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The efficacy of glucagon-like peptide 1 receptor agonists in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis of randomized controlled trials

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ABSTRACT

Background: non-alcoholic fatty liver disease (NAFLD) is considered as the hepatic manifestation of metabolic syndrome and is highly prevalent all over the world. New drugs are urgently needed for the treatment of NAFLD. The aim of this meta-analysis was to assess the efficacy of glucagon-like peptide 1 receptor agonists (GLP-1RAs) in

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patients with NAFLD.

Method: English language publications in the PubMed, Cochrane Library, Embase and Web of Science databases were searched from inception to October 2019. All randomized controlled trials (RCTs) of GLP-1RAs treatment for NAFLD were considered. Standardized mean difference (SMD) with 95% confidence intervals (CIs) were pooled using the fixed-effects or random-effects model.

Results: six RCTs, involving 406 patients, were included in the analysis. A significant improvement was found in liver fat fraction (LFF) (SMD = -0.33, 95% CI, -0.64 to -0.03, p = 0.034), body mass index (BMI) (SMD: -0.89, 95% CI: -1.60 to -0.19, p = 0.012) and adiponectin (SMD: 0.66, 95% CI: 0.37 to 0.95, p = 0.000) with GLP-1RAs treatment. There were no significant differences in serum alanine aminotransferase (ALT) (SMD: -0.52, 95% CI: -1.04 to 0.01, p = 0.054) and aspartate transaminase (AST) (SMD: -0.20, 95% CI: -0.54 to 0.15, p = 0.134) reduction between the GLP-1RAs and control groups. In the subgroup analysis, exenatide was associated with an improvement in serum ALT (SMD = -1.25, 95% CI: -1.68 to -0.82, p = 0.000) and AST (SMD = -0.62, 95% CI: -1.16 to -0.08, p = 0.024). Liraglutide was associated with a reduction in BMI (SMD = -0.44, 95% CI: -0.77 to -0.11, p = 0.010) and an increase in adiponectin (SMD = -0.33, 95% CI, -0.64 to -0.03, p = 0.034).

Conclusion: our study suggested that GLP-1RAs may improve LFF, BMI and adiponectin in patients with NAFLD. Furthermore, the potential efficacy to treat NAFLD was also shown. More high-quality RCTs are needed to validate our findings.

Keywords: Glucagon-like peptide 1 receptor agonists. Non-alcoholic fatty liver disease. Systematic review. Meta-analysis.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathological syndrome characterized by an excessive accumulation of fat (> 5% hepatic steatosis) in hepatocytes, while no other causes for secondary hepatic fat accumulation (e.g., heavy alcohol consumption, hypothyroidism and drugs, etc.) are present. NAFLD ranges from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH),

the latter being a more progressive type of liver disease (1). Nowadays, NAFLD has become a common cause of chronic liver disease. The prevalence of NASH among NAFLD patients who have undergone a liver biopsy due to a "clinical indication" is estimated to be 59.10% (2) and approximately 9 to 20% of NASH patients will progress to cirrhosis. One-third of these will die from liver failure and hepatocellular carcinoma (3). In addition to the liver-related consequences, NAFLD can also contribute to the burden of extra-hepatic chronic complications. It is now clear that NAFLD is a risk factor for type 2 diabetes mellitus (T2DM) and cardiovascular disease. Up to 70% of people with T2DM suffer from NAFLD, and cardiovascular disease has become the most common cause of death in patients with NAFLD (1). More importantly, the prevalence of NAFLD is expected to increase in the near future due to our increasingly unhealthy lifestyle and diet. (4).

Currently, the first line of treatment for patients with NAFLD is weight loss and metabolic improvement via lifestyle intervention. However, most patients cannot achieve the required degree of weight loss or have trouble maintaining weight loss in the long term (5). There are no approved pharmacotherapies for the treatment of NAFLD (4). Thus, identifying new pharmacotherapies, which can be used to improve NAFLD and its consequences, has attracted much attention.

Glucagon-like peptide 1 receptor agonists (GLP-1RAs) are new hypoglycemic agents for the treatment of T2DM, which also have significant effects on weight loss (6). Recently, GLP-1RAs have been found to improve NAFLD. There is now growing evidence that GLP-1RAs can ameliorate liver inflammation, steatosis and oxidative stress in animal models (7-9). Numerous observational studies have also shown that GLP-1RAs may improve hepatic enzyme levels, liver fat content and liver fibrosis. Cuthbertson DJ et al. (10) studied the efficacy of GLP-1RAs in patients with NAFLD and T2DM and showed that the levels of alanine aminotransferase (ALT), γ-glutamyl transferase (GGT) and intrahepatic lipid were significantly decreased. An open-labeled, prospective case series (11) showed that three out of eight patients with T2DM and biopsy-proven NAFLD had an improvement verified by liver histology after 28 weeks of exenatide therapy. There have been many randomized controlled trials (RCTs) to determine whether GLP-1RAs improve NAFLD, but the results are not

consistent. The objective of this meta-analysis was to explore the efficacy of GLP-1RAs in patients with NAFLD.

