The effects of GLP-1 receptor agonists on histopathological and secondary biomarkers of non-alcoholic steatohepatitis: A systematic review and meta-analysis

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Short running title: GLP-1RA for NASH: A systematic review and meta-analysis

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1. Background

Non-alcoholic steatohepatitis (NASH) is manifested by ectopic fat accumulation on the liver tissue without a secondary cause. NASH cardinal features include increased hepatic tissue inflammatory activity and hepatocyte injury (1). Diagnosis is substantiated when fat liver accumulation exceeds 5% and alcoholic intake is below 20 g/day for men and 10 g/day in women (1,2). NASH histolopathological characteristics include macrovesicular steatosis, inflammation, and liver cell ballooning, typically with a predominantly centrilobular (acinar zone 3) distribution in hepatic tissue (2). Approximately 30% of patients with NASH progress to cirrhosis, potentially leading to liver transplantation (1). Recent reports estimate the demand for liver transplants to rise by 20% in the next 15 years, and the overall cost of the procedure within the global health care sector to almost double in the same amount of time (3). Additionally, patients with NASH have higher liver-related morbidity and mortality as well as metabolic and cardiovascular disease, with the former reaching a 10-fold higher likelihood compared to the general population (4). NASH has statistically become the second commonest cause of liver transplantation after hepatitis C in the US alone (4) and its underestimated prevalence in the general population (5) calls for a deeper investigation of the available pharmacological treatments.

Glucagon-like peptide receptor agonists (GLP-1 RA) may represent a solution to this conundrum through their pleiotropic actions. GLP-1RA are known to mimic the actions of GLP-1 incretin hormone secreted from the small intestine in response to orally ingested carbohydrates, which promotes secretion of insulin and induces satiety (6). Consequently, GLP-1 RA have been utilized primarily in the management of the dysmetabolic diseases, such as type 2 diabetes mellitus (T2DM) and obesity, exhibiting significant effects in glycaemic control and weight loss (7). Despite the well documented outcomes in both diabetic and non-

diabetic patients (8), GLP-1 use has not been extended to NASH management but with preclinical data looking promising (5,9).

2. Methodology

Our systematic review and subsequent meta-analysis were performed in accordance with the PRISMA guidelines. This systematic review has been registered with PROSPERO (National Institute of Health Research - International Prospective Register of Systematic Reviews – University of York) with ID number CRD42021240058.

2.1 Literature Search and Selection Criteria:

One author (P.B) conducted systematic searches of eligible studies published from the inception date between January 1st 2000 and March 20th 2021 on the Medical Literature Analysis and Retrieval System Online (MEDLINE), Excerpta Medica (EMBASE), Cochrane Central Register of Controlled Trials (CENTRAL), ClinicalTrials.gov and SCOPUS.

The key MeSH search terms included relevant terminology regarding NASH and GLP-1 receptor agonists and are presented in their entirety below:

(Non-alcoholic steatohepatitis[Title/Abstract])) OR (Metabolic NASH[Title/Abstract])) OR (Type I NASH Title/Abstract])) OR (liver cirrhosis [Title/Abstract])) OR (hepatic ballooning [Title/Abstract])) OR (Hepatic Fibrosis[Title/Abstract])) AND (GLP-1 RA [Title/Abstract])) OR (exenatide [Title/Abstract])) OR (exendin-4 [Title/Abstract])) OR (liraglutide[Title/Abstract])) OR (albiglutide [Title/Abstract])) OR (glucagon-like peptide [Title/Abstract])) OR (Dulaglutide [Title/Abstract])) OR (Ozempic[Title/Abstract])) OR (Incretin Hormones[Title/Abstract])) OR (Victoza [Title/Abstract])) OR (Trulicity [Title/Abstract])) OR(Saxenda [Title/Abstract])) OR (Lixisenatide [Title/Abstract])) OR

(Lyxumia [Title/Abstract])) OR (Byetta [Title/Abstract])) OR (Bydureon [Title/Abstract])) OR (Tanzeum [Title/Abstract])) AND (random [Title/Abstract])).

* Details concerning the methodology of this systematic review and meta-analysis can be found in the supplementary appendix.

