Efficacy of peroxisome proliferator-activated receptor agonists, glucagon-like peptide-1 receptor agonists or sodium-glucose cotransporter-2 inhibitors for treatment of non-alcoholic fatty liver disease: A systematic review

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Word count: 153 abstract; 4461 text (*excluding* title page, abstract, references, tables and figure legends). **Figures**: 1, **Tables**: 3 + online-only supplementary material (**supplementary Tables**: 4, **supplementary Figures**: 3). **References**: 70

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SUMMARY

There are no licensed treatments for nonalcoholic fatty liver disease (NAFLD), but three different classes of anti-hyperglycaemic agents [peroxisome proliferator-activated receptor (PPAR) agonists, glucagon-like peptide-1 receptor agonists (GLP-1RAs) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors] show promise in the treatment of NAFLD. We undertook an updated systematic review of randomised controlled trials examining the efficacy of PPAR agonists, GLP-1RAs or SGLT2 inhibitors to specifically treat NAFLD in adults with or without type 2 diabetes. A total of 25 active-controlled or placebo-controlled trials met our inclusion criteria [PPAR agonists (n=8), GLP-1RAs (n=10) or SGLT-2 inhibitors (n=7)]. 2,597 individuals (53% men; mean (SD) age: 52±6 years; BMI: 32±3 kg/m^2; 62% with diabetes) were included. Pioglitazone, lanifibranor and GLP1-RAs (mostly liraglutide and semaglutide) improved individual histologic scores of NASH (steatosis, ballooning, lobular inflammation) or achieved resolution of NASH without worsening of fibrosis. SGLT-2 inhibitors (mostly empagliflozin and dapagliflozin) improved liver fat content, as assessed by magnetic resonance-based techniques.

1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a public health problem across the globe. Accumulating evidence supports that NAFLD is a "multi-system" disease requiring a multidisciplinary and holistic approach[1,2]. Its natural history, the development of nonalcoholic steatohepatitis (NASH) and fibrosis, is highly variable, prone to multiple genetic and environmental disease modifiers, and may fluctuate over time[3]. The complexity of the pathophysiology of this common and burdensome liver disease is clearly reflected by the multitude of pharmacological targets in development.

The incidence of NAFLD is rapidly increasing worldwide in parallel to the epidemics of obesity and type 2 diabetes mellitus (T2DM)[4]. Furthermore, it is known that T2DM is one of the strongest clinical risk factors for a faster progression of NAFLD to NASH, cirrhosis and hepatocellular carcinoma[5-8].

To date, the management of NAFLD is mainly based on lifestyle interventions and early treatment of coexisting cardiometabolic risk factors[2,8,9]. However, several drugs with different mechanisms of action are now in phase 2 and 3 development for treating this metabolic liver disease and could enter clinical practice in the near future[10]. Although there are currently no approved pharmacotherapies to specifically treat NAFLD or NASH, because T2DM is closely linked to NAFLD and its more severe histologic forms, an ever-increasing number of randomised controlled trials (RCTs) and non-randomised intervention studies have focused on the efficacy of newer anti-hyperglycaemic drugs, such as peroxisome proliferator-activated receptor (PPAR) agonists (mostly pioglitazone, but also other newer dual- or pan-PPAR agonists, such as elafibranor, saroglitazar or lanifibranor), glucagon-like peptide-1 receptor agonists (GLP-1RAs) and sodium-glucose cotransporter-2 (SGLT-2) inhibitors, amongst individuals with NAFLD or NASH, regardless of the

presence of T2DM (as discussed in detail below). The PPAR-gamma agonist pioglitazone is the best-studied pharmacological drug in NASH[11-13]. However, promising hepato-protective effects have been recently reported in some phase-2 RCTs using GLP-1RAs or SGLT-2 inhibitors. In addition, recent large RCTs on pioglitazone, GLP-1RAs or SGLT-2 inhibitors have also shown that these drugs exert beneficial effects on long-term risk of major adverse cardiovascular events[14-16], which is a leading cause of morbidity and mortality in people with NAFLD[17]. This may, therefore, represent an attractive bonus for the long-term use of these agents in individuals with NAFLD or NASH, especially in those who are obese or have T2DM.

