FGF-21 analogues for treatment of non-alcoholic steatohepatitis and fibrosis: a meta-analysis with fragility index of phase 2 randomised placebo-controlled trials

We have read with interest the excellent review by Trauner and Fuchs on the novel therapeutic approaches that are currently developed for treating non-alcoholic steatohepatitis (NASH). Although there are no licensed pharmacotherapies for NASH, long-acting fibroblast growth factor-21 (FGF-21) analogues are being evaluated to treat NASH because FGF-21 is a pleotropic liver-derived hormone regulating lipid metabolism, insulin sensitivity and energy homeostasis, all mechanisms closely implicated in NASH development.

To quantify the magnitude of the possible hepatoprotective effects of FGF-21 analogues, we systematically searched three electronic databases from the inception date to 1 September 2023 to identify phase-2 randomised controlled trials (RCTs) examining the efficacy of FGF-21 analogues on the US Food and Drug Administration defined histological endpoints for conditional approval of new drugs for NASH, that is, NASH resolution without worsening of fibrosis or ≥1 stage fibrosis improvement without worsening of NASH. We also calculated the fragility index (FI) to establish the robustness of the trial’s data. More details of the systematic review are reported in online supplemental material.

We included five phase-2 placebo-controlled RCTs involving 602 obese adults with biopsy-confirmed NASH and stages F1–F4 fibrosis, most of whom had diabetes (446 randomly assigned to FGF-21 analogues and 156 assigned to placebo). Online supplemental figure 1 shows the results of the literature research and study selection. The main characteristics of the eligible RCTs are summarised in table 1. Three RCTs were in phase 2b, whereas two were in phase 2a with histological endpoints available only for a subset of individuals. Figure 1 shows the treatment effects of FGF-21 analogues on resolution of NASH without worsening of fibrosis (figure 1A, n=3 RCTs) or ≥1 stage fibrosis improvement without worsening of NASH (figure 1B, n=5 RCTs) compared with placebo. Treatment with once-weekly subcutaneous FGF-21 analogues for 16–48 weeks resulted in a significantly higher percentage of patients with NASH resolution with no worsening of fibrosis, or ≥1 stage fibrosis improvement without worsening of NASH than placebo (especially using common-effects models). However, it should be noted that the FI for both histological endpoints was small, suggesting that the findings are weak. Specifically, the FI for the ≥1 stage fibrosis improvement was 3 (ie, only three participants from the active-comparator arm should be reassigned to the placebo arm to change the result from significant to non-significant), while the FI for the resolution of NASH was 8.

The FI measures the robustness (or fragility) of the results from a clinical trial using dichotomous outcomes. The FI represents the minimum number of participants whose status needs to change from an ‘event’ to a ‘non-event’ (or vice versa) so that the results switch from statistically significant to non-significant. Whether few participants are needed to hamper the significance of a result, the strength of evidence for affirming the superiority of a specific treatment over a placebo might be questionable. When we looked at the efficacy of pioglitazone or semaglutide in achieving resolution of NASH or ≥1 stage fibrosis improvement without worsening of NASH, we found that the FI of pioglitazone for NASH resolution with no worsening of fibrosis was 11, while the FI for ≥1 stage fibrosis improvement was

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<th>Table 1 Main characteristics of the phase-2 RCTs examining the efficacy of long-acting FGF-21 analogues for treating adult individuals with biopsy-confirmed NASH and different stages of fibrosis included in the meta-analysis</th>
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Ref. #4 and #5 did not provide mean age, mean BMI and percentage of men and known T2DM because the histological endpoints were available only in a small subset of individuals. In these two trials, the primary study endpoints were changes from baseline in liver fat content (as assessed by MRI-PDFF), liver stiffness and other non-invasive biomarkers of liver fibrosis.

*Risk of bias was assessed using the Cochrane risk-of-bias tool for each eligible RCT.*

FGF-21, fibroblast growth factor-21; NASH, non-alcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.
In a phase-2b placebo-controlled RCT testing semaglutide, we found that the FI of semaglutide for the resolution of NASH without worsening of fibrosis was 8. In conclusion, the results of this meta-analysis suggest that FGF-21 analogues are a promising treatment option for adults with biopsy-confirmed NASH and fibrosis. However, based on the FI of the trials' data, uncertainty remains about the robustness and clinical benefit of FGF-21 analogues. Future large phase-3 RCTs with long-term follow-up are needed to have more robust findings.

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REFERENCES


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Figure 1 Forest plots and pooled estimates of the effects of long-acting FGF-21 analogues on the histological resolution of NASH with no worsening of fibrosis (A), n=3 RCTs 6–8) or the improvement in fibrosis of one stage or more without worsening of NASH (B, n=5 RCTs 9–11) compared with placebo. The pooled and individual effect sizes for all RCTs are expressed as OR and 95% CIs, as estimated by both common-effects and random-effects models. For the active-comparator group (ie, participants using FGF-21 analogues), we calculated the individual effect sizes of each RCT by combining the treatment effect data of variable dosages of FGF-21 analogues into a single group (to avoid including individuals in the placebo group several times in the analysis). With regard to this, it is important to note that before combining the data of the active-comparator group for each RCT, we have previously tested in a multivariate meta-regression analysis that neither the different type nor the different dosage of FGF-21 analogue used in each RCT were significant predictors of the observed effects of this drug class on the histological liver endpoints. FGF-21, fibroblast growth factor-21; NASH, non-alcoholic steatohepatitis; RCT, non-alcoholic steatohepatitis.
METHODS

