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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

Lise L. Gluud[1,2], Filip K. Knop[1], Tina Vilsbøll[1]

[1]Diabetes Research Center, Department of Medicine, Gentofte Hospital, University of Copenhagen, Denmark

[2]Department of Gastroenterology, Hvidovre Hospital, University of Copenhagen, Denmark

L.L. Gluud, Consultant, DMSc

F.K. Knop, Associate Professor, Specialist Registrar, PhD

T. Vilsbøll, Professor, Consultant, DMSc

Running Head: Lixisenatide for type 2 diabetes and elevated liver blood tests

Correspondence to: Gluud, liselottegluud@yahoo.dk

Department of Gastroenterology, Copenhagen University Hospital Hvidovre, Kettagaards Alle, DK-2650 Hvidovre, Denmark.

Key words

Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, meta-analysis, insulin resistance, type 2 diabetes, alanine amino transferase.

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What we already know

Patients with type 2 diabetes (especially those who are overweight) have a high risk of developing non-alcoholic fatty liver disease (NAFLD). Alanine aminotransferase (ALT) is associated with NAFLD, especially in the early stages. Interventions that improve glycaemic control may have beneficial effects on NAFLD in type 2 diabetes. The glucagon-like peptide-1 receptor agonist lixisenatide improves glycaemic control in type 2 diabetes.

What this study adds

Lixisenatide increases the proportion of patients with normalisation of ALT compared with placebo or active comparators.

The effect of lixisenatide on normalisation of ALT was confirmed in subgroup analyses on patients who were obese or overweight, but not for patients with a normal weight.

No beneficial or detrimental effects of lixisenatide on aspartate aminotransferase, alkaline phosphatase or bilirubin were identified.

Abstract

Objective To evaluate the effects of the glucagon-like peptide-1 receptor agonist lixisenatide on elevated liver blood tests in patients with type 2 diabetes.

Design Systematic review.

Data sources Electronic and manual searches were combined.

Study selection Randomised controlled trials (RCTs) on lixisenatide versus placebo or active comparators for type 2 diabetes were included.

Participants Individual patient data were retrieved to calculate outcomes for patients with elevated liver blood tests.

Main outcome measures Normalisation of alanine aminotransferase (ALT) and aspartate aminotransferase.

Data Synthesis The results of included trials were combined in meta-analyses. Sequential, subgroup and regression analyses were performed to evaluate heterogeneity and bias.

Results Included RCTs compared lixisenatide versus placebo (n=12) or the active comparators (N=3) liraglutide, exenatide and sitagliptin. The mean treatment duration was 29 weeks. Lixisenatide increased the proportion of patients with normalisation of ALT (risk difference 0.07, 95% confidence interval 0.01 to 0.14; number needed to treat 14 patients). The effect was not confirmed in sequential analysis. No effects of lixisenatide were identified on aspartate aminotransferase, alkaline phosphatase or bilirubin. No evidence of

bias was identified. Mixed effect multilevel meta-regression analyses suggested that the benefit of lixisenatide was limited to patients who were overweight or obese.

Conclusion This review suggests that lixisenatide increases the proportion of obese or overweight patients with type 2 diabetes who achieve normalisation of ALT. Additional research is needed to determine if the findings translate to clinical outcome measures.

Strengths and limitations of this study

- This systematic review of randomised controlled trials evaluates if lixisenatide has a beneficial effect of liver blood tests associated with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatosis (NASH).
- Based on analyses of individual patient data, lixisenatide increases the proportion of patients with normalisation of alanine aminotransferase (ALT) compared with placebo or active comparators. In subgroup analyses, the effect was verified for patients who were obese or overweight, but not for patients with a normal weight.
- The analyses include data from published and unpublished trials with intention to treat analyses of all patients included irrespective of compliance or follow up. The bias control was classed as adequate in all trials based on four or five of the five components included in the Cochrane bias assessment tool.
- Although ALT is the most sensitive biochemical marker of NAFLD and NASH, important effects may be overlooked because patients with severe liver disease were excluded from the trials.
- The available data did not allow for assessment of clinical outcome measures such as development of cirrhosis or hepatocellular carcinoma.

Introduction

The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing and the costs to are considerable.[1-3] About ten per cent of patients with NAFLD develop non-alcoholic steato-hepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. Obesity and decreased insulin sensitivity increase the risk of these complications. The risk of NAFLD and NASH is associated with type 2 diabetes and obesity. NAFLD is generally an asymptomatic disease. Liver blood tests are linked with metabolic risk factors and are independent predictors of NAFLD although the sensitivity is low.[4] A systematic review on observational studies found that routinely available biochemical markers may be used in the assessment of NAFLD.[5] Elevation of alanine aminotransferase (ALT) is more common than aspartate aminotransferase (AST).[6] The gold standard for the assessment of patients with NAFLD is to perform a liver biopsy but the procedure is associated with risks and potential sampling error. Biopsy-related complications including bleeding still occur in ultrasonically-guided techniques.[7 8]

A systematic review from 2007 found three randomised controlled trials (RCTs) on drugs that aim to improve insulin resistance in patients with NAFLD or NASH.[9] Based on a meta-analysis including two of these trials, treatment with metformin increased the proportion of patients with normalisation of ALT compared with diet alone or vitamin E. A subsequent health technology assessment on insulin sensitizers for NAFLD[10] included RTCs on metformin, rosiglitazone and pioglitazone. Pioglitazone improved liver histology. The effect of metformin on histology was less convincing, but both metformin and pioglitazone reduced ALT. A systematic review on the glucagon-like peptide-1 receptor agonists (GLP-1RAs) liraglutide and exenatide for patients with diabetes found beneficial

effects on body weight, ALT and AST.[11] Unfortunately, subgroup analyses on patients with increased liver blood tests were not available.

Patients with type 2 diabetes and elevated transaminases have a considerable risk of NAFLD and subsequently NASH. The risk of developing NASH and the severity of the disease is closely linked with impaired insulin sensitivity and overweight. Lixisenatide is a GLP-1RA that improves glycaemic control and reduces body weight in patients with type 2 diabetes.[12 13] These effects suggest a potential benefit in patients with type 2 diabetes and NAFLD. At present, there are no RCTs on lixisenatide or other GLP1RAs for patients with NAFLD. We therefore conducted a systematic review with individual patient meta-analyses on patients with type 2 diabetes and elevated liver blood tests randomised to lixisenatide, placebo, no intervention or active controls.

Methods

This review is based on a registered protocol (CRD42013005779). The review methods follow the recommendations described in the Cochrane Handbook for Reviews on Interventions (www.cochrane.org). RCTs were included irrespective of blinding, language or publication status. Adult patients with elevated liver blood tests were included irrespective of gender or body weight. The intervention comparisons included lixisenatide versus placebo, no intervention or other active comparators.

Based on previous evidence,[6] the primary outcome measures were normalisation of ALT and AST. Secondary outcome measures included normalisation of alkaline phosphatase

and bilirubin as well as a normalisation of the composite outcome measure combining all liver blood tests. The pharmaceutical company producing lixisenatide (Sanofi-Aventis) provided data and additional information on the design of included trials. All outcomes were recalculated based on individual patient data.

All authors participated in the identification and selection of trials. Excluded trials were listed with the reason for exclusion. Eligible trials were identified through electronic and manual searches. Electronic searches were performed in MEDLINE, Cochrane Library, Embase and Web of science. The Cochrane Library was searched using the strategy: lixisenatide ti, ab, kw (with automated word variations included). Additional manual searches were performed in reference lists of relevant papers, correspondence with experts, the pharmaceutical company producing lixisenatide and the World Health Organisation Trial Search Database (http://apps.who.int/trialsearch/).

