PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Effects of lixisenatide on elevated liver transaminases: systematic review with individual patient data meta-analysis of randomised controlled trials on patients with type 2 diabetes
AUTHORS	Gluud, Lise; Knop, Filip; Vilsbøll, Tina

VERSION 1 - REVIEW

REVIEWER	Siyan Zhan Peking University Science Center, China
REVIEW RETURNED	05-Jun-2014

GENERAL COMMENTS	This meta-analysis with individual patient-level data about the effect of lixisensatide on liver enzymes seems very interesting. However, there are a few questions in this manuscript: Major comments: 1. The authors did not clearly and logically introduce the reasons why they have to do this study in the Introduction section. I think the second paragraph is irrelevant, especially the content about pioglitazone and metformin, which should be deleted from the manuscript. 2. The discussion section seems not so logical and not rich enough. The authors should conclude the results and findings concisely first, then state the strengths and limitations of this study, with additional comparison and explanation of the findings. The explanation of the findings in this manuscript is not rich enough to make sense. Minor comments: 1. There was no trial with “no intervention” as comparison, so “no intervention” should be deleted from the Introduction section and Method section. 2. The authors should clearly state the inception and closing date about the electronic search in the Method section. 3. The parameters in the trial sequential analysis, such as relative risk reduction and control incidence rate, should be clearly stated in Figure 3. 4. Table 2 and Table 3 should include the unit of weight and liver enzymes. 5. There are some grammatical errors in the manuscript: 1) The first sentence in the Introduction section: ‘The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing and the costs to are considerable’, the word ‘to’ should be deleted. 2) Et al

REVIEWER	Carlo Bruno Giorda Metabolism and Diabetes Unit

REVIEW RETURNED	06-Jun-2014

GENERAL COMMENTS	This paper addresses an important issue i.e. the effects of agents for type 2 diabetes treatment on liver and on NASH and NAFLD in particular. It is well-written and clear.The findings are worth publication even though they are somewhat weak. I valued the discussion because it is very cautious on the intepretation of results and stresses the need for other research in this field. I suggest adding explainatory legends to figure 2-5 to help the readers understand.

REVIEWER	Yutaka Seino Kansai Electric Power Hospital, Osaka Japan
REVIEW RETURNED	07-Jun-2014

GENERAL COMMENTS	The manuscript entitled “Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on non-alcoholic fatty liver disease: systematic review with individual patient data meta-analysis of randomised controlled trials” by Gluud et al describes the meta analysis of RCTs comparing effects of lixi on blood liver enzymes levels with those of placebo or active comparators. They found that lixi treatment associates better with ALT normalization in obese or overweight people with type 2 diabetes. While the issue reported in this manuscript is of great interest, there are several problems that need to be revised for any consideration in its publication. 1. As they pointed out in the manuscript, changes in ALT levels are not necessarily linked to NAFLD. Thus, it is too much to discuss effects of lixi on NAFLD with their reporting data on ALT. Especially, the title should be revised such as “Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on ALT levels in the meta analysis of randomized controlled trials: Implications for non-alcoholic fatty liver disease”. 2. The reviewer concerns how the authors adjust effects of alcohol consumption in RCTs. Nausea and/or vomiting frequently associated with GLP-1RA possibly associates with reduced alcohol consumption, which then affects ALT levels. 3. GLP-1R activation has been suggested to improve NAFLD (J Diabetes Investig. 2013; 4(2):108-130; J Diabetes Complications. 2013; 27(4):401-6). The reviewer wonders why they observe superior ALT normalization in lixi, when compared not only to placebo control but also to GLP-1RA such as lira and exe. They should discuss these issues in discussion.

REVIEWER	Dr Yannan Jiang The University of Auckland New Zealand
REVIEW RETURNED	22-Jun-2014

GENERAL COMMENTS	This study investigated the effect of glucagon-like peptide-1 receptor agonist lixisenatide on normalisation of ALT in Type 2 diabetes patients with mildly elevanted liver blood tests. Although this patient