3. Results

3.1 Search results and description of selected studies

A total of 4 studies were included in the quantitative synthesis for a meta-analysis. Two were double-blinded RCTs examining the effects of liraglutide (10) and semaglutide (12), while the remaining studies were carried out on a single patient cohort with an unblinded open label design and investigated the efficacy of liraglutide and dulaglutide in NASH patients with T2DM (13,21).

The LEAN trial conducted by *Armstrong et. al* involved a total of 56 patients divided into 2 arms, with one receiving a daily subcutaneous 1.8mg dose of liraglutide, and the other receiving placebo for 48 weeks. The second eligible RCT was a phase II study, carried out by *Newsome et. al*. It contained 4 arms, with patients in each arm receiving daily subcutaneous doses of either 0.1, 0.2, 0.4mg of semaglutide, or placebo for 72 weeks, while a total of 320 participants took part in the study.

Eguchi et al. study from 2015 investigated a cohort of 19 diabetic Japanese participants, who subcutaneously received 0.9mg of liraglutide daily for a 24-week period, after completing a preliminary 24-week lifestyle intervention.

Finally, *Seko et al.* performed a non-controlled, open label study including a single patient group (n=15) to receive dulaglutide subcutaneously at 0.75mg per day for 12 weeks. The patient group were Japanese, diagnosed with T2DM. and primarily female (n=12).

Table 1: Summary of Study Design and Patient Characteristics from the studies selected for meta-analysis (n=4)

Study	*Newsome 2020*	*Eguchi 2015*	*Armstrong 2016*	*Seko 2017*
Study Type	RCT	Observation Cohort Study	RCT	Observational Cohort Study
GLP-1RA used	Semaglutide	Liraglutide	Liraglutide	Dulaglutide
Dosage (mg/day)	0.1,0.2,0.4	0.9	1.8	0.75
Control Treatment	Placebo	Lifestyle Intervention	Placebo	None
Follow-up Duration (weeks)	64	24	48	12

Total Number of Participants	320	19	52	15
Participants with T2DM	199	All	17	All
Sex (Male/Female)	126/194	11/16	31/21	3/12
Mean Age	55.0±10.6	54.1±12.3	50±11-Intervention arm; 52±12-Control arm	66.8±2.7
Participant Ethnicity	248-White 48-Asian 24-Other	19-Japanese	46-White 2-Asian 4-Other	15-Japanese
Adverse Effects Reported	18/32 AEs leading to treatment discontinuation in semaglutide arms were gastrointestinal (GI)	None	38/52 patients reported GI-related AE	None

Key: T2DM=type 2 diabetes mellitus, GLP-1RA=glucagon-like peptide-1 receptor agonist, RCT=randomized control trial, AE=adverse effect

3.2 Bias Assessment:

The bias assessment of the listed studies revealed no significant risks for the Newsome's and Armstrong's trials, which were graded as low risk of bias by two independent reviewers. Alternatively, the studies of Eguchi *et. al* and Seko *et. al* received a higher grade of bias

qualification due to the absence of placebo arms as well as other parameters which are discussed in more detail in other sections of this paper (Tables 2A,2B).

3.3 Effects of GLP-1RA on liver histology

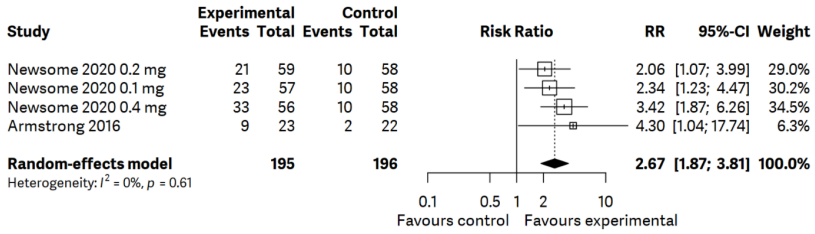
The calculated Relative Risks (RR) indicate the higher efficacy of GLP-1RA on the improvement of NASH as measured by a decrease in the overall NAS score, compared to placebo (RR= 2.67, 95% CI= [1.87; 3.81] Figure 2A) as well as improvement of hepatic fibrosis by at least 1 point according to the Kleiner classification (RR=1.29, 95% CI=[0.99; 1.70]- Figure 2B). A similar trend was observed for lobular inflammation (RR=1.44, 95% CI=[1.11; 1.86]- Figure 2C). The I^2 tests indicated low levels of heterogeneity in the observed parameters except for lobular inflammation, where borderline moderate heterogeneity was observed (I^2=26%- Figure 2C).