The bias risk assessment followed the recommendations described in the Cochrane Handbook for Reviews of Interventions and included the domains selection bias (random sequence generation, allocation concealment), performance bias (blinding of participants and personnel, blinding of outcome assessment), attrition bias (incomplete outcome data), reporting bias (selective reporting), and other sources of bias.

Statistical Analysis

The analyses were performed in Stata version 13 (STATA Corp, Texas, USA). Random effects meta-analyses were performed due to an expected between study heterogenetiy. The results were expressed as risk differences with 95% confidence intervals and I^2 as a measure of heterogeneity and with the number needed to treat for statistically significant outcome measures. We defined I^2 values below 30% as unimportant, 30-50% as moderate heterogeneity, 50-75% as substantial heterogeneity and >75% as considerable heterogeneity. All patients were included in the analysis irrespective of compliance or follow-up and with imputation of outcomes for patients with missing outcome data (intention to treat).

Mixed effect multilevel meta-regression and subgroup analyses were performed to evaluate heterogeneity. The meta-regression analysis evaluated the influence of the metabolic regulation (HbA_{1c} ≤8.5% (69 mmol/mol)), duration of diabetes (≥5 years), and body mass index (BMI) (normal weight ≤25 kg/m^2, overweight >25 kg/m^2 or obese >30 kg/m^2). Post-hoc analyses were performed to evaluate the effect of the change in bodyweight and ALT. The subgroup analyses evaluated the influence of publication status (full paper articles compared with abstracts and unpublished trials), control groups and collateral interventions. Since all trials had a low risk of bias, we did not perform subgroup analyses on bias control. Publication bias and other small study effects were estimated in regression analyses (Harbord's test) and funnel plots. We performed sequential analyses to evaluate the robustness of results from meta-analyses with a statistically significant result. The analysis was performed with alpha set to 5%, power to 80%, model-based

diversity correction and the relative risk reduction and control group incidence rate determined in the meta-analysis.

Results

The initial searches identified 531 potentially eligible records (figure 1). After reading the titles and abstracts, duplicates and records that clearly did not describe RCTs on lixisenatide were excluded. One ongoing trial was excluded because data were not yet available. The remaining records referred to 15 multicentre RCTs that were included in the qualitative and quantitative analyses (table 1). Eleven trials were published as full paper articles,[14-24] three were published as abstracts[25-27] and one was unpublished.

None of the included trials found statistically significant differences between patient characteristics in the lixisenatide and control groups. The proportion of men was 50% and the mean age in the lixisenatide and control group ranged from 43 to 61 years (table 2). For the lixisenatide group, mean BMI ranged from 25.1 to 36.8 kg/m^2 and the mean HbA_{1c} from 7.2 to 8.5% (53 to 69 mmol/mol). For the control group, the mean BMI ranged from 25.2 to 36.8 kg/m^2 and the HbA_{1c} 7.4 to 8.9% (56 to 74 mmol/mol). Table 3 shows the mean baseline liver blood tests in the lixisenatide and control groups. The proportion of patients with elevated ALT ranged from 20 to 77% for the lixisenatide group and from 19 to 75% for the control groups.

The duration of therapy ranged from 4 to 76 weeks (mean 29 weeks). Two trials (one unpublished)[14] were designed to evaluate dose titration. The dose of lixisenatide was 20 microgram once-daily in the remaining trials. Twelve trials compared lixisenatide versus placebo and three trials compared lixisenatide versus active controls administered once-daily (liraglutide and sitagliptin) or twice daily (exenatide). The collateral interventions (background therapy) were metformin (five trials), metformin plus sulphonylurea (four trials), metformin plus pioglitazone (one trial), insulin (three trials) or diet (one trial). The collateral interventions were administered equally to the lixisenatide and control groups.

All trials used a parallel group design with patients randomised 1:1 or 2:1. All trials used adequate allocation sequence generation and allocation concealment (central randomisation of computer-generated random numbers). All trials with a placebo control were double blind (with blinding of patients and investigators including outcome assessors). Trials with an active control group were open label. No evidence of attrition bias (incomplete outcome data), reporting bias (selective reporting) or other biases were identified.

In total, 1,070 patients had elevated ALT at baseline (figure 2). Lixisenatide had a beneficial effect on normalisation of ALT (risk difference 0.073, 0.01 to 0.14; I^2=23%; number needed to treat 14 patients). The sequential analysis confirmed the primary meta-analysis when using the traditional 5% level of statistical significance (figure 3), but not after adjusting for multiple testing (the trial monitoring boundary was not crossed). Mixed effect multilevel meta-regression analyses of double blind trials (figure 4) found no effect of

the metabolic regulation, duration of diabetes or BMI on the overall result (P>0.05 for all analyses). When the analyses were repeated for RCTs with an active control group, lixisenatide had a beneficial effect on normalisation of ALT among patients who were obese (P=0.01) or overweight (P=0.004), but not among normal weight patients (P=0.98). There was a moderate correlation between change in bodyweight and change in ALT (regression coefficient = 0.38). The baseline metabolic regulation and duration of diabetes did not predict the intervention effect. No evidence of small study effects was seed in regression analysis (Harbord's test P=0.26) or funnel plots (figure 5). Subgroup analyses showed no differences between trials stratified by the publication status, control groups or collateral interventions.

In total, 191 of 303 (37%) patients randomised to lixisenatide and 128 of 216 (41%) controls achieved normalisation of AST after treatment (0.04; -0.04 to 0.13; I^2=9%). Lixisenatide had no effect on alkaline phosphatase (-0.10; -0.23 to 0.03), bilirubin (-0.12, -0.30 to 0.07) or normalisation of all liver blood tests (0.01, -0.01 to 0.03). No differences between subgroups were identified.

Discussion

This systematic review found a potential beneficial effect of lixisenatide compared with placebo or active glucose-lowering comparators on ALT levels among patients with type 2 diabetes. Our analysis suggests that for every 14 patients treated with lixisenatide for about 29 weeks, one additional patient will achieve normalisation of ALT. The risk difference was small and the sequential analyses did not confirm the findings.

Furthermore, none of the trials were specifically designed to evaluate the effects of lixisenatide on liver blood tests. Liver blood tests were only mildly elevated and patients with severe liver disease were excluded from the trials. Although the results are promising, additional evidence is needed to determine if our findings translate to clinical outcome measures.

ALT is included in the diagnostic evaluations and follow-up of patients with NAFLD and NASH in clinical practice. Nevertheless, the value of this surrogate outcome is debatable and previous evidence suggests that the sensitivity is low. The strength of our findings is mainly related to the fact that we were able to retrieve all the necessary data from included trials. Information on the design and conduct of the trials as well as all outcome measures were obtained. Our primary outcome measure is objective, which also strengthens our findings. Lixisenatide only appeared to have an effect on ALT, which is the most sensitive biochemical marker of NAFLD. Although the included trials were large, only a proportion of patients had elevated liver blood tests at baseline. In spite of the hypothetical benefit of lixisenatide on AST, we only found a non-significant trend on this outcome measure. As expected, no beneficial or detrimental effects were identified when analysing the remaining liver blood tests.