Thus, we undertook an updated systematic review of active-controlled or placebo-controlled randomised trials that have examined the efficacy and safety of PPAR agonists, GLP-1RAs or SGLT2 inhibitors to specifically treat NAFLD or NASH in adults with or without established T2DM.

2. METHODS

We performed a systematic review of studies that assessed the effects of PPAR agonists, GLP-1RAs or SGLT2 inhibitors for the treatment of NAFLD or NASH. We reported the results according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement[18]. The protocol for the systematic review was registered in advance on Open Science Framework registries (no: osf.io/ner56).

2.1 Eligibility criteria

Studies were included in the systematic review if they met the following criteria: (1) they were phase 2 or phase 3 active-controlled or placebo-controlled RCTs that used a PPAR agonist, GLP-

1RA or SGLT2 inhibitor for the treatment of NAFLD or NASH; (2) enrolled individuals with NAFLD or NASH, where the diagnosis of this liver disease was based on either liver biopsy or magnetic resonance-based techniques (i.e., magnetic resonance-protein density fat fraction [MRI-PDFF] or magnetic resonance spectroscopy [MRS]); and (3) enrolled at least 15 subjects per each treatment arm of interest. Studies were excluded if they were: (1) observational or non-randomised intervention studies; (2) trials enrolling children or adolescents (<18 years old); (3) trials of futile therapy based on the European Association for the Study of the Liver (EASL), the American Association for the Study of Liver Diseases (AASLD) practice guidelines or recent systematic reviews (e.g., metformin, sulphonylureas and dipeptidyl peptidase–4 inhibitors)[12,13,19,20]; (4) trials testing the efficacy of rosiglitazone on NASH, because this drug was withdrawn from the market in Europe and other countries in 2010; or (5) trials using liver ultrasonography, computed tomography, vibration-controlled transient elastography or blood biomarkers/scores for the diagnosis of NAFLD.

2.2 Search strategy

We systematically searched four large electronic databases (PubMed, Scopus, Web of Science and ClinicalTrials.gov), using pre-defined keywords, in order to identify relevant active-controlled or placebo-controlled randomised trials of adults with NAFLD or NASH, published until May 1, 2021. The search strategy and the search free text terms used for the systematic review are available in **supplementary Tables 1-3**. Searches were restricted to human studies. Studies in languages other than English were also excluded.

2.3 Study selection

Two independent investigators (AM and GT) reviewed the titles and abstracts of all citations identified by the search. Full-text articles were retrieved for the included abstracts and were subsequently screened for eligibility (according to the aforementioned inclusion criteria) by two independent investigators (AM and GT). Disagreements at this level were resolved by consensus and a third reviewer if needed (CDB).

2.4 Data extraction and quality assessment

Data extraction was done individually by two investigators (AM and GT). For all RCTs, we extracted information on first author, publication year, study country, main participants' characteristics, types of interventions (and daily dosages of drugs used), duration of treatment, methods used for the diagnosis of NAFLD or NASH, and results for effectiveness and harms outcomes. The primary outcomes of interest of this systematic review were the changes in liver fat content (as assessed by MRI-PDFF or MRS), changes in histologic features of NASH, or resolution of NASH without worsening of fibrosis or improvement in fibrosis stage without worsening of NASH. We also extracted information on changes in serum aminotransferase levels, body weight, haemoglobin A1c and serious adverse events, as well as percentage of withdrawals due to adverse events.

Each eligible RCT was assessed for quality by two independent reviewers (AM and GT), with disagreements resolved through consensus. Risk of bias for each study was assessed using the Cochrane risk of bias assessment tool in which studies were deemed to be at low, high, or unclear risk[21].