The study by Eguchi *et. al* could not be included in this section of the meta-analysis due to the absence of data from a control group, however the available data on liver histology were the following:

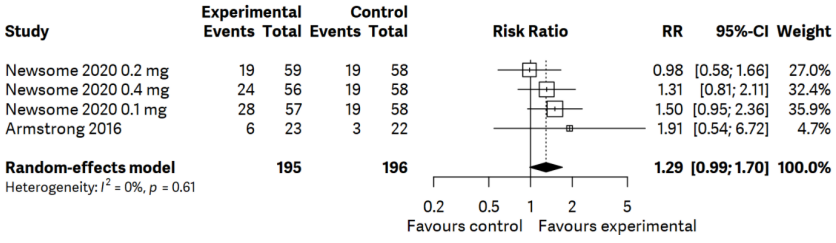
NASH Resolution (defined as NAS Score of 3 or less and absence of hepatocyte ballooning [10]) (n=0) patients, improvement in fibrosis by at least 1 scale (n=6), improvement in steatosis (n=7), improvement in lobular inflammation (n=2), and improvement in hepatocyte ballooning (n=5).

Figures 1A-E: Forest Plots summarizing:

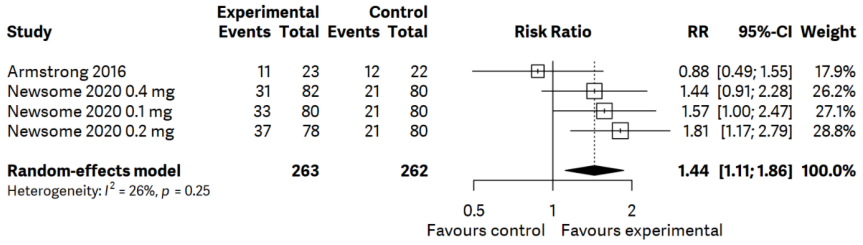
A) Effects of GLP-1RA on NASH Resolution



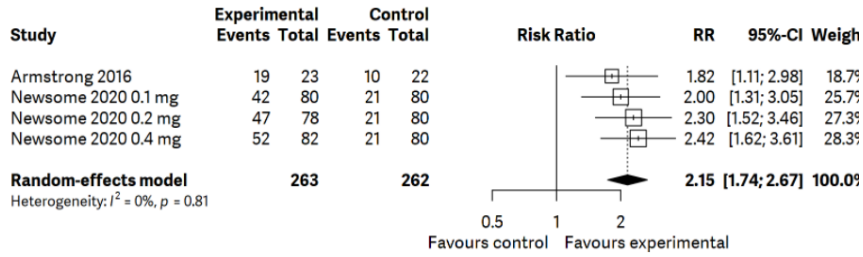
B) Effects of GLP-1RA on Fibrosis Improvement by at least 1 scale of the Kleiner classification



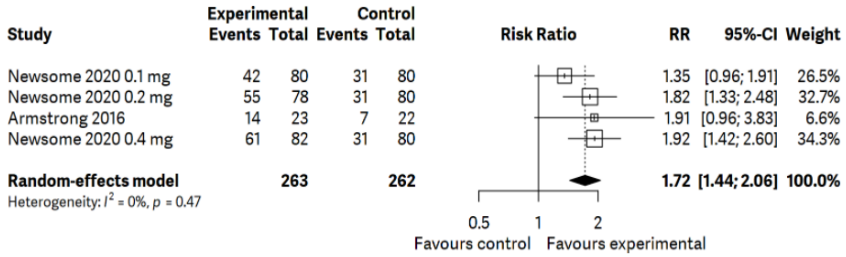
C) Effects of GLP-1RA on lobular inflammation