Individual patient data meta-analyses are based on the original research data instead of data extracted from published reports. The benefits of this approach include a reduced risk of errors during reporting and data collection as well as the ability to perform the relevant subgroup and sensitivity analyses. These data can then be re-analysed centrally and, if

appropriate, combined in meta-analyses. The quality of such analyses is high.[28 29] For this reason they are considered to be a 'gold standard' of systematic reviews.[28]

Incretin-based therapies such as lixisenatide and other GLP-1RAs are an important part of the pharmacological treatment of patients with type 2 diabetes. The beneficial effects include improved glycaemic control as well as beneficial effects on bodyweight, blood pressure, cholesterol and cardiovascular biomarkers. Lixisenatide is a once-daily GLP-1RA. The included RCTs found beneficial effects of lixisenatide used as monotherapy or in combination with metformin, sulphonylureas, thiazolidinediones or basal insulin glargine. The improved glycaemic control was mainly demonstrated in RCTs with a placebo control. Conversely, we found that the benefit of lixisenatide on liver blood tests was more pronounced in RCTs with an active control group. This result suggests that improved metabolic regulation and reductions in body weight may not be the only reason for the potentially beneficial effect on lixisenatide on NAFLD.

In conclusion, the use of lixisenatide seems to have beneficial effects on liver blood tests and could possibly have a role in the treatment of patients with NAFLD.

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Contributorship Statement: LG drafted the paper and performed the statistical analyses. TV and FK revised the paper. All authors participated in the interpretation of the results and have approved of the final version.

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and received fees for speaking from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Novartis, Sanofi, Takeda and Zealand Pharma, and received research support from Novo Nordisk.

Data Sharing Statement: Data will be available on request

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Legends to figures

Figure 1 Trial flow chart.

Figure 2 Random effects meta-analysis of randomised controlled trials on normalisation of alanine aminotransferase (ALT). The intervention comparisons are lixisenatide versus placebo or active interventions. The included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT.

Figure 3 Sequential analysis of risk ratios (random effects) in randomised controlled trials on lixisenatide versus placebo or active interventions for patients with type 2 diabetes and elevated alanine aminotransferase (ALT) at baseline. The outcome measure is normalisation of ALT. The analysis shows that lixisenatide has a beneficial effect on normalisation of ALT when assessed using the traditional 5% level of significance (the horizontal line), but not after adjusting for cumulative assessment (the trial monitoring boundary).

Figure 4 Mixed model meta-regression analysis of the effect of lixisenatide versus placebo on normalisation of alanine aminotransferase (ALT). Included patients have type 2 diabetes and elevated ALT at baseline. The figure shows the estimated intervention effect (normalisation of ALT) on the log-odds ratio scale in relation to the baseline body weight of included patients from 12 placebo controlled randomised controlled trials. The size (area) of each circle is inversely proportional to the variance of the log-odds ratio (the larger the circle the less the variance).

Figure 5 Funnel plot including randomised controlled trials on lixisenatide for patients with type 2 diabetes. The outcome measure is normalisation of alanine aminotransferase (ALT). The figure shows the estimated intervention (Risk Difference) effect with its associated standard error (seRD).

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Table 1 Characteristics of included trials

Trial	Publication status	Control	Collateral interventions*	Duration
Lorenz 2013 ACT6011 QD	Full paper	Placebo	Sulfonulureas ± metformin	4
Ratner 2010 DRI6012 QD	Full paper	Placebo	Metformin	24
Pan 2012 GetGoal-M-Asia	Abstract	Placebo	Sulfonulureas ± metformin	24
Riddle 2013 GetGoal-Duo1	Full paper	Placebo	Insulin	24
Riddle 2013 GetGoal-L	Full paper	Placebo	Insulin	24
Seino 2012 GetGoal-L-Asia	Full paper	Placebo	Insulin	24
Ahren 2013 GetGoal-M	Full paper	Placebo	Metformin	24
Fonseca 2012 GetGoal-Mono	Full paper	Placebo	Diet	24
Ratner 2012 GetGoal-S	Abstract	Placebo	Sulfonulureas ± metformin	76
PDY6797 QD	Unpublished	Placebo	Sulfonulureas ± metformin	76
Rosenstock 2013 GetGoal-X	Full paper	Exenatide	Metformin	24
Kapitza 2013 PDY10931	Full paper	Liraglutide	Metformin	4
Seino 2012 EFC10780	Full paper	Sitagliptin	Metformin	24
Pinget 2013 GetGoal-P	Full paper	Placebo	Pioglitazone ±metformin	24
Bolli 2013 GetGoal-F1	Full paper	Placebo	Metformin	24

*Collateral interventions were administered equally to the lixisenatide and control groups

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Table 2 Characteristics of included patients (mean and standard deviation)

Trial	Body Mass Index				Weight				Glycated haemoglobin			
	Lixisenatide		Controls		Lixisenatide		Controls		Lixisenatide		Controls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lorenz 2013 ACT6011 QD	31.38	4.05	29.77	3.76	89.40	14.66	83.80	14.68	8.54	1.07	8.87	1.07
Ratner 2010 DRI6012 QD	32.01	4.28	31.74	4.15	89.37	17.00	87.68	13.63	7.58	0.65	7.53	0.63
Bolli 2013 GetGoal-F1	32.53	5.36	32.37	5.45	88.81	17.98	87.87	17.37	8.05	0.88	8.03	0.82
Riddle 2013 GetGoal-Duo1	31.99	6.63	31.65	6.01	87.31	21.76	86.75	20.41	7.56	0.55	7.60	0.54
Seino 2012 GetGoal-L-Asia	25.36	3.69	25.15	3.94	65.93	13.00	65.60	12.47	8.54	0.73	8.52	0.78
Pan 2012 GetGoal-M-Asia	26.75	3.86	27.08	3.75	73.18	13.93	72.74	13.64	7.95	0.81	7.85	0.71
Ahren 2013 GetGoal-M	32.84	6.34	33.12	6.45	89.57	20.91	90.15	20.14	8.06	0.89	8.06	0.90
Ratner 2012 GetGoal-S	30.13	6.62	30.42	6.64	82.30	21.76	84.42	22.83	8.28	0.86	8.21	0.84
Riddle 2013 GetGoal-L	31.91	6.17	32.56	6.32	87.10	20.01	88.94	20.84	8.42	0.88	8.37	0.84
Pinget 2013 GetGoal-P	33.66	6.71	34.44	7.04	92.93	22.90	96.74	25.58	8.08	0.90	8.06	0.79

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Fonseca 2012	31.99	6.66	31.76	6.69	87.77	21.58	86.08	22.21	8.03	0.89	8.07	0.91
GetGoal-Mono PDY6797	25.09	3.65	26.39	3.50	71.54	16.77	76.92	16.07	8.19	0.82	8.38	0.75
Rosenstock 2013	33.68	6.27	33.51	6.54	94.01	19.63	96.09	22.52	7.95	0.81	7.97	0.78
GetGoal-X												
Seino 2012	36.76	7.25	36.76	6.34	98.51	23.48	100.56	23.77	8.16	0.89	8.09	0.96
EFC10780												
Kapitza 2013	31.23	3.93	31.33	4.08	91.16	15.28	92.88	16.59	7.20	0.63	7.41	0.81
PDY10931												