3. RESULTS

Figure 1 shows the PRISMA flow diagram summarizing the results of the literature research and study selection. After systematically searching four large electronic databases from the inception date to May 1, 2021, we found 25 active-controlled or placebo-controlled phase-2 RCTs that met our inclusion criteria. Overall, these RCTs included a total of 2,597 middle-aged individuals (53% men; mean (SD) age: 52±6 years; BMI: 32±3 kg/m^2; 62% had pre-existing T2DM), who were treated with PPAR agonists, GLP-1RAs or SGLT-2 inhibitors for the treatment of NAFLD or NASH. **Table 1**, **Table 2** and **Table 3** summarize the baseline characteristics of the included RCTs using PPAR agonists (*n*=8 [22-29]), GLP-1RAs (*n*=10 [30-39]), or SGLT-2 inhibitors (*n*=7 [40-46]), respectively. As also shown in the PRISMA flow diagram, three phase 2 RCTs[47-49] were excluded at the stage of eligibility for reasons mainly due to unsatisfactory study design or unsatisfactory inclusion criteria. These excluded studies are listed in **Supplementary Table 4**.

Five RCTs were conducted in the United States, eight studies were performed in Asia (China, Singapore and India), seven were conducted in Europe (Sweden, Germany, Netherlands and UK), and five RCTs enrolled multinational cohorts of individuals with NASH. Overall, most of the aforementioned eligible RCTs were small (*n* <50 per each treatment arm) with a relatively short follow-up duration (i.e., with a median period of 24 weeks [interquartile range (IQR): 24-50 weeks]; only six RCTs had a follow-up duration ≥52 weeks) and in about two thirds of cases did not have liver histology endpoints (except for seven RCTs using PPAR agonists and two RCTs using GLP-1RAs). Sixteen of these RCTs enrolled exclusively patients with T2DM.

Supplementary Figure 1 (for RCTs using PPAR agonists), **Supplementary Figure 2** (for RCTs using GLP-1RAs) and **Supplementary Figure 3** (for RCTs using SGLT-2 inhibitors) summarize the quality

assessment for the included RCTs. Overall, most of the eligible RCTs (especially those using PPARs) were considered at relatively low risk of bias for all potential sources of bias, except for those RCTs that did not perform liver biopsies (the gold standard); in such cases, these RCTs that used magnetic resonance-based techniques were arbitrarily considered at unclear risk of bias regarding the "other bias" item of the Cochrane risk of bias assessment tool.

3.1 PPAR agonists

As shown in **Table 1**, we found a total of eight active-controlled or placebo-controlled phase-2 RCTs that used either the PPAR-gamma agonist pioglitazone (five studies), the dual PPAR-alpha/delta agonist elafibranor (one study), the dual PPAR-alpha/gamma agonist saroglitazar (one study), or the pan-PPAR agonist lanifibranor (one study) to specifically treat NAFLD or NASH. Overall, these 8 phase-2 RCTs included 1,162 individuals (50% men; age 49±5 years; BMI 32±3 kg/m^2; ALT 74±15 IU/L; AST 52±9 IU/L), who were treated for a median period of 38 weeks (IQR 24-57 months). Most of these participants (~68%) did not have established T2DM. Four RCTs were conducted in the United States[22,24,26,28], one in Europe[23], one in Asia[25], whereas two RCTs were international[27,29]. Among these RCTs, only one RCT [28] involved patients with NAFLD as assessed by MRI-PDFF, whereas all other seven RCTs enrolled patients with biopsy-proven NASH.