MATERIALS AND METHODS

Protocol and registration

This systematic review and meta-analysis was reported in accordance with the PRISMA guidelines (12). Our meta-analysis protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) as number CRD42019118849.

Search strategy

Two reviewers (S.F. and X.S.) independently searched English language publications in the PubMed, Embase, Cochrane Library and Web of Science databases from inception to October 2019. The main key search words were: “exenatide” OR “liraglutide” OR “Albiglutide” OR “Lixisenatide” OR “Dulaglutide” OR “Gastric Inhibitory Polypeptide” OR “Glucagon-Like Peptide 1” OR “Rglp-1 protein” AND “non-alcoholic fatty liver disease” OR “non alcoholic fatty liver disease” OR “nonalcoholic fatty liver disease” OR “NAFLD” OR “nonalcoholic fatty liver*” OR “non-alcoholic steatohepatiti*” OR “nonalcoholic steatohepatiti*” OR “non alcoholic steatohepatiti*” OR “NASH” OR “fatty liver*”. The reference lists of reviewed articles were manually searched for additional relevant studies. In the case of incomplete information, attempts were made to contact the study investigators for additional information. A third reviewer (J.Y.) was involved in the discussion of any disagreements.

Study selection

The criteria for study inclusion were as follows: a) study design: RCTs with any follow-up duration and sample size were allowed; b) population: adult (age ≥ 18 year) patients with a definitive diagnosis of NAFLD or NASH by histologic or imaging evidence (ultrasound, computed tomography or magnetic resonance imaging); c) intervention: GLP-1RAs (liraglutide, exenatide, albiglutide, lixisenatide and

dulaglutide) at any dose and route; d) control: placebo or other active agents; and e) outcomes: changes from baseline in liver fibrosis, liver fat content, serum ALT and aspartate transaminase (AST) level after the follow up period.

The exclusion criteria were as follows: a) not RCTs (e.g., animal, *in vitro* study, observational studies, etc.); b) studies that did not assess primary data; and c) letter to the editor, conference papers and articles only available in abstract form.

Data extraction

Two reviewers (S.F. and X.S.) independently reviewed all identified data based on the exclusion and inclusion criteria and duplicate literature was removed. Publications were assessed according to their titles, abstracts and full texts in subsequent stages. Two reviewers (S.F. and X.S.) independently extracted data for review using a predefined data extraction sheet. A third reviewer (J.Y.) was involved in the discussion of any disagreements. The following information was extracted from the included studies: first author, published year, study location, study design, inclusion/exclusion criteria, sample size, participants' baseline characteristics, intervention characteristics, control and outcome data. The figures in the study were consulted if the raw data was not directly provided in the text or tables. If relevant details were insufficiently reported in studies, the authors were contacted and the ClinicalTrials.gov register was searched for further information. If unsuccessful, the missing data were calculated from the raw numbers and reported p-values (13,14).

Quality assessment

Methodological quality of the included RCTs was assessed by two authors (S.F. and X.S.) independently using the Cochrane risk of bias tool (13), which identified the quality of studies as high, low, or unclear risk of bias based on seven items: random sequence generation, allocation concealment, blinding of participants and personnel, incomplete outcome data, selective outcome reporting and other bias. Disagreements were resolved by mutual discussion or referral to a third reviewer (J.Y.), as appropriate. The results of quality assessment are shown in table 1.

Statistical analysis

All statistical analyses were performed using the STATA 12.0 software. The primary outcomes were the changes in liver fibrosis, liver fat fraction (LFF), ALT and AST after GLP-1RAs treatment. Total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-L), high-density lipoprotein cholesterol (HDL-L), body mass index (BMI) and adiponectin were also analyzed in these RCTs. All data was described and analyzed as the mean ± standard deviations (SDs). The change of the mean ± SD was calculated using established methods if only the median and interquartile range were provided (14), or using a formula recommended in the Cochrane Handbook Version 5.1.0 (13) if only (means ± SD) pre- and post-intervention period data was provided. Since the studies by Yan et al. (15) and Feng et al. (16, 17) had two control groups, the two control groups were combined into one control group using the formula recommended in the Cochrane Handbook Version 5.1.0 (13), and then the experimental group was compared with the combined control group. All outcomes were presented as the standardized mean difference (SMD) and 95% confidence intervals (CIs). Statistical heterogeneity was assessed using the Cochran's Q-test and I^2 statistic (p-value < 0.10 or I^2 statistic > 50% was defined as substantial heterogeneity). A random-effects model was applied in the presence of heterogeneity. In other cases, the fixed-effects model was used. Sensitivity analysis was performed by removing a single trial each time and repeating the meta-analysis to assess the impact of each study on the overall effect size. In addition, subgroup analysis was performed by the type of intervention drugs (liraglutide or exenatide). Publication bias was assessed via Egger's test and Begg's test with $p < 0.05$ considered as statistically significant.