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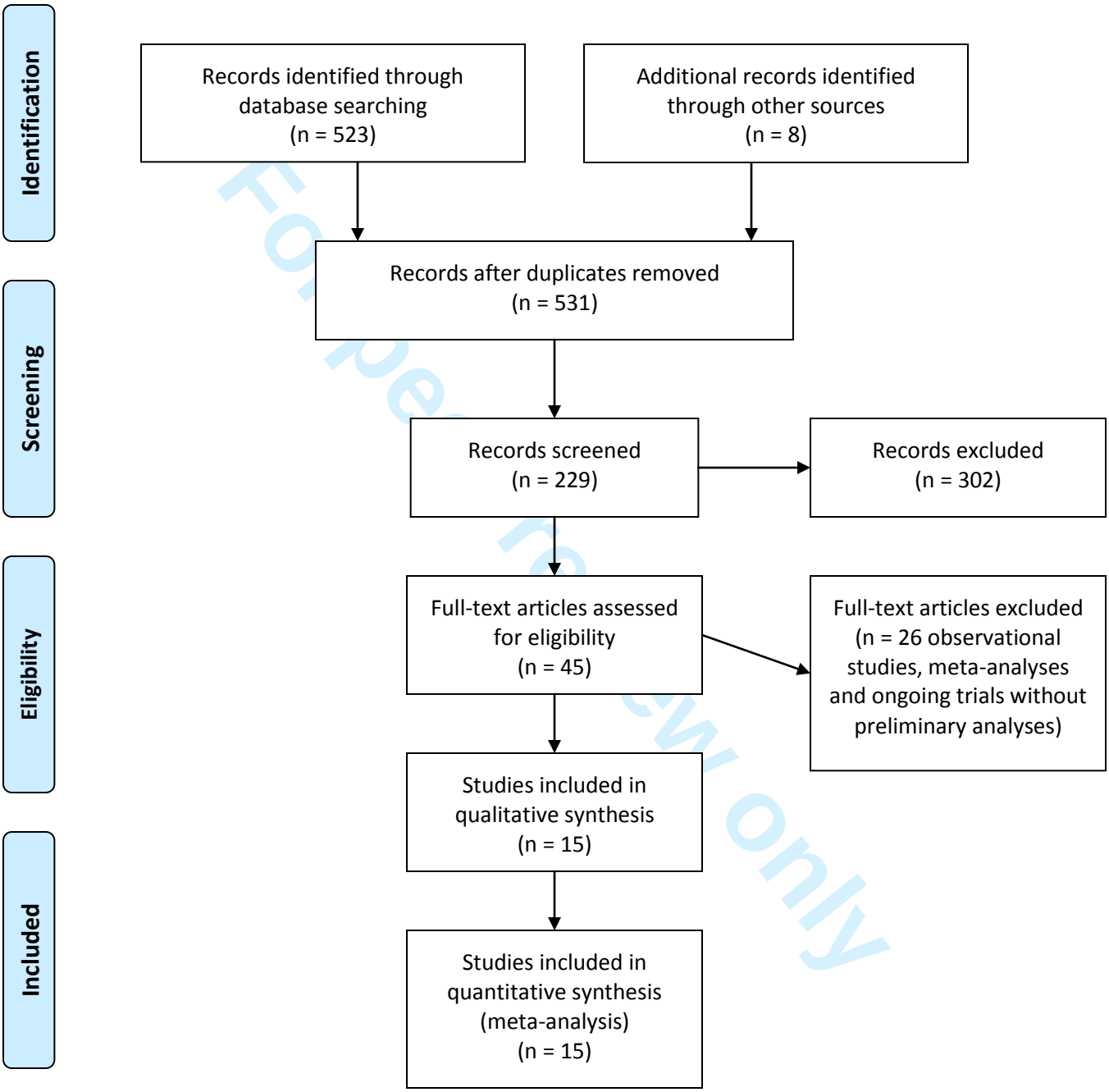
Table 3 Baseline liver blood tests (mean and standard deviation, SD)

Trial	Alanine aminotransferase				Aspartate aminotransferase				Alkaline phosphatase			
	Lixisenatide		Controls		Lixisenatide		Controls		Lixisenatide		Controls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lorenz 2013 ACT6011 QD	25.48	8.18	25.91	12.50	19.43	4.95	19.23	4.62	83.52	27.00	83.55	19.43
Ratner 2010 DRI6012 QD	28.31	19.42	25.24	13.94	24.65	15.38	23.49	11.35	73.60	28.28	75.47	41.00
Bolli 2013 GetGoal-F1	30.69	16.34	29.34	15.41	23.93	10.30	23.62	10.68	72.83	21.58	74.54	21.23
Riddle 2013 GetGoal-Duo1	23.93	17.09	24.71	12.90	21.16	8.81	21.67	8.64	71.18	21.65	70.95	23.29
Seino 2012 GetGoal-L-Asia	25.19	12.85	23.03	10.34	22.78	7.56	21.43	7.79	71.90	17.59	71.01	20.11
Pan 2012 GetGoal-M-Asia	31.36	19.32	32.82	20.69	24.13	11.07	25.14	11.08	78.89	21.60	80.48	26.75
Ahren 2013 GetGoal-M	31.08	16.58	33.48	46.58	24.38	11.74	26.12	22.07	78.88	24.24	74.65	25.15
Ratner 2012 GetGoal-S	26.93	12.56	27.72	12.64	21.45	7.09	21.54	7.20	73.40	21.41	72.69	22.69
Riddle 2013 GetGoal-L	26.57	17.61	25.92	12.57	22.88	12.13	22.31	7.76	81.38	25.84	78.56	25.43
Pinget 2013	24.66	12.01	24.59	10.86	21.46	8.93	21.48	8.27	75.24	29.68	73.13	22.49

Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on liver blood tests: systematic review with individual patient data meta-analysis of randomised controlled trials. Gluud et al

GetGoal-P												
Fonseca 2012	29.74	16.05	25.43	11.40	23.71	11.78	21.91	7.40	78.95	21.10	80.07	26.42
GetGoal-Mono												
PDY6797	23.72	11.15	27.00	14.67	23.97	15.33	23.73	9.52	61.59	14.64	70.73	18.19
Seino 2012	35.70	20.63	39.83	23.64	25.50	13.76	28.74	20.54	81.97	25.11	83.61	28.13
EFC10780												
Rosenstock 2013	28.50	13.06	30.65	15.83	22.17	7.92	23.28	9.27	72.43	22.13	72.15	19.86
GetGoal-X												
Kapitza 2013	33.88	16.36	34.23	16.99	27.31	13.47	26.45	9.07	67.56	16.62	67.58	20.07
PDY10931												