3.1.1 Pioglitazone

All the five active-controlled or placebo-controlled phase 2 RCTs using pioglitazone (at a variable daily dosage of 30 mg or 45 mg) showed that pioglitazone use was significantly associated with an improvement in individual histologic components of NASH (steatosis, ballooning, lobular inflammation) vs. 2 points of NAFLD activity score (NAS), or NASH resolution vs. NASH resolution without fibrosis improvement (as specified in **Table 1**). In most cases, pioglitazone use also

improved serum aminotransferase levels, insulin resistance and plasma lipid profile. Only one placebo-controlled RCT using pioglitazone at a dose of 45 mg/day for 72 weeks showed that compared to placebo, treatment with pioglitazone was associated with an improvement in liver fibrosis in patients with NASH and prediabetes or T2DM[26]. As regards to this, a previous small meta-analysis of phase-2 RCTs reported that pioglitazone treatment (for a duration of 6 to 24 months) was associated with improvement in advanced fibrosis and fibrosis of any stage in patients with biopsy-proven NASH, regardless of the presence of T2DM[50]. In all the five phase-2 RCTs included in our systematic review, pioglitazone treatment had a similar adverse event profile to placebo or reference therapy, with the exception of a moderate weight gain (~2.5 kg at a dose of 45 mg/day for 72 weeks).

3.1.2 Saroglitazar

The placebo-controlled phase 2 RCT using the dual PPAR-alpha/gamma agonist saroglitazar (i.e., a drug approved for treatment of T2DM and atherogenic dyslipidaemia in India) enrolled 106 obese patients with NAFLD or NASH, who were randomised to receive saroglitazar 1 mg, saroglitazar 2 mg, saroglitazar 4 mg/day or placebo for 16 weeks[28]. Compared to placebo, saroglitazar 4 mg/day significantly improved liver fat content, as assessed by MRI-PDFF (mean changes from baseline: +4.1% vs. -19.7%, respectively), homeostasis model assessment-estimated insulin resistance, serum triglyceride and aminotransferase levels. Treatment with saroglitazar was well tolerated. A mean weight gain of 1.5 kg was observed with saroglitazar 4 mg/day vs. 0.3 kg with placebo.

3.1.3 Elafibranor

The placebo-controlled phase-2 RCT using the dual PPAR-alpha/delta agonist elafibranor included 274 overweight or obese patients with biopsy-confirmed NASH, who were randomly assigned to

receive elafibranor 80 mg, elafibranor 120 mg daily or placebo for 52 weeks. In intention-to-treat analysis, there was no significant difference between the elafibranor and placebo groups in the protocol-defined primary outcomes. However, NASH resolved without fibrosis worsening in a higher proportion of patients in the 120-mg elafibranor group vs. the placebo group (19% vs. 12%), based on a post-hoc analysis for a modified definition of treatment response[27]. Treatment with elafibranor was well tolerated and improved serum liver enzyme levels, lipid parameters, as well as fasting plasma glucose (−0.98 mmol/L at 120 mg/day) and HbA1c levels (−0.46%) in patients with T2DM (40% of total). Elafibranor produced a mild, reversible increase in serum creatinine levels. However, it is important to note that the *interim analysis* from the RESOLVE-IT phase 3 trial (NCT02704403) evaluating once-daily 120 mg of elafibranor in individuals with NASH neither achieved the primary NASH endpoint (i.e., NASH resolution without worsening of fibrosis was 19.2% for elafibranor vs. 14.7% for placebo) nor improved metabolic parameters, as recently announced by the GENFIT biopharmaceutical company that abandoned the development of this drug for NASH treatment (https://www.globenewswire.com).

3.1.4 Lanifibranor

The placebo-controlled phase-2 RCT using the pan-PPAR agonist lanifibranor[29], i.e. the NATIVE (NAsh Trial to Validate IVA337 Efficacy) trial, enrolled 247 obese patients with biopsy-proven NASH, who were randomly assigned to receive lanifibranor 800 mg, lanifibranor 1200 mg/day or placebo for 24 weeks. For data extraction, we contacted the authors, who kindly provided the full set of slides presented at the recent 2020 AASLD meeting. 24-week's treatment with lanifibranor was significantly superior to placebo on NASH resolution with no worsening of fibrosis, as well as improvement of ≥1 stage of liver fibrosis with no worsening of NASH. In addition, treatment with lanifibranor was associated with significant improvements in serum aminotransferase levels and

biomarkers of liver damage, inflammation and fibrosis, as well as improvements in HbA1c, plasma lipid profile and insulin levels. Treatment with lanifibranor had a favorable safety and tolerability profile compared to placebo. At 24 week, a mean weight gain of ~2.5 kg was observed with lanifibranor compared to placebo.