RESULTS

Search results and study characteristics

The flowchart of the study criteria for inclusion in the meta-analysis is shown in figure 1. The initial search yielded 1,349 records (PubMed: 253; Embase: 447; Cochrane Library: 36; Web of Science: 613). After duplicates were removed, 746 records remained. Following the analysis of their titles and abstracts, 31 publications

underwent a full-text review. Of the 31 publications, 23 were excluded and the reasons for exclusion are provided in figure 1. Finally, eight publications (six unique studies) were included (15-22), enrolling 406 adult patients.

The characteristics of the included studies are summarized in table 2. The included RCTs were published from 2013 to 2019. The Fan et al. study (18) randomly assigned 144 NAFLD patients with T2DM to receive either exenatide (20 ug/d) or metformin (2.0 g/d) with lifestyle interventions for 12 weeks. The Shao et al. study (19) randomly assigned 60 NAFLD patients with obesity and T2DM to receive either exenatide (20 ug/d) and insulin glargine or intensive insulin (insulin aspart and insulin glargine) for 12 weeks. The Armstrong et al. study (20,21) randomly assigned 52 patients with biopsy-proven NASH to receive either liraglutide (1.8 mg/d) or placebo for 48 weeks. The Khoo et al. study (22) randomly assigned 24 NAFLD patients with obesity but no T2DM to receive either liraglutide (3 mg/d) or structured lifestyle modification for 26 weeks. The Feng et al. study (16,17) randomly assigned 93 NAFLD patients with T2DM to receive either liraglutide (1.8 mg/d), metformin (2 g/d), or gliclazide (120 mg/d) for 24 weeks. The Yan et al. study (15) randomly assigned 75 NAFLD patients with T2DM to receive either liraglutide (1.8 mg/d) or sitagliptin (100 mg/d), insulin glargine along with metformin therapy (1.5 g/d) for 24 weeks. Of the six RCTs included, four evaluated liraglutide (15-17,20-22) and two evaluated exenatide (18,19). Trial durations were 12 to 48 weeks, with daily dosages ranging from 1.8 mg to 3 mg for liraglutide and 20 ug for exenatide. Four hundred and six adult patients were included (63% male, 37% female), with a mean age range of 41-53 years, mean BMI range of 28.1-35.9 kg/m^2 and 354 complicated with diabetes mellitus.

Primary results

Effect of GLP-1RAs on liver fibrosis

Two studies (15,20) had data on the effect of GLP-1RAs on liver fibrosis. The Armstrong et al. study (20) showed that nine of 23 (35%) patients that received liraglutide *vs* two of 22 patients (8%) that received the placebo had a resolution of NASH, defined as the disappearance of hepatocyte ballooning without worsened

fibrosis (relative risk [RR], 4.5; 95% CI, 1.1 to 18.9; p = 0.02). Meanwhile, fewer patients with liraglutide treatment had fibrosis progression (RR, 0.2; 95%CI, 0.1 to 1.0; p = 0.04) compared with the placebo group. Furthermore, a greater proportion of patients with liraglutide treatment had improvements in hepatocyte ballooning (RR, 1.9; 95% CI, 1.0 to 3.8; p = 0.05) and steatosis (RR, 1.8; 95% CI, 1.1 to 3.0; p = 0.009) than in the placebo group. However, no differences were seen in NAFLD activity score (NAS) (RR, 1.2; 95% CI, 0.8 to 1.7; p = 0.46) and lobular inflammation (RR, 0.9; 95% CI, 0.5 to 1.6; p = 0.65). The Yan et al. study (15) showed that there were no significant differences in the fibrosis-4 index and NAFLD fibrosis score (non-invasive biomarkers for detecting liver fibrosis) in the liraglutide, sitagliptin and insulin glargine groups compared with baseline. Due to the limited number of studies, it was impossible to perform a meta-analysis to assess the effects of GLP-1RAs on liver fibrosis.