Figure 1



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Figure 2

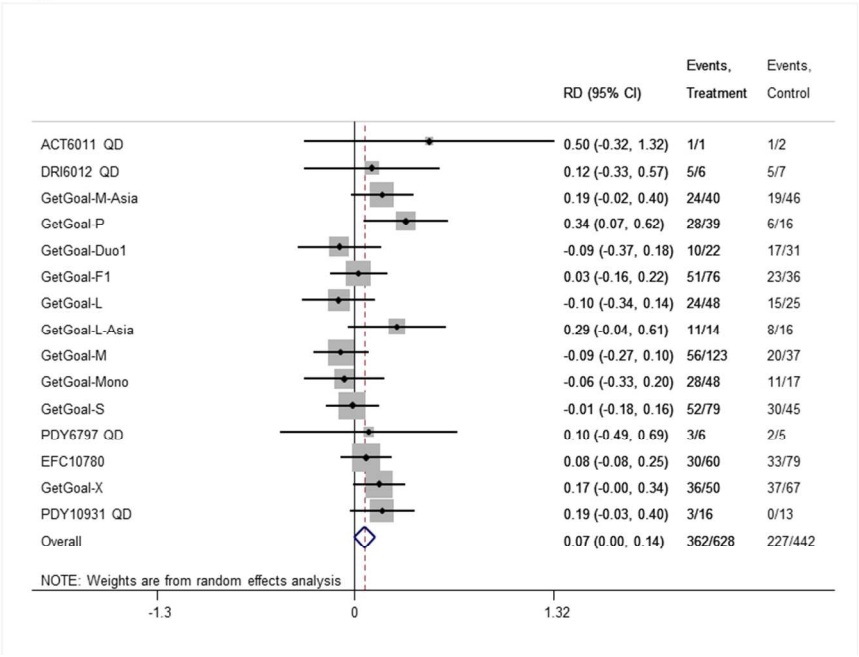

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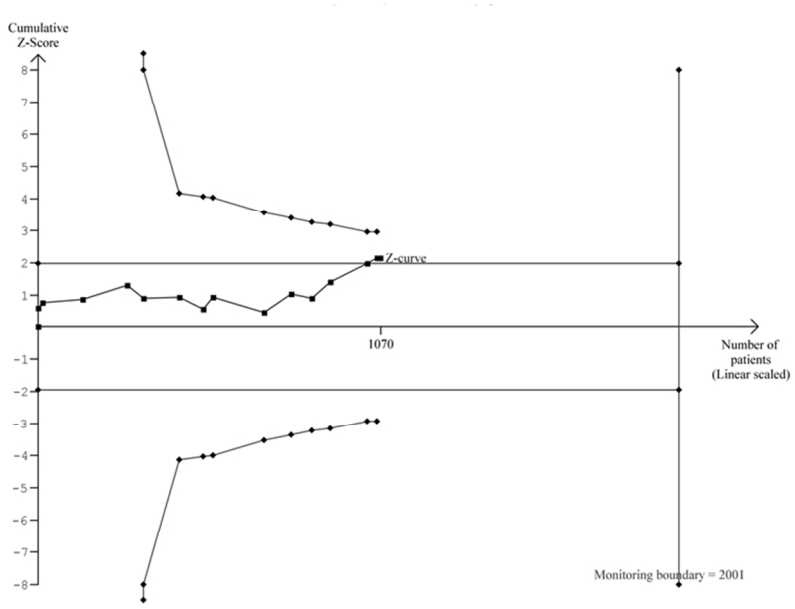
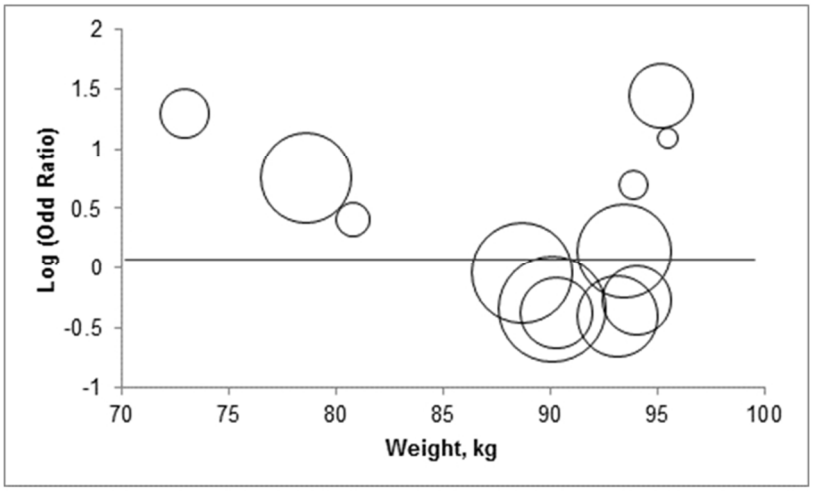
Figure 3



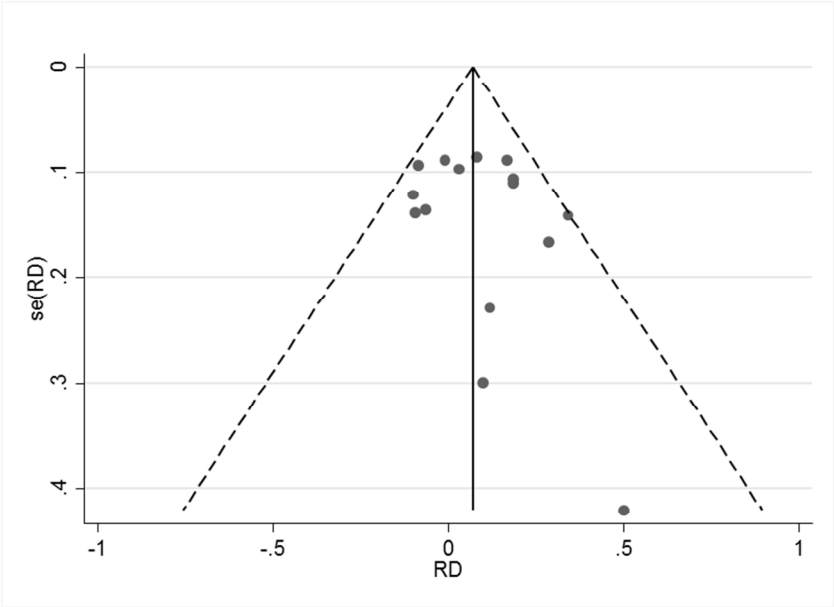
254x190mm (96 x 96 DPI)

Figure 4



254x190mm (96 x 96 DPI)

Figure 5



254x190mm (96 x 96 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6-7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8-9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8-9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8-9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8-9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9-10 and tables
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10-11 and figures
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11 and figures
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11-13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11-13
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	14

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097.

PRISMA 2009 Checklist

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BMJ Open

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

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Secondary Subject Heading:	Gastroenterology and hepatology
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Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on liver blood tests: systematic review with individual patient data meta-analysis of randomised controlled trials. Gluud et al

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

Lise L. Gluud[1,2], Filip K. Knop[1], Tina Vilsbøll[1]

[1]Diabetes Research Centre, Department of Medicine, Gentofte Hospital, University of Copenhagen, Denmark

[2]Department of Gastroenterology, Hvidovre Hospital, University of Copenhagen, Denmark

L.L. Gluud, Consultant, DMSc

F.K. Knop, Associate Professor, Specialist Registrar, PhD

T. Vilsbøll, Professor, Consultant, DMSc

Running Head: Lixisenatide for type 2 diabetes and elevated liver blood tests

Correspondence to: L.L. Gluud, Department of Gastroenterology, Copenhagen University Hospital Hvidovre, Kettegaards Alle 30, DK-2650 Hvidovre, Denmark; liselottegluud@yahoo.dk

Key words: Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, meta-analysis, insulin resistance, type 2 diabetes, alanine aminotransferase.

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Word count: Abstract: 241; Text: 3324

Abstract

Objective To evaluate the effects of the glucagon-like peptide-1 receptor agonist lixisenatide on elevated liver blood tests in patients with type 2 diabetes.

Design Systematic review.

Data sources Electronic and manual searches were combined.

Study selection Randomised controlled trials (RCTs) on lixisenatide versus placebo or active comparators for type 2 diabetes were included.

Participants Individual patient data were retrieved to calculate outcomes for patients with elevated liver blood tests.

Main outcome measures Normalisation of alanine aminotransferase (ALT) and aspartate aminotransferase.

Data Synthesis The results of included trials were combined in meta-analyses. Sequential, subgroup and regression analyses were performed to evaluate heterogeneity and bias.