	population has a considerable risk of NAFLD and subsequenlty NASH, there are no RCTs on lixisenatide for patients with NAFLD at present as the authors stated. Therefore, the title on "Effects of ... lixisenatide on non-alcoholic fatty liver disease" is overstated. For meta-analysis, none of the included trials were specifically designed to evaluate the effects of lixisenatide on liver blood tests. In addition, those patients with severe liver disease were excluded from the trials. Eligible patients with elevant liver blood tests were selected for the study, which were only sub-samples of the randomised cohorts and might not be balanced between the treatment and control groups. As shown in Figure 2, the study sample sizes varied dramatically across all trials (e.g. the first trial only had 3 patients). These issues have raised considerable concerns on the validity of this meta-analysis, whether the study design is appropriate to address the research question of interest. Some specific comments are listed below. Abstract - Study design involves both systematic review and meta-analysis. Participants' selection criteria should be presented clearly. Both primary and secondary outcome measures considered in this study need to be stated. More numeric results may be reported, including the total sample size on the primary outcome and level of significance. Method - In first paragraph, the last sentence mentioned "no intervention". Is this correct? Also, were both ALT and AST the primary outcomes, with the fact that elevation of ALT is more commmon than AST? Statistical Analysis - How missing data were imputed? Results - More details should be given on the characteristics of included trials such as the patients' selection criteria, total sample size, follow up duration (with unit) and outcome assessments. For the purpose of this study, eligible patients were selected for comparison based on their baseline liver blood tests and weight status. The group sizes and distribution of data across relevant categories should be provided, rather than mean/SD only as overall. The wide range of therapy duration from 4 to 76 weeks is of some concern. The p-values should be reported together with 95% confidence interval on the primary outcome, same for the number needed to treat. The effect sizes should also be reported for those patients with different weight status. Additional forest plots on the normalisation of AST and other important secondary outcomes may be useful. Discussion - The main strength, as highlighted by the authors, is the individual patients' data retrieved from included trials. With the richness of data, more in-depth analyses could be conducted using mixed effect regression models on important individual patients' characteristics and potentially a continuous outcome. The paper is well written, and the strength of individual patient data meta-analyses is noticed. However, the study design is limited by the patient populations available from included trials. As discussed, none of the individual trials were specifically designed to evaluate the effects of lixisenatide on liver blood tests. In addition, those patients with severe liver disease were excluded from the trials. The

	eligible patients selected for this study were no longer a representative sample of original randomised cohorts, which was considered important in RCTs. More convincing evidence on this meta-analysis is needed to support final conclusions and recommendations.

VERSION 1 – AUTHOR RESPONSE

Reviewer Name Siyan Zhan
Institution and Country Peking University Science Center, China
Please state any competing interests or state 'None declared': None declared

This meta-analysis with individual patient-level data about the effect of lixisensatide on liver enzymes seems very interesting. However, there are a few questions in this manuscript:
Major comments:
1. The authors did not clearly and logically introduce the reasons why they have to do this study in the Introduction section. I think the second paragraph is irrelevant, especially the content about pioglitazone and metformin, which should be deleted from the manuscript.
Reply: We have revised the introduction to clarify why we performed the review and have deleted the second paragraph as suggested.
2. The discussion section seems not so logical and not rich enough. The authors should conclude the results and findings concisely first, then state the strengths and limitations of this study, with additional comparison and explanation of the findings. The explanation of the findings in this manuscript is not rich enough to make sense.
Reply: We have rewritten most of our discussion to make it more logical. We have reported our findings and main conclusions first then stated the strengths and limitations of our study.
Minor comments:
1. There was no trial with "no intervention" as comparison, so "no intervention" should be deleted from the Introduction section and Method section.
Reply: We wrote our protocol before completing the trial searches and did not know in advance if RCTs with a 'no intervention' group exist. We therefore included 'no intervention' as a potential comparator. However, we acknowledge that the wording may seem confusing and have changed the wording as suggested.
2. The authors should clearly state the inception and closing date about the electronic search in the Method section.
Reply: We have added information about the searches (please also see our reply to the editorial comments).
3. The parameters in the trial sequential analysis, such as relative risk reduction and control incidence rate, should be clearly stated in Figure 3.
Reply: We have added the information to the label in figure 3 (relative risk reduction and control incidence rate).
4. Table 2 and Table 3 should include the unit of weight and liver enzymes.
Reply: We have included the unit for weight and liver enzymes.
5. There are some grammatical errors in the manuscript:
1) The first sentence in the Introduction section: 'The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing and the costs to are considerable', the word 'to' should be deleted.
Reply: We have deleted the word 'to'.
2) Et al
Reply: We have revised the reference list.

Reviewer Name Carlo Bruno Giorda
Institution and Country Metabolism and Diabetes Unit, ASL Torino 5, 10023 Chieri, ITALY
Please state any competing interests or state 'None declared': None declared

This paper addresses an important issue i.e. the effects of agents for type 2 diabetes treatment on liver and on NASH and NAFLD in particular. It is well-written and clear.The findings are worth publication even though they are somewhat weak. I valued the discussion because it is very cautious on the intepretation of results and stresses the need for other research in this field.