Effect of GLP-1RAs on LFF

Three studies (15,17,22) reported results for LFF. The Yan et al. study (15) found that liraglutide and sitagliptin both significantly decreased LFF from baseline to 26 weeks, but this effect was not seen with insulin glargine. The Feng et al. study (17) showed that LFF significantly decreased in all treatment groups (liraglutide group, gliclazide group and metformin group). The reduction in LFF following liraglutide treatment was more significant than following gliclazide treatment. The Khoo et al. study (22) showed that LFF significantly decreased from baseline after liraglutide therapy. However, this change was not significantly different between the liraglutide and diet-exercise groups. When the meta-analysis was performed, there was no significant heterogeneity among the studies (I^2 = 0.0%, p = 0.374). Using a fixed-effect model, our meta-analysis showed significant overall effects of GLP-1RAs on LFF (SMD = -0.33, 95% CI, -0.64 to -0.03, p = 0.034) (Fig. 2A).

Effect of GLP-1RAs on hepatic enzyme parameters

Six studies (15,16,18-20,22) reported results for ALT. The average baseline serum ALT ranged from 49.73 ± 5.79 U/l to 169.54 ± 18.23 U/l in the GLP-1RAs group.

Similar baseline serum ALT levels were observed in the control groups. After GLP-1RAs therapy, the serum ALT of the intervention group had decreased significantly in two studies compared to the control group (18,19), whereas the remaining four studies (15,16,20,22) showed no differences. When the meta-analysis was performed, significant heterogeneity was found among the studies (I^2 = 83.3%, p = 0.000). Using a random-effect model, it was found that GLP-1RAs had no significant effect on ALT compared with the control group (SMD: -0.52, 95% CI: -1.04 to 0.01, p = 0.054) (Fig. 2B). In the subgroup analysis performed according to the type of intervention, a significant difference in the reduction of serum ALT (SMD = -1.25, 95% CI: -1.68 to -0.82, p = 0.000; I^2 = 37.5%, p = 0.206) (Table 3) with exenatide therapy was found as compared to the control group. However, this effect was not seen for liraglutide therapy (SMD = -0.12, 95% CI: -0.39 to 0.15, p = 0.382; I^2 = 0.0%, p = 0.469) (Table 3).

Six studies (15,16,18-20,22) reported results for AST. The average baseline serum AST ranged from 31.22 ± 2.56 U/l to 125.18 ± 16.38 U/l in the GLP-1RAs group. Similar baseline serum AST level was observed in the control groups. After GLP-1RAs therapy, serum AST of the intervention group had decreased significantly compared to the control group in two studies (18,19). The remaining four studies (15,16,20,22) showed no differences. When the meta-analysis was performed, significant heterogeneity was found among the studies (I^2 = 63.3%, p = 0.266). According to a random-effect model, there were no significant differences in the GLP-1RAs group compared with the control group (SMD: -0.20, 95% CI: -0.54 to 0.15, p = 0.134) (Fig. 2C). However, according to the subgroup analysis, exenatide therapy had a significant reduction effect on serum AST (SMD = -0.62, 95% CI: -1.16 to -0.08, p = 0.024; I^2 = 64.0%, p = 0.096) (Table 3). This effect was not seen for liraglutide therapy (SMD = 0.06, 95% CI: -0.21 to 0.33, p = 0.573; I^2 = 0%, p = 0.660) (Table 3).

Secondary results

Six studies (15,16,18-20,22) had data for inclusion in the analysis of BMI. There was significant heterogeneity among these studies (I^2 = 90.0%, p = 0.000). The random-effect model showed that GLP-1RAs had a significant effect on BMI (SMD: -0.89, 95%

CI: -1.60 to -0.19, p = 0.012) (Fig. 3A). Three studies (15,18,21) had data for inclusion in the analysis of the effect of liraglutide on adiponectin and there was no significant heterogeneity among these studies (I^2 = 0.0%, p = 0.613). The fix-effect model showed that GLP-1RAs had a significant effect on adiponectin (SMD: 0.66, 95% CI: 0.37 to 0.95, p = 0.000) (Fig. 3B). Five studies (15,16,18-20) reported results for TC and TG, and four studies (15,16,18,20) reported results for HDL-L and LDL-L. No significant heterogeneity was found among the studies (TC: I^2 = 0.0%, p = 0.862; TG: I^2 = 0.0%, p = 0.922; HDL-L: I^2 = 25.4%, p = 0.259; LDL-L: I^2 = 0.0%, p = 0.555). A fix-effect model did not show a significant difference in the GLP-1RAs group compared with the control group for TC (SMD: 0.00, 95% CI: -0.21 to 0.21, p = 0.990), TG (SMD: -0.12, 95% CI: -0.33 to 0.09, p = 0.261), HDL-L (SMD: 0.10, 95% CI: -0.12 to 0.33, p = 0.370) and LDL-L (SMD: 0.05, 95% CI: -0.17 to 0.28, p = 0.650) (Fig. 3C-F).