Results We included 12 RCTs on lixisenatide versus placebo and three RCTs with the active comparators liraglutide, exenatide, or sitagliptin. The mean treatment duration was 29 weeks. Lixisenatide increased the proportion of patients with normalisation of ALT (risk difference: 0.07; 95% confidence interval: 0.01 to 0.14; number needed to treat: 14 patients, p=0.042). The effect was not confirmed in sequential analysis. No effects of lixisenatide were identified on AST, alkaline phosphatase or bilirubin. No evidence of bias

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was identified. Mixed effect multilevel meta-regression analyses suggested that the benefit of lixisenatide on ALT was limited to patients who were overweight or obese.

Conclusion This review suggests that lixisenatide increases the proportion of obese or overweight patients with type 2 diabetes who achieve normalisation of ALT. Additional research is needed to determine if the findings translate to clinical outcome measures.

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Strengths and limitations of this study

- This systematic review of randomised controlled trials evaluates if lixisenatide has a beneficial effect of liver blood tests associated with non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH).
- Based on analyses of individual patient data, lixisenatide increases the proportion of patients with normalisation of alanine aminotransferase (ALT) compared with placebo or active comparators. In subgroup analyses, the effect was verified for patients who were obese or overweight, but not for normal weight patients.
- The analyses include data from published and unpublished trials with intention-to-treat analyses of all patients included irrespective of compliance or follow up. The bias control was classed as adequate in all trials based on four or five of the five components included in the Cochrane bias assessment tool.
- Although ALT is the most sensitive biochemical marker of NAFLD and NASH, important effects may be overlooked because patients with severe liver disease were excluded from the trials.
- The available data did not allow for assessment of clinical outcome measures such as development of cirrhosis or hepatocellular carcinoma.

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What we already know

Patients with type 2 diabetes (especially those who are overweight) have a high risk of developing non-alcoholic fatty liver disease (NAFLD). Alanine aminotransferase (ALT) is associated with NAFLD, especially in the early stages. Interventions that improve glycaemic control may have beneficial effects on NAFLD in type 2 diabetes. The glucagon-like peptide-1 receptor agonist lixisenatide improves glycaemic control in type 2 diabetes.

What this study adds

Lixisenatide increases the proportion of patients with normalisation of ALT compared with placebo or active comparators.

The effect of lixisenatide on normalisation of ALT was confirmed in subgroup analyses on patients who were obese or overweight, but not for patients with a normal weight.

No beneficial or detrimental effects of lixisenatide on aspartate aminotransferase, alkaline phosphatase or bilirubin were identified.

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Introduction

The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing and the costs are considerable.[1-3] About ten per cent of patients with NAFLD develop nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. Obesity and decreased insulin sensitivity are associated with NAFLD and NASH, which common in patients with type 2 diabetes.[4-6] NAFLD is generally an asymptomatic disease. Elevated transaminases are independent predictors of NAFLD although the sensitivity is low.[7] A large proportion of patients with type 2 diabetes and elevated transaminases have NAFLD or NASH. A systematic review on observational studies found that routinely available biochemical markers may be used in the assessment of NAFLD.[8] Elevation of alanine aminotransferase (ALT) is more common than aspartate aminotransferase (AST).[9] The gold standard for the assessment of patients with NAFLD is to perform a liver biopsy, but the procedure is associated with risks and potential sampling error. Biopsy-related complications including bleeding still occur in ultrasonically guided techniques.[10 11]

Treatment of NAFLD and NASH is important. Anti-diabetic interventions have been assessed as a potential treatment option. A systematic review from 2007 found three randomised controlled trials (RCTs) on metformin and pioglitazone in patients with NAFLD or NASH.[12] The review found that the interventions increased the proportion of patients with normalisation of ALT. A subsequent health technology assessment on insulin sensitizers for NAFLD reached a similar conclusion.[13] However, pioglitazone is associated with a considerable risk of serious adverse events including cardiovascular disease and bladder cancer.[14 15] Alternative treatment options are therefore needed.

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Recent studies suggest that glucagon-like peptide-1 receptor agonists (GLP-1RA) improve insulin sensitivity and resistance - most likely via their body weight-lowering effect.[16] In addition, GLP-1RAs may have a direct effect on hepatocytes reducing hepatic steatosis via glucagon-like peptide-1 receptors in the liver.[17] A review on the GLP1-RA liraglutide and exenatide found that the interventions reduce ALT and AST in patients with type 2 diabetes or obesity.[18] Unfortunately, analyses of patients with elevated liver blood tests were not available. Lixisenatide is a GLP-1RA that improves glycaemic control and reduces body weight in patients with type 2 diabetes.[19 20] There are no RCTs on lixisenatide for patients with NAFLD. However, several trials on lixisenatide included patients who were overweight and allowed inclusion of patients with mildly elevated liver blood tests. The trials were therefore likely to include a relatively large proportion of patients with NAFLD. We therefore conducted a systematic review with outcomes recalculated based on individual patient data from RCTs to determine the effect of lixisenatide on elevated liver blood tests in patients with type 2 diabetes.

Methods

This review is based on a registered protocol (CRD42013005779). The review methods follow the recommendations described in the Cochrane Handbook for Reviews on Interventions (www.cochrane.org). The main objective was to compare the effect of lixisenatide versus placebo or other active interventions on normalisation of liver transaminases. RCTs were included irrespective of blinding, language or publication status. Adult patients with type 2 and elevated liver blood tests were included irrespective of gender or body weight.

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Based on previous evidence,[9] the primary outcome measures were normalisation of ALT and AST. Secondary outcome measures included normalisation of alkaline phosphatase and bilirubin as well as a normalisation of the composite outcome measure combining all liver blood tests. The pharmaceutical company producing lixisenatide (Sanofi-Aventis) provided data and additional information on the design of included trials. All outcomes were recalculated based on individual patient data.

All authors participated in the identification and selection of trials. Excluded trials were listed with the reason for exclusion. Eligible trials were identified through electronic and manual searches. Electronic searches were performed without language restrictions in MEDLINE (1946-Feb 2014), Cochrane Library (Issue 2, 2014), Embase (1974 to Feb 2014) and Web of science (1900-Feb 2014). The search strategy in the Cochrane library was lixisenatide, ti,ab,kw (Word variations have been searched) in the Cochrane Library. In Medline, the search strategy was (lixisenatide AND (("randomized controlled trial"[Publication Type]) OR ("controlled clinical trial"[Publication Type])). Additional manual searches were performed in reference lists of relevant papers, correspondence with experts, the pharmaceutical company producing lixisenatide and the World Health Organisation Trial Search Database (http://apps.who.int/trialsearch/).

Two authors extracted data in an independent manner (LG and TV). Disagreements were resolved through discussion. The bias risk assessment was based on the Cochrane Collaboration Risk of Bias Assessment tool (www.cochrane.org). The assessment included the separate domains random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete

outcome data, selective bias and other biases. Each domain was classed as having a high, uncertain or low risk of bias.

Random sequence generation: Low risk' of bias: The investigators describe a random component in the sequence generation process such as: referring to a random number table or using a computer random number generator. Unclear risk of bias: Insufficient information about the sequence generation process to permit judgment of 'low risk' or 'high risk'. High risk of bias: The investigators describe a non-random component in the sequence generation process.

Allocation concealment: Low risk of bias: Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: Central allocation (including telephone, web-based and pharmacy-controlled). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: Participants or investigators enrolling participants could possibly foresee assignments.

Blinding of participants and personnel: Low risk of bias: No blinding or incomplete blinding, but the outcome is not likely to be influenced by lack of blinding (e.g., objective outcome measures such as blood tests). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding.