Withdrawals due to serious adverse effects were not increased in any of the aforementioned phase 2 RCTs using pioglitazone or other PPAR agonists, compared to placebo or other active agents.

3.2 GLP-1RAs

As shown in **Table 2**, we found a total of ten active-controlled or placebo-controlled phase-2 RCTs that used liraglutide (six studies), exenatide (two studies), dulaglutide (one study) or semaglutide (one study) to specifically treat NAFLD or NASH. Overall, these 10 phase-2 RCTs included 866 individuals (53% men; mean age 50±5 years; BMI 32±3 kg/m^2; ALT 49±24 IU/L; AST 37±10 IU/L), most of whom had known T2DM (78% of total), and who were treated for a median period of 26 weeks (IQR 24-31 weeks). Seven RCTs were undertaken exclusively in patients with T2DM[31,32,34-38]. Three RCTs included European individuals (France, Netherlands and UK), five RCTs involved Asian individuals (China, Singapore and India), and one RCT included a multi-national cohort of subjects with NASH. Notably, only two of these RCTs included subjects with biopsy-confirmed NASH[30,39], whereas the remaining eight RCTs tested the efficacy of these drugs on NAFLD by using magnetic resonance-based techniques (MRI-PDFF or MRS).

The two placebo-controlled phase 2 RCTs using liver biopsy for diagnosing NASH[30,39] showed that treatment with GLP-1RAs (once-daily subcutaneous liraglutide 1.8 mg for 48 weeks, or

semaglutide at dose of 0.1 mg, 0.2 mg or 0.4 mg/day for 72 weeks) resulted in a significantly higher percentage of patients with NASH resolution. However, both of these trials failed to show any significant improvement in liver fibrosis, and both trials did not adjust for weight changes in reporting the effect of treatment on the primary outcome. Among the active-controlled or placebo-controlled phase 2 RCTs that used by magnetic resonance-based techniques (n=8), treatment with GLP-1RAs was significantly associated with an improvement in liver fat content in five RCTs, whereas in the remaining three RCTs treatment with GLP-1RAs (liraglutide) showed significant, but similar reductions in liver fat content when compared to reference therapy.

In all RCTs, treatment with GLP-1RAs was associated with significant reductions of serum aminotransferase levels, as well as improvements in body weight (up to ~5 kg) and HbA1c levels (up to ~1%). GLP-1RAs were well tolerated and had a similar adverse event profile to placebo (or reference therapy), except for an increased frequency of gastro-intestinal disorders, such as loss of appetite, nausea, constipation or diarrhoea. However, these gastro-intestinal disorders tended to be transient and mild-to-moderate in severity across all of the included RCTs.

3.3 SGLT-2 inhibitors

As reported in **Table 3**, we found a total of seven active-controlled or placebo-controlled phase-2 RCTs that used empagliflozin (two studies), dapagliflozin (four studies), or canagliflozin (one study) to specifically treat NAFLD. Overall, these phase-2 RCTs included 569 individuals with established T2DM (59% men; mean age 59±5 years; BMI 32±1 kg/m^2; ALT 41±14 IU/L; AST 30±8 IU/L), who were treated for a median period of 24 weeks (IQR 16-28 months). Two RCTs included international cohorts of individuals with NAFLD, one RCT was performed in Asia (India), one in United States and three in Europe (Sweden and Germany). In these seven RCTs, the drug-induced

changes of NAFLD were assessed by using either MRI-PDFF (n=5) or MRS (n=2). None of these trials used liver biopsy (the gold standard) to test the hepatic effects of SGLT-2 inhibitors.