Reply: We agree that it is important to acknowledge the potential limitations in the interpretation of our results. In the revision of our discussion (suggested by reviewer Siyan Zahn), we have therefore remained cautious.
I suggest adding explainatory legends to figure 2-5 to help the readers understand.
Reply: We have revised the legends to figures 2-5 as suggested.
Reviewer Name Yutaka Seino
Institution and Country Kansai Electric Power Hospital, Osaka Japan
Please state any competing interests or state 'None declared': None declared
The manuscript entitled "Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on non-alcoholic fatty liver disease: systematic review with individual patient data meta-analysis of randomised controlled trials" by Gluud et al describes the meta analysis of RCTs comparing effects of lixi on blood liver enzymes levels with those of placebo or active comparators. They found that lixi treatment associates better with ALT normalization in obese or overweight people with type 2 diabetes. While the issue reported in this manuscript is of great interest, there are several problems that need to be revised for any consideration in its publication.
1. As they pointed out in the manuscript, changes in ALT levels are not necessarily linked to NAFLD. Thus, it is too much to discuss effects of lixi on NAFLD with their reporting data on ALT. Especially, the title should be revised such as "Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on ALT levels in the meta analysis of randomized controlled trials: Implications for non-alcoholic fatty liver disease".
Reply: We agree and have revised our title accordingly. Furthermore, we have attempted to remain cautious in the interpretation of our result.
2. The reviewer concerns how the authors adjust effects of alcohol consumption in RCTs. Nausea and/or vomiting frequently associated with GLP-1RA possibly associates with reduced alcohol consumption, which then affects ALT levels.
Reply: We agree that changes in alcohol consumption may change ALT. None of the included trials registered the exact daily intake in alcohol during the trial. However, considering that none of the included patients had an ongoing alcohol abuse or alcoholic liver disease, we are not convinced that our findings simply reflect confounding due to changes in the intake of alcohol. We have included information about these aspects in the results as well as the discussion section.
3. GLP-1R activation has been suggested to improve NAFLD (J Diabetes Investig. 2013; 4(2):108-130; J Diabetes Complications. 2013; 27(4):401-6). The reviewer wonders why they observe superior ALT normalization in lixi, when compared not only to placebo control but also to GLP-1RA such as lira and exe. They should discuss these issues in discussion.
Reply: As outlined in the review by Yutaka Seino and colleagues (Seino Y, Yabe D. Glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1: Incretin actions beyond the pancreas. J Diabetes Investig. 2013 Mar 18;4(2):108-30), experimental studies found that activation of GLP-1 receptors may prevent diabetes-related comorbidity including obesity and NASH. As suggested by Samson and colleagues (Samson SL, Bajaj M. Potential of incretin-based therapies for non-alcoholic fatty liver disease. J Diabetes Complications. 2013 Jul-Aug;27(4):401-6), GLP-1 analogues may improve hepatic steatosis. At present we have no clear evidence that can explain why we found a beneficial effect of lixisenatide in trials with an active comparator, but not in placebo controlled trials. One potential explanation could be that the trials with an active comparator included a larger proportion of patients who were overweight. None of the other potential patient characteristics seemed to predict the intervention effect. On the other hand, the analyses only include a small number of patients. Although the analyses were defined a priory, subgroup analyses in meta-analyses should always be interpreted with caution. We have included the suggested references and clarified the issues addressed in the discussion.

Reviewer Name Dr Yannan Jiang
Institution and Country The University of Auckland, New Zealand
Please state any competing interests or state 'None declared': None declared
This study investigated the effect of glucagon-like peptide-1 receptor agonist lixisenatide on normalisation of ALT in Type 2 diabetes patients with mildly elevanted liver blood tests. Although this patient population has a considerable risk of NAFLD and subsequenlty NASH, there are no RCTs on lixisenatide for patients with NAFLD at present as the authors stated. Therefore, the title on "Effects of ... lixisenatide on non-alcoholic fatty liver disease" is overstated.
Reply: We have changed our title as suggested.
For meta-analysis, none of the included trials were specifically designed to evaluate the effects of

lixisenatide on liver blood tests. In addition, those patients with severe liver disease were excluded from the trials. Eligible patients with elevant liver blood tests were selected for the study, which were only sub-samples of the randomised cohorts and might not be balanced between the treatment and control groups. As shown in Figure 2, the study sample sizes varied dramatically across all trials (e.g. the first trial only had 3 patients). These issues have raised considerable concerns on the validity of this meta-analysis, whether the study design is appropriate to address the research question of interest.