Blinding of outcome assessment: Low risk of bias: No blinding of outcome assessment, but the outcome measurement is not likely to be influenced by lack of blinding (e.g., blood tests). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high

risk'. High risk of bias: No blinding of outcome assessment and the outcome measurement is likely to be influenced by lack of blinding.

Incomplete outcome data: Low risk of bias: No missing outcome data; reasons for missing outcome data unlikely to be related to true outcome. Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups, The proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; missing data have been imputed using appropriate methods. Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: Reason for missing outcome data likely to be related to true outcome; the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; 'as-treated' analysis done with substantial departure of the assigned intervention; potentially inappropriate application of simple imputation.

Selective reporting: Low risk of bias: The study protocol is available and all of the study's pre-specified outcomes (primary and secondary) that are of interest in the review have been reported in the pre-specified way; The study protocol is not available, but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: Not all of the study's pre-specified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data that were not pre-specified; one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse

effect); one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; the study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Other bias: Low risk: The study appears to be free of other sources of bias. Unclear risk of bias: Insufficient information to assess whether an important risk of bias exists. High risk: The study had a potential source of bias related to the specific study design used, or has been claimed to have been fraudulent, or had some other problem.

Statistical analysis

The analyses were performed in Stata version 13 (STATA Corp, Texas, USA). Random effects meta-analyses were performed due to an expected between study heterogeneity. The results were expressed as risk differences with 95% confidence intervals (CI) and p values and I^2 as a measure of heterogeneity and with the number needed to treat for statistically significant outcome measures. We defined I^2 values below 30% as unimportant, 30-50% as moderate heterogeneity, 50-75% as substantial heterogeneity and >75% as considerable heterogeneity. All patients were included in the analysis irrespective of compliance or follow-up and with imputation of outcomes for patients with missing outcome data (intention to treat). Mixed effect multilevel meta-regression and subgroup analyses were performed to evaluate heterogeneity. The meta-regression analysis evaluated the influence of the metabolic regulation (HbA_{1c} ≤8.5% (69 mmol/mol)), duration of diabetes (≥5 years), and body mass index (BMI) (normal weight ≤25 kg/m^2, overweight >25 kg/m^2 or obese >30 kg/m^2). Post hoc analyses were performed to evaluate the effect of the change in bodyweight and ALT. The subgroup analyses evaluated the influence of publication status (full paper articles compared with abstracts and unpublished trials),

control groups and collateral interventions. Since all trials had a low risk of bias, we did not perform subgroup analyses on bias control. Publication bias and other small study effects were estimated in regression analyses (Harbord's test) and funnel plots. We performed sequential analyses to evaluate the robustness of results from meta-analyses with a statistically significant result. The analysis was performed with alpha 5%, power 80%, model-based diversity correction 12%, relative risk reduction 8% and control group incidence rate 51%.

Results

The initial searches identified 531 potentially eligible records (figure 1). After reading the titles and abstracts, duplicates and records that clearly did not describe RCTs on lixisenatide were excluded. One ongoing trial was excluded because data were not yet available. The remaining records referred to 15 multicentre RCTs that were included in the qualitative and quantitative analyses (table 1). Eleven trials were published as full paper articles,[21-31] three were published as abstracts[32-34] and one was unpublished.

The trials included patients with type 2 diabetes diagnosed based on glycated haemoglobin or fasting glucose with inadequate metabolic control on current intervention regimens. The exclusion criteria varied, but overall, none of the trials included patients with an ongoing drug or alcohol abuse, or patients with pancreatitis, gastric surgery, inflammatory bowel disease or other severe systemic illnesses such as alcoholic liver disease. All trials were designed to evaluate the effect of lixisenatide on metabolic regulation.

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None of the included trials found statistically significant differences between patient characteristics in the lixisenatide and control groups. The proportion of men was 50% and the mean age in the lixisenatide and control group ranged from 43 to 61 years (table 2). For the lixisenatide group, mean BMI ranged from 25.1 to 36.8 kg/m^2 and the mean HbA_{1c} from 7.2 to 8.5% (53 to 69 mmol/mol). For the control group, the mean BMI ranged from 25.2 to 36.8 kg/m^2 and the HbA_{1c} 7.4 to 8.9% (56 to 74 mmol/mol). Table 3 shows the mean baseline liver blood tests in the lixisenatide and control groups. The proportion of patients with elevated ALT ranged from 20 to 77% for the lixisenatide group and from 19 to 75% for the control groups. All trials randomised patients based on computer-generated random numbers with central randomisation. RCTs with a placebo control group were double blind with blinding of participants and personnel. RCTs with an active control group were open. All outcomes were objective (blood tests). We estimated that the outcomes were not likely to be influenced by lack of blinding and therefore classed all trials as having a low risk of bias in the domains blinding of participants and personnel and blinding of outcome assessment. All patients were accounted for and included in the analyses and all clinically relevant outcomes were defined and reported. No discrepancies were detected between the protocol and reported outcomes. No other biases were detected. We therefore classed all RCTs as having a low risk of bias.

The duration of therapy ranged from 4 to 76 weeks (mean 29 weeks). Two trials (one unpublished)[21] were designed to evaluate dose titration. The dose of lixisenatide was 20 microgram once-daily in the remaining trials. Twelve trials compared lixisenatide versus placebo and three trials compared lixisenatide versus active controls administered once-daily (liraglutide and sitagliptin) or twice daily (exenatide). The collateral interventions

(background therapy) were metformin (five trials), metformin and sulphonylurea (four trials), metformin and pioglitazone (one trial), insulin (three trials) or diet (one trial). The collateral interventions were administered equally to the lixisenatide and control groups.

In total, 1,070 patients had elevated ALT at baseline (figure 2). Lixisenatide had a beneficial effect on normalisation of ALT (risk difference 0.07; 95% CI 0.01 to 0.14; I^2=23%; number needed to treat 14 patients, p=0.042). The sequential analysis confirmed the primary meta-analysis when using the traditional 5% level of statistical significance (figure 3), but not after adjusting for multiple testing (the trial monitoring boundary was not crossed). Mixed effect multilevel meta-regression analyses of double blind trials (figure 4) found no effect of the metabolic regulation, duration of diabetes or BMI on the overall result (p>0.05 for all analyses). There was no difference between patients with a BMI<25 or a BMI≥25. When the analyses were repeated for RCTs with an active control group, lixisenatide had a beneficial effect on normalisation of ALT among patients who were obese (p=0.01) or overweight (p=0.004), but not among normal weight patients (p=0.98). Random effects subgroup meta-analyses of RCTs with an active comparator found a beneficial effect of lixisenatide in patients with a BMI≥25 (0.07; 0.02 to 0.12; p=0.004), but not in patients with a BMI<25 (-0.04; -0.36 to 0.27; p=0.28). There was a moderate correlation between change in bodyweight and change in ALT (regression coefficient=0.38). The baseline metabolic regulation and duration of diabetes did not predict the intervention effect. No evidence of small study effects was seen in regression analysis (Harbord's test, p=0.26) or funnel plots (figure 5). Subgroup analyses showed no differences between trials stratified by the publication status, control groups or collateral interventions.

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In total, 191 of 303 (37%) patients randomised to lixisenatide and 128 of 216 (41%) controls achieved normalisation of AST after treatment (figure 5). Random effects meta-analysis found no effect of lixisenatide on AST (0.06; -0.04 to 0.17), alkaline phosphatase (-0.10; -0.23 to 0.03), bilirubin (-0.12, -0.30 to 0.07) or normalisation of all liver blood tests (0.01, -0.01 to 0.03). No differences between subgroups were identified.