Overall, when compared to placebo or reference therapy, treatment with SGLT-2 inhibitors (especially empagliflozin and dapagliflozin) was associated with an improvement in liver fat content (that reached statistical significance in four RCTs[41,42,44,45] and borderline significance in the other three RCTs), along with a significant reduction of body weight (up to ~3.5 kg) and HbA1c level (~0.5%). In all RCTs, treatment with SGLT-2 inhibitors was also associated with a significant reduction of serum aminotransferase levels. SGLT-2 inhibitors had a similar adverse event profile to placebo (or reference therapy), except for increased risk of genitourinary infections.

4. DISCUSSION

This updated systematic review included 25 active-controlled or placebo-controlled phase-2 RCTs (published until May 1, 2021) using PPAR agonists, GLP-1RAs or SGLT2 inhibitors for the treatment of NAFLD or NASH that reported biopsy-based histological or magnetic resonance-based non-invasive endpoints (as MRS accurately quantifies hepatic fat content and MRI-PDFF correlates with histologic improvements)[51-53]. Overall, these 25 included RCTs had a total of 2,597 middle-aged overweight or obese individuals with and without T2DM (mean age: 52±6 years; BMI: 32±3 kg/m^2; 62% had known T2DM), who were treated for a median period of 24 weeks (IQR: 24-50 weeks).

We did not attempt to pool these phase-2 RCTs into a meta-analysis due to the highly variable mechanisms of actions of the drugs used, as well as the high heterogeneity of study design, patient demographics, interventions and comparisons, and outcome assessment, which limit the

comparability of published trials. In addition, we did not perform a network meta-analysis to assess the comparative efficacy of these pharmacological therapies, due to the almost complete absence of direct (head-to-head) comparative RCTs so far, making difficult a direct and reliable comparison between these separate classes of drugs.

In this systematic review, we were able to make some key observations: (1) pioglitazone, lanifibranor and some injectable GLP-1RAs (i.e. once-daily subcutaneous liraglutide and semaglutide) were significantly better than placebo in improving the individual histologic features of NASH (steatosis, ballooning and lobular inflammation) or achieving resolution of NASH without worsening of fibrosis; (2) compared to placebo, pioglitazone and lanifibranor seem also to exert some beneficial effects on fibrosis stage (although longer RCTs are needed to corroborate this finding); (3) in the RCTs using magnetic resonance-based techniques, treatments with either GLP-1RAs (mostly liraglutide, exenatide and dulaglutide) or SGLT-2 inhibitors (mostly empagliflozin and dapagliflozin) were associated with a significant improvement in liver fat content, as assessed by MRI-PDFF or MRS; and (4) despite some preliminary hepato-protective effects were observed in a phase 2 RCT, elafibranor failed to reach the primary histological endpoint of NASH resolution without a worsening of fibrosis in the phase 3 RESOLVE-IT trial and, therefore, this trial has been halted early (as recently announced by the GENFIT biopharmaceutical company; https://www.globenewswire.com).

The results of this updated systematic review provide new supportive evidence on pioglitazone use as one of the best long-term drug-treatment of choice for NASH, irrespective of T2DM. As mentioned above, a prior meta-analysis of pioglitazone use in NASH (including five phase-2 RCTs with a total of nearly 500 patients with biopsy-proven NASH, who were treated up to 24 months

