statistically significant reduction in levels of PROC3 (MD -6.12; 95% CI -8.05 to -4.20; p=0.09; I^2=46%; Figure 3B). Furthermore, there was no significant difference in NASH resolution between both groups (RR 3.57; 95% CI 0.91 to 13.94; p=0.07; I^2=54%; Figure 4). On sensitivity analysis, we removed FALCON 1 due to the high risk of bias. After its removal, statistically significant results were found (RR 7.14; 95% CI 2.27 to 22.44; I^2=0%; Figure S14).

3B: Pro C3 was significantly lower in the FGF21 analog group. IV: Inverse Variance; CI = Confidence Interval; M-H: Mantel Haenszel; SD = Standard Deviation.

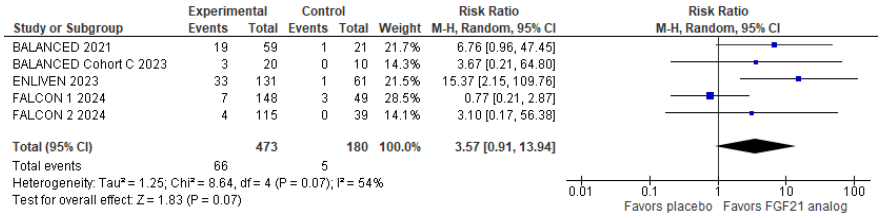


Figure 4. NASH resolution was not significantly different between FGF21 analog and placebo. M-H = Mantel-Haenszel method; CI = Confidence Interval.

FGF21 therapy led to substantial reductions in HbA1c levels among patients (MD -0.20; 95% CI -0.37 to -0.02; p=0.03; I^2=22%; Figure S2). In addition, weight loss was not significantly different between groups (MD -0.33; 95% CI -1.36 to 0.70; p=0.55; I^2=9%; Figure S3). However, the rate of adverse events was significantly higher in the FGF21 analog group in comparison to placebo (RR 1.08; 95% CI 1.01 to 1.14; p=0.02; I^2=0%; Figure 5), although there was no statistically significant difference in serious adverse effects between groups (RR 1.05; 95% CI 0.58 to 1.90; p=0.87; I^2=10%; Figure S4).

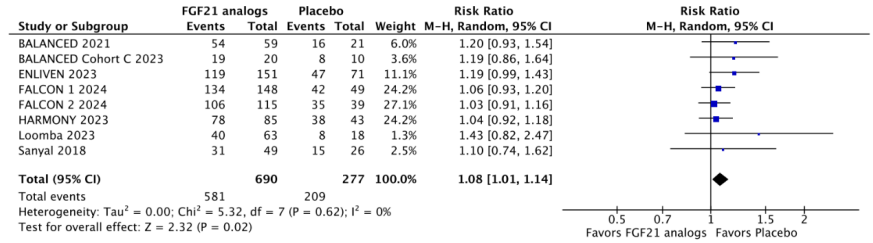


Figure 5. There was a higher risk of adverse events in patients who underwent FGF21 analog compared to placebo. M-H = Mantel-Haenszel method; CI = Confidence Interval.

3.3 Subgroup analyses in selected populations

In the subgroup analyses, the outcomes stratifying the different dosages of the drug are consistent with the overall analysis. There was no difference between the groups in the resolution of NASH at low (RR 4.34; 95% CI 0.60 to 31.27; p=0.14; I^2=62%; Figure S5), medium (RR 3.91; 95% CI 0.85 to 16.06; p=0.08; I^2=52%; Figure S5) or high doses (RR 3.54; 95% CI 0.86 to 14.49; p=0.03; I^2=44%; Figure S5). The removal of each study from the pooled analysis did not affect the NASH resolution endpoint, except for FALCON-1. The withdrawal of this study increased the frequency of resolution of NASH for low (RR 12.20, 95% CI 1.89-78.71; p=0.009; I2= 12%; Figure S15), medium (RR 8.73, 95% CI 2.42-31.48; p=0.0009; I2= 0%) and high doses (RR 7.39, 95% CI 2.30-23.79; p=0.0008; I2= 0%).