D) Effects of GLP1RA on Steatosis



E) Effects of GLP1RA on Hepatocellular Ballooning



4. Conclusions

All GLP-1RA showed significant improvements in NASH resolution as well as fibrosis (Figures 2A-C), matching the clinical definition outlined by Sanyal *et al*. The outcomes of our meta-analysis support the findings presented in other systematic reviews and meta-analyses on the pooled effects of GLP-1RA in NAFLD/NASH, highlighting a greater effect of the drug class in alleviating NAFLD/NASH and fibrosis compared to placebo treatments (1,14). In addition, this report evaluated the detailed impact of GLP-1 RA on individual parameters of the NAS scoring system. The key finding in the following section was the calculated combined beneficial impact of GLP-1RA in the reduction of hepatocyte ballooning (Figure 2C), the disappearance of which is a requirement for the therapeutic resolution of NASH (9). Additionally, 1.8mg/day of liraglutide (11) and 0.4mg/day semaglutide (12) demonstrated the greatest reduction in hepatocyte ballooning. However, the effect distribution was more linear for semaglutide, with improvements observed consistently for each concentration of the drug in a non-parameter-specific fashion, while liraglutide exhibited a more pronounced effect on hepatocyte ballooning and steatosis, without producing significant changes in the levels of lobular inflammation compared to placebo (Figure 2E). A similar observation was made in the data from Eguchis *et al.* trial (16) which reported the least number of improvements in lobular inflammation. LEAN trial reported a significant reduction of insulin resistance induced by liraglutide compared to placebo (Figure 7A), with this effect reversed as the treatment period

was prolonged from 48 to 60 weeks accompanied by the loss of significance. More specifically, the calculated insulin resistance index (HOMA-IR) and adipose tissue insulin resistance index (ADIPO-IR) with mean changes compared to placebo treatment ($MC_{Placebo}$) from baseline after 60 weeks were reported in the supplementary appendix of the LEAN trial as follows: ($MC_{Placebo}$ =0.04; 95%CI=[-2.06;2.15]- for HOMA-IR); ($MC_{Placebo}$ =1.59; 95%CI=[-5.03;8.20]-for ADIPO- IR), indicating an increase in insulin resistance after 60 weeks of liraglutide administration.

The individual comparisons between the GLP-1RA included in this study suggest that semaglutide achieved the greatest beneficial effect on individual histological parameters of NASH (Figures 2C-E). The calculated effect sizes of GLP-1 RA on the secondary markers of NASH also suggested superiority of semaglutide as it was able to generate the largest favourable effect on secondary markers such as BMI (Figure 6E *), CCCK-18 M30 (Figure 4A*) and AST (Figure 5B*). Coupled with good tolerability from patients as well as strong impact on NASH resolution (Figure 2A), the above provides a good overall effect profile, boosting the therapeutic potential of semaglutide. In contrast, despite being the most effective at resolving NASH in terms of the number of patients with complete NASH resolution (RR=4.30; 95% CI= [1.04; 17.74]- Figure 2A), liraglutide failed to produce a significant impact on any secondary parameters other than ALT, AST, and HbA1c in both Armstrong's and Eguchi's trials (Figures 5A-B, 6A*). However, there are several observations that should be addressed with the trial design for this study (13). Firstly, the mean participant age as well as the female to male ratio differed significantly from the other studies included in our systematic review (Table 1). Given the 3:2 male to female risk ratio of developing NASH (17), the previous may in turn account for crucial differences in the effects of dulaglutide on the secondary biomarkers of NASH compared to other GLP-1 RA. For instance, the results of the study indicated that dulaglutide decreased HDL, while an increase in this parameter was

observed in all other trials included in this review (Figure 6D*). This change may be explained by the age-related changes in the structure of HDL and its metabolism, which decrease the anti-oxidant property of HDL as well as the degree of its saturation with cholesterol, leading to a fall in its concentration in the presence of inflammation present in NASH (18). Nevertheless, such methodological steps may impede comparability to other trials, as demonstrated by high degrees of heterogeneity for several secondary parameters, including BMI and HOMA-IR (Figures 6B,E*). A possible suggestion for future trials may involve stratifying the results by sex and age groups (i.e 18-35, 35-50 and 50-65) in order to assess the patient groups most likely to benefit from dulaglutide. Further studies are required to elucidate dulaglutide's role in NASH management.