with pioglitazone) also showed that this drug was associated with improvement in liver fibrosis stage, both in patients with and without T2DM[50]. However, longer RCTs are needed to further confirm the possible beneficial effects of pioglitazone on liver fibrosis. It is well known that pioglitazone also has beneficial effects on risk of adverse cardiovascular outcomes in patients with and without T2DM[54-57]. Adverse events precluding its wider clinical use, include moderately increased body weight (by promoting accumulation of triglyceride in subcutaneous fat depots) and an increased risk of non-osteoporotic fractures (especially in women). Given that there is only one RCT for lanifibranor, liraglutide or semaglutide that tested the efficacy of these agents to specifically treat NASH, we believe that there is not sufficient evidence supporting the efficacy of these three aforementioned drugs and further validation in larger phase 3 trials is required. In line with our data, some recent meta-analyses showed that compared to placebo/reference therapy, treatments with either GLP-1RAs or SGLT-2 inhibitors were associated with an improvement in the absolute percentage of liver fat content. Assessed by magnetic resonance-based techniques, these improvements in the absolute percentage of liver fat content were -3.92% (95% CI -6.27% to -1.56%) for the RCTs using GLP-1RAs; and -2.05% (95%CI -2.61 to -1.48%) for the RCTs using SGLT-2 inhibitors[58,59]. However, it is important to underline that no robust data from sufficiently large RCTs with liver histological endpoints are currently available to comment on the long-term efficacy of GLP-1RAs and SGLT-2 inhibitors as a treatment for NASH.

It is established that lifestyle interventions (consisting of hypocaloric diet, exercise and weight loss) are the recommended treatment for NAFLD or NASH, as no pharmacotherapies are approved by regulatory agencies[10]. Furthermore, pharmacological treatments aimed primarily at improving liver disease should generally be targeted at individuals with more severe liver disease, such as those with biopsy-proven NASH and fibrosis. Drug development targeting multiple pathological

pathways in NASH have exploded in the last decade, with numerous new drugs under investigation[8,60-62] (for example, obeticholic acid and other selective farnesoid X receptor (FXR) agonists, chemokine receptor inhibitors, thyroid hormone receptor-ß agonists, modulators of lipid metabolism or antifibrotic drugs). The current National Institute for Health and Care Excellence (NICE), AASLD and EASL practice guidelines recommended the use of pioglitazone in both diabetic and nondiabetic adults with biopsy-confirmed NASH[11-13]. PPAR-receptors represent interesting therapeutic targets both in a liver-centred and systemic approach to NAFLD, in terms of improving liver function and liver, cardiovascular and diabetes-related clinical outcomes[63]. PPARs are key regulators of glucose and lipid metabolism, and regulate many inflammatory and fibrotic processes in different tissues[63]. Risks and benefits for long-term use of pioglitazone should be discussed with each subject before starting therapy. That said, pioglitazone is to date a cost-effective but 'forgotten' drug for treatment of both T2DM and NAFLD[64,65]. As metformin use does not improve liver histology in adult patients with NAFLD or NASH, all the aforementioned scientific guidelines recommend against using metformin as a specific treatment for NASH. Regarding treatment with GLP-1RAs, the AASLD practice guidelines in 2018 suggested that it is premature to consider this class of drug to specifically treat liver disease in patients with NAFLD or NASH[13]. The results of our systematic review suggest that GLP-1RAs (especially liraglutide and semaglutide) are attractive treatment options for NAFLD or NASH, particularly in patients with T2DM or obesity.

The major strengths of our study lie in the use of a systematic review methodology to identify all relevant RCTs (published up to May 1, 2021) that meet predefined inclusion criteria. To our knowledge, this is the largest and most updated assessment to date on the efficacy of PPAR agonists, GLP-1RAs or SGLT2 inhibitors to specifically treat NAFLD or NASH in patients with and without T2DM. In addition, we included RCTs using liver biopsy, which is the 'gold standard'

method for testing NASH resolution with or without coexisting improvement in fibrosis stage, and magnetic resonance-based techniques, which have been shown to quantify accurately changes in liver fat content (although the accuracy of MRI-PDFF or MRS for detecting NASH and assessing changes in liver fibrosis stage is somewhat limited).