In the subgroup analysis of the secondary results grouped by the intervention drugs, a significant reduction in BMI (SMD = -0.44, 95% CI: -0.77 to -0.11, p = 0.010; I^2 = 27.6%, p = 0.246) (Table 3) was observed with liraglutide use. However, no significant differences in TC (SMD = 0.05, 95% CI: -0.24 to 0.34, p = 0.738; I^2 = 0.0%, p = 0.586), TG (SMD = -0.17, 95% CI: -0.46 to 0.12, p = 0.25; I^2 = 0.0%, p = 0.922), HDL-L (SMD = 0.17, 95% CI: -0.12 to 0.46, p = 0.25; I^2 = 43.3%, p = 0.171) and LDL-L (SMD = 0.11, 95% CI: -0.18 to 0.40, p = 0.447; I^2 = 0.0%, p = 0.437) (Table 3) were observed in the liraglutide group compared with the control group. In addition, no significant differences in TC (SMD = -0.05, 95% CI: -0.35 to 0.25, p = 0.741; I^2 = 0.0%, p = 0.922), TG (SMD = -0.06, 95% CI: -0.36 to 0.24, p = 0.680; I^2 = 0.0%, p = 0.473) and BMI (SMD = -1.95, 95% CI: -4.54 to 0.65, p = 0.141; I^2 = 97.2%, p = 0.000) (Table 3) were observed in the exenatide group compared with the control group.

Heterogeneity and sensitivity analysis

There was some heterogeneity in some parameters of our analysis, including ALT, AST and BMI. Sensitivity analysis was performed and the results for AST and BMI remained consistent with a pooled effect size. However, the pooled SMD for ALT showed a significant difference between pre-sensitivity pooled SMD and post-sensitivity pooled SMD after excluding the Yan et al. (15) and Khoo et al. studies (22).

Both of these studies showed no differences on reducing ALT between the liraglutide and control groups. The trial by Yan et al. (15) compared the effects of liraglutide with the control group plus metformin therapy, whereas Khoo et al. (22) compared the effects of liraglutide on NAFLD with obesity but not diabetes with structured lifestyle modification, aiming for 7% weight loss, which is the only recommendation for improvement of NAFLD. Moreover, subgroup analysis based on intervention drugs indicated that exenatide further decreased ALT and AST levels, while liraglutide significantly decreased BMI. Thus, in our opinion, the heterogeneity might be due to intervention drugs, control groups and the presence or absence of diabetes. The variations in the study population, gender, the health status of patients and the quality of studies may also be sources of heterogeneity.

Publication bias

Both the Begg's test (ALT: p = 0.707, AST: p = 1.000) and Egger's test (ALT: p = 0.619, AST: p = 0.754) showed no significant publication bias.

DISCUSSION

This meta-analysis of six RCTs evaluated the efficacy of GLP-1RAs in patients with NAFLD. Only liraglutide and exenatide were evaluated in the treatment of NAFLD. The pooled results showed that GLP-1RAs had significant effects on LFF, BMI and adiponectin.

As a well-recognized serum biomarker of liver damage, serum aminotransferase is recommended to monitor disease development in NAFLD (23) and has recently been shown to be a significant predictor of histological changes over a period of years (24). A previous meta-analysis of Dong et al. (25) only included three RCTs and showed that GLP-1RAs significantly reduced GGT levels in NAFLD patients compared with placebo and positive agents. However, due to the limited sample size, they failed to estimate the effects of GLP-1RAs on ALT and AST levels. Our meta-analysis indicated that there was no significant difference in ALT and AST reduction between GLP-1RAs and control groups. This is inconsistent with the meta-analysis by Carbone (26), which pooled results from three cohort studies and showed a significant

reduction in serum ALT following GLP-1RAs treatment compared with baseline levels. The inconsistencies may be due to the inclusion of different types of studies (RCTs *vs* cohort studies) and comparisons (controls *vs* baseline). Based on the subgroup analysis, the overall estimate of pooled data from two low-quality RCTs demonstrated that the administration of exenatide significantly improved ALT and AST level. However, the effect of liraglutide on ALT and AST levels was not satisfactory. This may be due to the higher efficacy of exenatide than liraglutide to reduce ALT and AST levels. However, the results need to be interpreted with caution, as there were limited studies included in the subgroup analysis.

The significant improvement in LFF that was measured by imaging to quantify hepatic steatosis in our trial analysis was encouraging. This is consistent with the meta-analysis by Dong et al. (25), which pooled results from one RCT and two observational studies of 64 patients. This analysis demonstrated that GLP-1RAs improved liver steatosis by liver biopsy from baseline. Moreover, GLP-1RAs could significantly reduce BMI according to our analysis. Previous studies had shown that a loss of at least 5% of the body weight might improve hepatic steatosis, while a weight loss of ≥ 7% was associated with NAS improvement (1,5). More studies are required to reveal the association between weight loss and hepatic steatosis in NAFLD.