The main limitations of the current systematic review were the paucity of available high-quality RCT's with longer follow up duration, as only Newsome's and Armstrong's trials were carried out using the following study design, while studies of Eguchi and Seko *et. al* were neither blinded nor randomized, with the latter following patients for 12 weeks, providing evidence of short effects of treatment with GLP-1RA on NASH parameters in this systematic review. Consequently, meta-analysis of histological parameters of NASH and fibrosis, was limited to the evaluation of only 2 studies eligible for statistical comparison of the effects of GLP-1RA (Figures 2A-E).

Furthermore, conclusions made from trials examining small samples and variable follow up periods, may not be directly extrapolated to the wider population of patients with NASH. (Table 1). There are currently several RCT's being conducted involving the drugs discussed in this systematic review (ClinicalTrials.gov ID: NCT04019561, NCT04166773) as well as an ongoing large stage 3 trial of semaglutide consisting of 1200 patients (NCT04822181), which is beyond the scope of this systematic review and meta-analysis.

Potential limitations of this study include the lack of homogeneity in the assessment of secondary markers of NASH among the selected studies. The following can be inferred from the calculated I^2 statistic for the combined effects on HbA1c and CCCK-18 M-30 (Figures 4C-D*). In addition, Eguchi *et al.,* and Seko *et al.* studies reported different secondary markers of NASH, namely liver stiffness assessed by Fibroscan, which has not been available to all participants of the other reviewed trials, and the outcomes were thus not reported in any of the published materials.

The main strength of the current systematic review and meta-analysis is the specific focus on liver histology as the primary measure of GLP-1RA efficacy in improving NASH. This was further reinforced by excluding trials of GLP-1RA in NAFLD patients, allowing us to examine clinical data relevant exclusively to NASH. Furthermore, this is the first systematic review providing a breakdown of effects of GLP-1RA on each of the parameters of the NAS scoring system. As a result, we were able to carry out a detailed comparison of GLP-1RA based on a greater number of histological NASH biomarkers, and derive a more precise effect profile of every drug in comparison to existing literature (14-15). In particular, the effects on lobular inflammation outlined before may provide additional insights into the influence of NASH on other metabolic parameters such as insulin resistance and how GLP-1RA treatment may affect these. In addition, we compared for the first time the secondary markers of NASH other than aminotransferase levels, that may be utilized to supplement the results of GLP-1RA treatment on histological aspects of NASH. Finally, we tried to evaluate the potential influence of ethnicity and age on the efficacy of GLP-1RA on NASH resolution and recommend future clinical trials to take the following factors into consideration.

In conclusion, we have evaluated the available clinical data of GLP-1RA efficacy on the improvement of NASH histopathology and secondary biomarkers excluding studies investigating patients with NAFLD. To our knowledge, this is the most comprehensive

systematic review on the effects of GLP-1 RA on histopathological and secondary NASH parameters to date. The results of our meta-analysis indicate that 0.4mg/day of semaglutide administered subcutaneously is the most effective regimen for the management of NASH when compared to other currently available treatment options involving GLP-1 RA, demonstrating a positive impact on most predictive secondary biomarkers of the condition discussed in this review. Further, high-quality population-based RCTs are a priority to inform around efficacy of other GLP-1RA in relation to optimal dose and treatment duration.

*****Please refer to supplementary appendix for details.

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Conflict of Interest:

No authors report a conflict of interest.

Author Contributions:

G.D, R.V and CWLR conceived the idea of this work. P.B, G.D, C.C, Z.Z, and W.S designed the protocol of the systematic review. Literature search was carried out by P.B. Study screening and selection was performed by all authors mentioned above plus V.R. E.O conducted the meta-analysis. P.B composed the first draft, which was interpreted and edited with the help of G.D, C.C, W.S, Z.Z, and E.O. The final version of the manuscript was reviewed and approved by all authors.

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