Data sources and searches
We performed a systematic review and meta-analysis following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (http://www.prisma-statement.org). We systematically searched in PubMed, Scopus, and Web of Science, from the inception date to 1 September 2023, using the terms “NAFLD” OR “non-alcoholic fatty liver disease” OR “non-alcoholic steatohepatitis” OR “NASH” AND “FGF-21 analogues” OR “efruxifermin” OR “pegbelfermin” OR “pegvozafermin” IN “humans”, to identify all published phase-2 randomized placebo-controlled trials (RCTs) examining the efficacy of FGF-21 analogues on the histological resolution of NASH without worsening of fibrosis or the improvement in liver fibrosis of one stage or more without worsening of NASH (i.e., the two FDA defined histological endpoints for conditional approval of new drugs for NASH). No language restrictions were imposed. In addition, we reviewed references from relevant original papers and reviews to identify further eligible RCTs not covered by the three large electronic databases.

Study selection
Studies were included if they met the following criteria: (1) phase-2 placebo-controlled RCTs examining the efficacy of FGF-21 analogues on histological resolution of NASH without worsening of liver fibrosis, or ≥1-stage fibrosis improvement without worsening of NASH in adults with biopsy-confirmed NASH and different stages of liver fibrosis; and (2) phase 2 RCTs reporting odds ratios (OR) with 95% confidence intervals for the two histological endpoints of interest. Phase-2 placebo-controlled RCTs examining the efficacy of FGF-21 analogues on hepatic fat content assessed by MRI-proton density fat fraction and non-invasive biomarkers of fibrosis that did not also have histological liver endpoints were excluded from the meta-analysis. Congress abstracts, theses, case reports, reviews, commentaries, editorials, practice guidelines, and observational studies were also excluded from the meta-analysis.

Data extraction and quality assessment
Two investigators (AM and GT) independently examined all titles and abstracts and obtained full texts of potentially eligible studies. Working independently and in duplicate, we analyzed whether these studies met the inclusion criteria mentioned above. Discrepancies were resolved by consensus. For all phase 2 RCTs, we extracted information on study design, sample size, and the number of successes and failures for each histological liver endpoint for both the active-comparator group (using FGF-21 analogues) and the placebo group. For the active-comparator group, in each eligible RCT, we combined treatment effects of variable weekly dosages of FGF-21 analogues into a single group (to avoid including subjects in the placebo group several times in the analysis). We did not contact any corresponding author of the eligible RCTs to obtain further information for the meta-analysis.

Two investigators (AM and GT) independently evaluated the risk of bias for each eligible RCT. For this purpose, we used the Cochrane Collaboration’s tool, which assesses the following seven potential sources of bias: random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other biases. For each of these domains, we categorized each eligible RCT into three categories: low, unclear, or high risk of bias (Cumpston M et al. Updated guidance for trusted systematic reviews: a

Data synthesis and analysis
The odds ratios with their 95% CIs were considered as the effect size for each eligible study. An overall effect size estimate was calculated using the common-effects model (i.e., the Mantel-Haenszel method) or the random-effects model (i.e., the DerSimonian-Laird method). Visual inspection of the forest plots was used to evaluate the possibility of statistical heterogeneity. Statistical heterogeneity was assessed by the $I^2$-statistics, which estimates the percentage of variability across the eligible RCTs due to heterogeneity rather than chance alone. $I^2$ values of about 25% represent low heterogeneity, about 50% represent medium heterogeneity, and about 75% represent high heterogeneity. We also performed multivariate meta-regression models adjusted for the different type and the different weekly dosage of FGF-21 analogue used in each single RCT.

The fragility index (FI) for meta-analysis was also calculated. In particular, the FI was defined as the minimum number of participants from trials included in the meta-analysis for which a modification of the event status (i.e., changing events to non-events or non-events to events) can change the statistical significance of the pooled treatment effect (see Atal I et al. The statistical significance of meta-analyses is frequently fragile: definition of a fragility index for meta-analyses. J Clin Epidemiol. 2019;111:32-40). The larger the FI, the more robust are the trial’s data. Given the small number of RCTs in the meta-analysis (n=5 studies), a formal assessment of the publication bias using the funnel plot was not performable according to the Cochrane guidance for systematic reviews (Cumpston M et al. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. Cochrane Database Syst Rev. 2019;10:ED000142).

All two-sided statistical tests used a significance level of P-value <0.05. We used STATA version 17.0 (StataCorp, College Station, Texas, USA) and its meta-analysis package and R version 4.2.2 for all statistical analyses.
**Supplementary Figure 1.** PRISMA flow chart of the study selection.

**PRISMA 2009 Flow Diagram**

- **Records identified through database searching on three large electronic databases, including articles published until September 1st, 2023 (n = 59)**

- **Additional records identified through other sources (n = 0)**

- **Records after duplicates removed (n = 59)**

- **Records screened (n = 59)**

- **Records excluded (n = 51)**

- **Full-text articles assessed for eligibility (n = 8)**

- **Full-text articles excluded, with reasons (n = 3)**

- **Studies included in qualitative synthesis (n = 5)**

- **Studies included in quantitative synthesis (meta-analysis) (n = 5)**

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