The increase in adiponectin in our trial analysis was significant. Patel SA et al. (27) found that adiponectin was an insulin-sensitizing hormone, which could improve skeletal muscle insulin sensitivity in rats. Lara-Castro C et al. (28) found that serum high molecular weight adiponectin was associated with increased insulin sensitivity and reduced abdominal fat and was involved in metabolic syndrome. Currently, insulin resistance is known as the pathophysiological hallmark and pathogenic factor of NAFLD (29,30). Taking this into account, we speculate that GLP-1RAs may improve insulin resistance via increased adiponectin, which has a beneficial effect on NAFLD.

There was no data on liver histology. Therefore, it was impossible to assess the efficacy of GLP-1RAs with regard to improved liver fibrosis. The meta-analysis by Dong et al. (25) included one RCT and two observational studies found that GLP-1RAs improved liver histology from baseline, including steatosis, lobular

inflammation, hepatocellular ballooning and fibrosis. Moreover, according to the high-quality evidence from one RCT (Table 2), liraglutide improved liver histology in patients with biopsy-proven NASH after 48-weeks treatment. A greater proportion of patients had improvements in hepatocyte ballooning and steatosis with liraglutide treatment and fewer patients had fibrosis progression than in the placebo group. More RCTs with complete histological outcomes are needed.

The exact mechanisms by which GLP-1RAs improves NAFLD have not been elucidated. So far, it has been demonstrated that GLP-1RAs can reduce hepatic steatosis by modulating elements of the insulin-signaling pathway and improving insulin sensitivity of hepatocytes (31,32). Meanwhile, GLP-1RAs can significantly increase the production of cyclic adenosine monophosphate (cAMP), leading to the phosphorylation of cAMP-activated protein kinase (AMPK) (33). Furthermore, it may reduce the expression of stearoyl-CoA desaturase 1 mRNA and genes associated with fatty acid synthesis (32), ultimately reducing *de novo* lipogenesis. In addition, GLP-1RAs protect hepatocytes from ischemia reperfusion injury by reducing necrosis and apoptosis (34) and prevent fatty acid-related hepatocyte death by inhibiting the endoplasmic reticulum (ER) stress response (35). In conclusion, GLP-1RAs decrease hepatic lipid accumulation and protect hepatocytes from oxidative stress and subsequent injury or death, eventually delaying the progression of NAFLD.

There are some limitations in our meta-analysis. First, a small quantity of RCTs was included and some data was calculated using a formula of the Cochrane Handbook or established methods and not the original data. Second, the heterogeneity between studies was significant for some parameters, which led to a high risk of outcome bias. Thirdly, the control group of some included studies was not a placebo but active drugs or structured lifestyle modification, which may have had an impact on the evaluation of the efficacy of GLP-1RAs.

CONCLUSIONS

This meta-analysis suggests that GLP-1RAs therapy may improve LFF, BMI and adiponectin in NAFLD patients, which are all related to NAFLD. Although the data are limited, GLP-1RAs show a potential efficacy in the treatment of NAFLD. More

research is required with RCTs of larger sample sizes and complete histological outcomes in patients with biopsy-proven NAFLD. Longer-term follow-up is needed to clarify the efficacy of GLP-1RAs for the improvement of liver fibrosis.

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Table 1. Risk of bias assessment in the included studies

Study (year)	*Random sequence generation*	*Allocation concealment*	*Blinding of participants and personnel*	*Blinding of outcome assessment*	*Incomplete outcome data*	*Selective reporting*	*Other bias*
Fan et al., 2013	U	U	H	L	L	H	U
Shao et al., 2014	U	U	H	L	L	U	U
Armstrong et al., 2015	L	L	L	L	L	L	U
Khoo et al., 2017	L	U	H	L	L	U	U
Feng et al., 2019	L	U	H	L	L	L	U
Yan et al., 2019	L	L	H	L	L	L	U

H: high risk; L: low risk; U: unclear risk.