The use of FGF21 in low (MD -0.75; 95% CI -0.80 to 2.31; p=0.96; I^2=0%; Figure S6), medium (MD -0.51; 95% CI -1.70 to 0.69; p=0.48; I^2=0%; Figure S6) or high doses (MD -1.26; 95% CI -3.93 to 1.42; p=0.04; I^2=70%; Figure S6) did not contribute to weight reduction, corroborating the global analysis. Regarding adverse events, there was also no significant difference between groups in low (RR 1.17; 95% CI 1.00 to 1.37; p=0.04; I^2=60%; Figure S7), medium (RR 1.09; 95% CI 1.00 to 1.19; p=0.24; I^2=25%; Supplementary Figure S8) or high doses (RR 1.07; 95% CI 1.00 to 1.15; p=0; I^2=80%; Supplementary Figure S9). The subanalysis results for serious adverse events revealed no significant association between the utilization of FGF21 analogs at low (RR 0.93; 95% CI 0.33 to 2.26; p=0.20; I^2=35%; Figure S10), medium (RR 1.10; 95% CI 0.58 to 2.08; p=0.53; I^2=0%; Figure S11) or high doses (RR 1.49; 95% CI 0.64 to 3.47; p=0.19; I^2=33%; Figure S12).

3.4 Quality assessment

Individual bias appraisal is reported in Supplementary Table 1. Two studies lost points in domains related to deviation from the intended result, due to changes in the pre-specified plan.[10,11]One study was considered at high risk of bias.[10] The remaining studies were considered low risk. In the analysis of the funnel chart, the outcome of adverse events was evaluated (Supplementary Figure 1). As illustrated in Supplementary Figure 1, a symmetrical distribution according to weight occurred, which converged to the combined effect of the treatment as the weights increased. There was no evidence of publication bias.

4. Discussion

In this systematic review and meta-analysis of 8 RCTs, including 967 patients, the safety and efficacy of FGF21 analogs were compared to placebo in patients with NASH. The main findings from the combined analysis were: (1) NASH resolution was not different between the two groups; (2) FGF21 analogs reduced glycated hemoglobin; (3) triglyceride levels were lower in patients using FGF21 analogs; (4) the intervention group had a higher incidence of adverse events.

The mechanism of NASH development, progression, and complications is likely to be related to metabolic syndrome, diabetes, and dyslipidemia.[21-23] Ideally, treatment for this condition would simultaneously address both local hepatic and metabolic factors.[24-26] In this regard, FGF21 analogs demonstrate efficacy in inhibiting adipose tissue lipolysis and liver lipogenesis, contributing to a significant decrease in intrahepatic triglyceride concentrations.[27,28] However, in the FALCON 1 trial, 197 patients with stage of III or IV fibrosis were randomized to receive once-weekly subcutaneous injections of pegbelfermin (PGBF) or placebo for 48 weeks. In the 148 patients who underwent PGBF, a higher NASH resolution was not found.[10] The study also did not meet its primary outcomes, such as 1-stage fibrosis improvement without NASH worsening or NASH improvement without fibrosis worsening probably due to a lack of dose-related changes in PGBF response rates. The effect of FGF21 analogs on NASH resolution in the remained trials, however, found a benefit in the same endpoint for patients who were randomized to receive Pegbelfermin,[11] Pegozafermin,[18] and Efruxifermin [15,16] as compared with placebo.