Our study has some limitations that are strictly inherent to the RCTs included. Firstly, most of the included RCTs had a relatively small sample size and a relatively short duration of treatment (only six RCTs had a follow-up duration ≥52 weeks). Secondly, liver histological endpoints as a primary outcome were available in the large majority of RCTs using PPAR agonists, but only in two RCTs using GLP-1RAs and in none of those using SGLT2 inhibitors. However, serial liver biopsies are not easy to perform in large RCTs to try and gauge responses to treatments. It is desirable that in the near future the use of magnetic resonance-based techniques (e.g. multi-parametric MRI and MR-elastography), as well as non-invasive tests based on omics and supervised learning will become a reliable and accurate, "non-invasive alternative" to liver biopsy that is currently the gold-standard for diagnosing and staging NAFLD[66-69]. Thirdly, most of the RCTs enrolled individuals with NAFLD/NASH and coexistent T2DM (~32% of cases in the RCTs with PPAR agonists, 78% of cases in the RCTs with GLP-1RAs and 100% of cases in the RCTs with SGLT-2 inhibitors), implying that larger RCTs in nondiabetic individuals with NAFLD or NASH are needed. Fourthly, although the approved doses of semaglutide for T2DM treatment are 0.5 mg or 1.0 mg once weekly, in the placebo-controlled RCT using semaglutide the patients with NASH were randomly assigned to receive once-daily subcutaneous semaglutide at a dose of 0.1 mg, 0.2 mg, 0.4 mg or placebo[39]. Therefore, the applicability and transferability of the findings of this RCT to clinical practice are uncertain. Moreover, in the treatment of obesity, the much higher dose of 2.4 mg of semaglutide once weekly has recently been tested[70]. Over 68 weeks of treatment, this dose of semaglutide was

shown to be very effective in promoting weight loss, with >10% weight loss in about two thirds of obese subjects[70]. However, no liver imaging data were available in this trial. Finally, the current lack of any formal head-to-head RCTs prevents us from recommending which of the three drug classes tested in our study is the most effective on NAFLD or NASH. Larger comparative RCTs are warranted to further establish the comparative efficacy of different interventions for NASH in demonstrating NASH resolution and/or ≥1 stage improvement in fibrosis.

In conclusion, our systematic review based on active-controlled or placebo-controlled randomised trials supports the efficacy of PPAR agonists (especially pioglitazone) and GLP-1RAs (especially liraglutide and semaglutide) in improving histological features of NASH. Our analysis also supports the efficacy of SGLT2 inhibitors (especially empagliflozin and dapagliflozin) in improving hepatic fat content, as assessed by MRI-PDFF or MRS. If these promising results are confirmed in larger phase-3 RCTs with liver histological endpoints, it is reasonable to suggest that PPAR agonists, GLP-1RAs and SGLT2 inhibitors will become important treatment options for individuals with NAFLD or NASH. Given the different mechanisms of actions of each of these three classes of drugs, combination therapy would seem to be a particularly attractive therapeutic option. To address this issue, we recommend that factorial trial designs are needed to test different combinations of treatments in NAFLD.

Disclosure statement: The Authors declared no conflicts of interest.

Acknowledgments: CDB is supported in part by grants from the Southampton National Institute for Health Research Biomedical Research Centre. GT is supported in part by grants from the University School of Medicine of Verona, Verona, Italy.

Contributors statement: The authors contributed equally to this manuscript.

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FIGURE LEGENDS

Figure 1. The PRISMA flow diagram for search and selection processes of the systematic review.

Supplementary Figure S1. Risk of bias summary (A) and graph (B) for each eligible RCT using PPAR agonists as assessed by the Cochrane Collaboration's tool.

Supplementary Figure S2. Risk of bias summary (A) and graph (B) for each eligible RCT using GLP-1RAs as assessed by the Cochrane Collaboration's tool.

Supplementary Figure S3. Risk of bias summary (A) and graph (B) for each eligible RCT using SGLT-2 inhibitors as assessed by the Cochrane Collaboration's tool.