Table 2. Characteristics of included studies

Study	*Study design*	*No. of patients (n)*	*Mean age (year)*	*Male (%)*	*Mean BMI (kg/m²)*	*No. of diabetes (n)*	*Agent (daily dosage)*	*Comparator (daily dosage)*	*Trial duration (weeks)*
Fan et al., 2013	RCT	117	53	56	27.9	117	Exenatide (max 20 ug)	Metformin (max 2.0 g)	12
Shao et al., 2014	RCT	60	43	48	30.5	60	Exenatide (max 20 ug) + Insulin glargine*	Insulin aspart* + Insulin glargine*	12
Armstrong et al., 2015	RCT MC DB	45	51	61	35.9	17	Liraglutide (max 1.8 mg)	Placebo	48
Khoo et al., 2017	RCT	24	41	92	33.1	0	Liraglutide (max 3 mg)	Structured lifestyle modification	26
Feng et al., 2019	RCT	85	47	69	28.1	85	Liraglutide (max 1.8 mg)	Metformin (max 2.0 g), Gliclazide (max 120 mg)	24
Yan et al., 2019	RCT MC	75	45	69	29.8	75	Liraglutide (max 1.8 mg) + Metformin (1.5 g)	Sitagliptin, (100 mg)+ Metformin (1.5 g), Insulin glargine* + Metformin (1.5 g)	26

BMI: body mass index; RCT: randomized controlled trial; MC: multiple center; DB:

double blind; max: maximum. *The adjustment of insulin was based on the monitored level of blood glucose.

Table 3. Subgroup meta-analysis of the included studies

	Subgroup	No. of studies	Heterogeneity		Model	Effect size (95% CI)	Q-statistics (p)
			I^2 (%)	p value			
ALT	Liraglutide	4	0.0	0.469	F	-0.12 (-0.39, 0.15)	0.469
	Exenatide	2	37.5	0.206	F	-1.25 (-1.68, -0.82)	0.000
AST	Liraglutide	4	0	0.660	F	0.06 (-0.21, 0.33)	0.573
	Exenatide	2	64.0	0.096	R	-0.62 (-1.16, -0.08)	0.024
TC	Liraglutide	3	0.0	0.586	F	0.05 (-0.24, 0.34)	0.738
	Exenatide	2	0.0	0.922	F	-0.05(-0.35, 0.25)	0.741
TG	Liraglutide	3	0.0	0.922	F	-0.17 (-0.46, 0.12)	0.25
	Exenatide	2	0.0	0.473	F	-0.06 (-0.36, 0.24)	0.680
LDL-L	Liraglutide	3	0.0	0.437	F	0.11 (-0.18, 0.40)	0.447
	Exenatide	/	/	/	/	/	/
HDL-L	Liraglutide	3	43.3	0.171	F	0.17 (-0.12, 0.46)	0.25
	Exenatide	/	/	/	/	/	/
BMI	Liraglutide	4	27.6	0.246	F	-0.44 (-0.77, -0.11)	0.010
	Exenatide	2	97.2	0.000	R	-1.95 (-4.54, 0.65)	0.141

ALT: alanine aminotransferase; AST: aspartate transaminase; TC: total cholesterol; TG: triglycerides; LDL-L: low-density lipoprotein cholesterol; HDL-L: high-density lipoprotein cholesterol; BMI: body mass index; FE: fixed-effects model; RE: random-effects model.