Reply: None of the included trials were specifically designed to determine the effect of lixisenatide on liver blood tests. One of the specific strengths of the meta-analysis is that it may allow assessments of questions not posed by the individual studies. In general, analyses of specific subgroups may be difficult in systematic reviews of randomized controlled trials that are based on published data. The difficulty is often related to the reporting. Accordingly, analyses of subsets of participants within studies are uncommon in reviews of the literature. By contrast, such subsets of participants can be analysed when individual patient data are collected (Deeks JJ, Higgins JPT, Altman DG (editors). Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 (updated March 2011)). The main strength of our analyses is the fact that we were able to retrieve data from all trials that allowed outcomes to be recalculated based on individual patient data. All our analyses were pre-specified and none of our subgroup analyses were post-hoc. We agree that the sample size of included trials is important as it has a considerable influence on aspects such as precision. We therefore performed our meta-analyses based on the weighted average of the intervention effects estimated in the individual studies. The weights reflect the amount of information that each study contains and therefore also reflects the sample size. We used both random and fixed effect models as well as sequential analyses to test the robustness of our result. These aspects are now clarified in our discussion section.

Results - More details should be given on the characteristics of included trials such as the patients' selection criteria, total sample size, follow up duration (with unit) and outcome assessments. For the purpose of this study, eligible patients were selected for comparison based on their baseline liver blood tests and weight status. The group sizes and distribution of data across relevant categories should be provided, rather than mean/SD only as overall. The wide range of therapy duration from 4 to 76 weeks is of some concern. The p-values should be reported together with 95% confidence interval on the primary outcome, same for the number needed to treat. The effect sizes should also be reported for those patients with different weight status. Additional forest plots on the normalisation of AST and other important secondary outcomes may be useful.

Reply: As requested, we have elaborated on the patient selection criteria. We have included information about the number of events and total number of patients in the forest plots and added information about the duration of follow up (with unit). We have clarified that the primary outcomes assessed in the principle trials was glycemic control and have added the p-values for the primary outcome measure. We have clarified in the results section that we found no clear association between the body weight status and the effect of lixisenatide when analyzing placebo-controlled trials. We have also reported the effect size for patients with a BMI≤25 kg/m2 for placebo-controlled trials. We have included a forest plot on the normalization of AST as requested.

Discussion - The main strength, as highlighted by the authors, is the individual patients' data retrieved from included trials. With the richness of data, more in-depth analyses could be conducted using mixed effect regression models on important individual patients' characteristics and potentially a continuous outcome.

Reply: We agree that the individual patient data was one of the main strengths of our work. The data allowed us to perform regression models on important individual patient characteristics. Based on our protocol, we used dichotomous outcome measures to facilitate the interpretation of our result. Theoretically, a continuous outcome measures could be more sensitive. On the other hand, the size of the effect may be difficult to determine because there is no clear evidence on the size of the decrease in ALT and the size of the intervention effect. Furthermore, additional post hoc analyses at the present stage will increase the risk of spurious results. We have elaborated on these issues in the discussion section.

The paper is well written, and the strength of individual patient data meta-analyses is noticed. However, the study design is limited by the patient populations available from included trials. As discussed, none of the individual trials were specifically designed to evaluate the effects of lixisenatide on liver blood tests. In addition, those patients with severe liver disease were excluded from the trials. The eligible patients selected for this study were no longer a representative sample of

original randomised cohorts, which was considered important in RCTs. More convincing evidence on this meta-analysis is needed to support final conclusions and recommendations.
Reply: We agree that the number of patients included limits the strength of our conclusions. The fact that we had access to the original trial data allowed us to address a clinical question not posed by the individual studies. However, apart from the limitation associated with the nature of the available outcomes (liver blood tests), our sequential analyses show that larger trials are needed. We have therefore been cautious in the interpretation of our result. Although our results are promising, additional trials are needed. This has been clarified in the discussion.

VERSION 2 – REVIEW

REVIEWER	Yutaka Seino Kansai Electric Power Hospital
REVIEW RETURNED	28-Jul-2014

GENERAL COMMENTS	The manuscript has been substantially improved. While there are still some concerns, due to limitations of meta analyses, these issues are difficult to solve and therefore, it should be recommended for its publication in BMJ Open.