There also exists uncertainty as to the optimal dose of FGF21 analogs for NASH disease. The dosage of FGF21 analogs varied substantially between the included studies, ranging from 3 mg to 70 mg. Accordingly, the lack

of clinical benefit with FGF21 analogs for NASH in previous studies could be related to the relatively reduced number of patients exposed to FGF21 analogs within groups by dose. Therefore, we performed a dedicated analysis of studies with low, medium, and high doses for NASH resolution, finding no difference between groups. However, a leave-one-out analysis excluding the FALCON-1 trial[10]showed a higher incidence of NASH resolution in patients receiving FGF21 analogs, regardless of the dose. In that trial, the study population had significant NASH disease activity and fibrosis at baseline in non-invasive assessments. In addition, the FALCON 1 study cirrhosis progression rate at week 24 was higher than those seen in previous studies at later time points [29,30] and in a natural history study.[31] Thus, in the absence of definitive improvement in clinical outcomes, our findings do not appear to support FGF21 analogs in all NASH patients, considering the administration of these therapeutic agents in non-severe fibrosis and NASH disease.

Furthermore, NAFLD is intrinsically linked to insulin resistance, with a prevalence about 2 to 5 times higher in individuals with type 2 diabetes compared to non-diabetics.[32-35] This association, in turn, amplifies the risks of hepatic fibrosis and mortality.[36,37] Therefore, controlling HbA1c is essential for a better prognosis among patients [38,39]Recent studies demonstrate benefits in insulin resistance, suggesting a potential positive impact on the development of type 2 diabetes (DM2).[15-17]As observed in the HARMONY study, by suppressing lipolysis, FGF21 analogs increase the action of insulin in adipose tissue.[17] These data corroborate the findings of our meta-analysis, in which the drug is associated with a reduction in glycated hemoglobin.

It is well established that the function of fibroblast growth factor 21 is related to the regulation of glucose and lipid metabolism, as well as the development of adaptive responses to stress and nutrient deprivation.[40] In addition to lipid metabolism, FGF-21 stimulates lipolysis in brown adipose tissue during fasting, hepatic ketogenesis, and free fatty acid oxidation, acting in an autocrine and paracrine manner in the liver.[41] The resulting free fatty acids from the lipolysis process provide substrates for these metabolic processes and stimulate PPARα activity in the liver, leading to increased protein and FGF21 gene transcription.[42,43] This is consistent with our analysis, which suggests that FGF21 analogs are associated with a reduction in triglyceride levels, probably due to free fatty acid oxidation.

Although the data is promising, addressing potential side effects is essential before considering these drugs as standard treatment for NASH. Overall, FGF21 analogs have been considered safe, with frequent but manageable adverse events such as gastrointestinal disorders and injection site reactions.[44] In a recently published RCT, adverse events were reported in 79% of patients receiving weekly pegofazemin. However, only one severe event, acute pancreatitis, was deemed by the investigator to be drug-related.[18] In the HARMONY study, among the 63 adverse events reported in patients in the intervention group, only one case of ulcerative esophagitis in a patient with a history of gastroesophageal reflux disease was considered severe and was attributed to the use of efruxifermin.[17]

Our study has some important limitations. First, the follow-up time differs significantly between studies. Second, the drug dosage used was also heterogeneous among studies. To address this, we performed a subgroup analysis by dosage (low, median, and high) for the main outcomes. Third, patients with different fibrosis stages were included. Unfortunately, the intended subgroup analysis by fibrosis stage was not possible due to lack of data, since the studies had a variety of fibrosis worsening and they did not report outcomes according to this baseline characteristic. These factors contributed to high heterogeneity in the pooled analysis. In light of this, leave-one-out sensitivity analyses were performed.

5. Conclusion

The use of FGF21 analogs reduced serum triglyceride levels, LDL levels, PRO-C3 levels, and glycated hemoglobin while increasing HDL levels. Despite no significant differences in NASH resolution and weight loss between the FGF21 analog-treated group and the control group, these findings suggest a potential dual benefit of FGF21 in liver health and glycemic control. We emphasize the need for larger RCTs and do not support the routine use of FGF21 analogs in all patients with NASH. Instead, FGF21 analogs may be reasonable among selected low stages of fibrosis and NASH disease, at the discretion of clinicians and patients through shared decision-making based on individualized considerations of treatment risks and benefits.

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