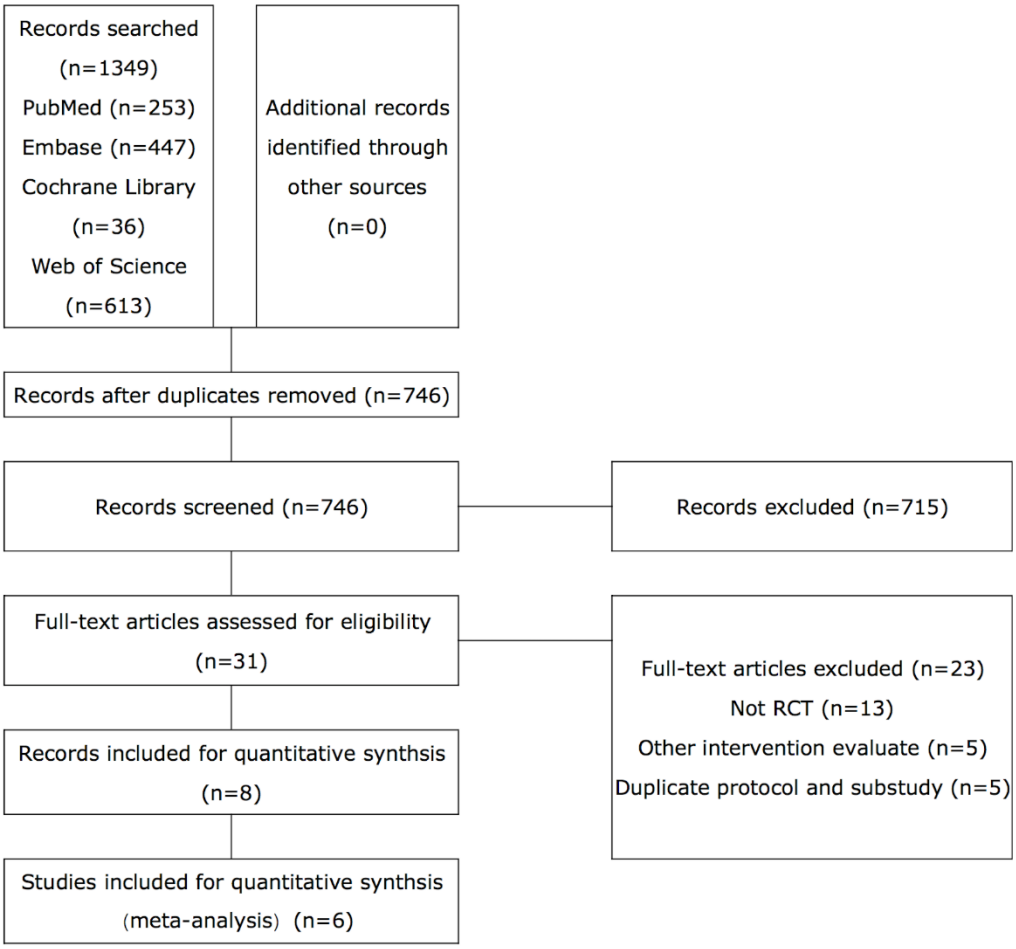

Fig. 1. Flow diagram of study selection.

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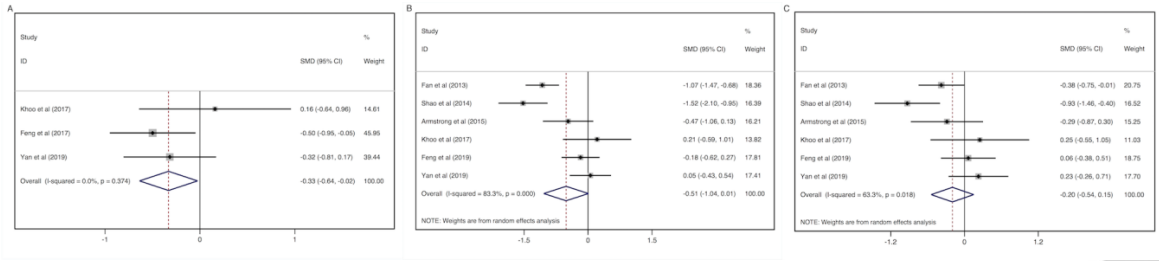

Fig. 2. Results of the effect of glucagon-like peptide 1 receptor agonists (GLP-1RAs) on (A) liver fat fraction (LFF), (B) alanine aminotransferase and (ALT) and (C) aspartate transaminase (AST).

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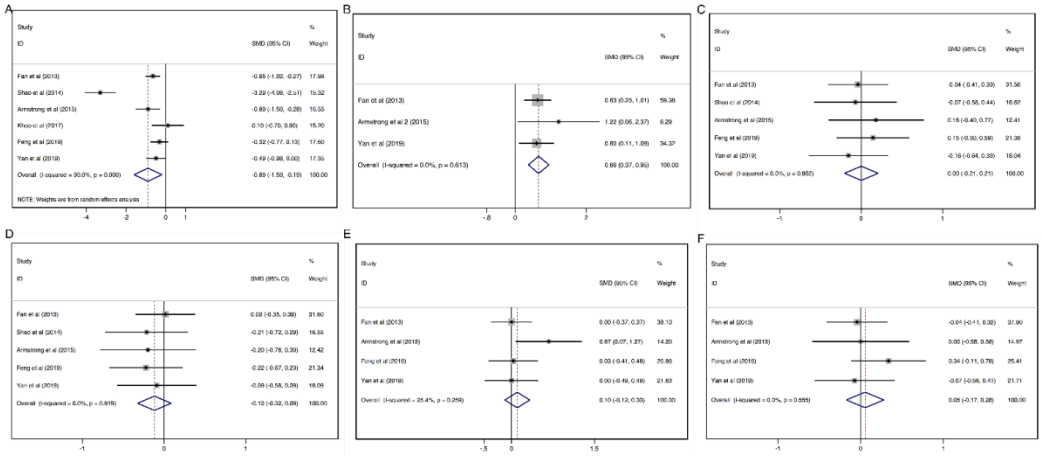

Fig. 3. Results of the effect of glucagon-like peptide 1 receptor agonists (GLP-1RAs) on (A) body mass index (BMI), (B) adiponectin, (C) total cholesterol (TC), (D) triglycerides (TG), (E) high-density lipoprotein cholesterol (HDL-L) and (F) low-density lipoprotein cholesterol (LDL-L).