Discussion

This systematic review evaluated the effects of lixisenatide on elevated transaminases among patients with type 2 diabetes and a high risk of NAFLD. Analyses of outcome measures recalculated based on individual patient data showed that lixisenatide increased the proportion of patients who achieved normal ALT levels compared with placebo or other glucose lowering agents. The number needed to treat was 14 patients suggesting that the size of the potential benefit is clinically relevant. Our subgroup analyses suggested that lixisenatide was more effective in obese patients. We also found that lixisenatide was more effective than other active controls, which included liraglutide, sitagliptin and exenatide. However, the number of patients in the subgroup analyses was small and the findings therefore uncertain. Furthermore, ALT is used in the diagnostic evaluations and follow-up of patients with NAFLD and NASH in clinical practice, but previous evidence suggests that the sensitivity is low. The low sensitivity suggests that we may overlook intervention benefits. Additional information about outcomes such as histological changes is needed.

Individual patient data meta-analyses are based on the original research data instead of data extracted from published reports. The benefits of this approach include a reduced risk of errors as well as the ability to perform the relevant subgroup and sensitivity analyses.

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The quality of such analyses is high and individual patient data meta-analyses are considered as a 'gold standard' of systematic reviews.[35 36] The main limitation of our review is related to the number of events and patients. As demonstrated in our sequential analyses, the available evidence cannot support or refute clinically relevant intervention effects. None the included trials were specifically designed to evaluate the effect of lixisenatide on patients with NAFLD or NASH. However, one of the specific strengths of the meta-analytic approach is that it allows an assessment of questions not posed by the individual studies. In general, analyses of specific subgroups may be difficult in systematic reviews of RCT that are based on published data. By contrast, such subsets of participants can be analysed when individual patient data are collected.[37] Lixisenatide only appeared to have an effect on ALT, which is the most sensitive biochemical marker of NAFLD. The objective nature of the outcome measure strengthens the validity of our findings. Theoretically, our analyses would have been more sensitive if we had analysed the change in ALT as a continuous outcome. However, there is no clear evidence between quantitative changes in ALT and intervention effects. We found no beneficial or detrimental effects when analysing the remaining liver blood tests.

Incretin-based therapies such as lixisenatide and other GLP-1RAs are an important part of the pharmacological treatment of patients with type 2 diabetes. Experimental studies suggest that activation of GLP-1 receptors may prevent diabetes-related comorbidity including obesity and NASH[38] and that GLP-1RAs may improve hepatic steatosis.[39] The beneficial effects of GLP-1RA include improved glycaemic control as well as beneficial effects on bodyweight, blood pressure, cholesterol and cardiovascular biomarkers. Lixisenatide is a once-daily GLP-1RA. The included RCTs found beneficial effects of

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lixisenatide used as monotherapy or in combination with metformin, sulphonylureas, thiazolidinediones or basal insulin glargine. The improved glycaemic control was mainly demonstrated in RCTs with a placebo control. Conversely, we found that the benefit of lixisenatide on liver blood tests was more pronounced in RCTs with an active control group. This result suggests that improved metabolic regulation and reductions in body weight may not be the only reason for the potentially beneficial effect on lixisenatide on NAFLD. Some experimental studies suggest that hepatocytes have specific GLP-1 receptors.[17 40] The findings are controversial. In theory, different GLP-1RA may vary in their receptor affinity. The differences between the intervention effects in RCTs with an active comparator and placebo-controlled trials could also reflect the proportion of patients who were overweight. However, at present there is no clear evidence to support or refute this theory. Although we found a potential difference between lixisenatide and other GLP-1RA, the number of trials and the number of patients was too small to make any definite conclusions.

It may be argued that the beneficial effect of lixisenatide reflects changes to the daily intake of alcohol due to gastrointestinal adverse effects. However, none of the primary RCTs included patients with an ongoing alcohol abuse or alcoholic liver disease. None of the RCTs collected data on the exact daily intake of alcohol during the trial. We were therefore unable to determine the potential influence of alcohol.

In conclusion, the risk of bias in this systematic review was small supporting the validity of our findings. The use of lixisenatide seems to have beneficial effects on elevated levels of ALT in patients with type 2 diabetes and could possibly have a role in the treatment of patients with type 2 diabetes and NAFLD. We found potential differences between patients

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who were obese or normal weight and trials with a placebo control or active comparator. However, additional trials are clearly needed to assess our findings. The evidence does not allow definite treatment recommendations.

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Contributorship statement

LG wrote the initial draft for the paper, extracted data and performed the statistical analyses. TV extracted data. LG, TV and FK participated in the selection of trials and revised the paper for important intellectual comments.

Competing interests

None of the authors have competing interests specifically related to the present work. L.L. Gluud has participated as an investigator in a trial funded by MSD.F.K. Knop has received research funding from Sanofi-Aventis and lecture fees from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Gilead Sciences, Merck Sharp & Dohme, Novo Nordisk, Ono Pharmaceuticals, Sanofi-Aventis, and Zealand Pharma, is part of the Advisory Boards of Eli Lilly, Bristol-Myers Squibb/AstraZeneca, Novo Nordisk and Zealand Pharma, and has consulted for AstraZeneca, Gilead Sciences, Ono Pharmaceuticals, Novo Nordisk, Sanofi-Aventis and Zealand Pharma. T. Vilsbøll has received fees for being part of an advisory board from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, GI Dynamics, Merck Sharp & Dohme, Novo Nordisk, Sanofi-Aventis and Takeda, and received fees for speaking from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Novartis, Sanofi-Aventis, Takeda and Zealand Pharma, and received research support from Novo Nordisk.

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Data sharing

Extra data is available by emailing LL Gluud (liselottegluud@yahoo.dk)

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Legends to figures

Figure 1 Trial flow chart.

Figure 2 Random effects meta-analysis of randomised controlled trials on normalisation of alanine aminotransferase (ALT). The result of the analysis is expressed as the risk difference (RD) with 95% confidence intervals (CI) and level of significance (p value). The intervention comparisons are lixisenatide versus placebo or active interventions. The included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT.

Figure 3 Sequential analysis of risk ratios (random effects) in randomised controlled trials on lixisenatide versus placebo or active interventions for patients with type 2 diabetes and elevated alanine aminotransferase (ALT) at baseline. The analysis was performed with alpha 5%, power 80%, model-based diversity correction 12%, relative risk reduction 8% and control group incidence rate 51%. The outcome measure is normalisation of ALT. The analysis shows that lixisenatide has a beneficial effect on normalisation of ALT when assessed using the traditional 5% level of significance (the horizontal line), but not after adjusting for cumulative assessment (the trial monitoring boundary).

Figure 4 Mixed model meta-regression analysis of the effect of lixisenatide versus placebo on normalisation of alanine aminotransferase (ALT). Included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT. The figure shows the estimated intervention effect (normalisation of ALT) on the log-odds ratio scale in relation to the baseline body weight of included patients from 12 randomised

placebo-controlled trials. The size (area) of each circle is inversely proportional to the variance of the log-odds ratio (the larger the circle the less the variance).

Figure 5 Random effects meta-analysis of randomised controlled trials on normalisation of aspartate aminotransferase (AST). The result of the analysis is expressed as the risk difference (RD) with 95% confidence intervals (CI) and level of significance (p value). The intervention comparisons are lixisenatide versus placebo or active interventions. The included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT.

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Table 1 Characteristics of included trials

Trial	Publication status	Control	Collateral interventions*	Duration (weeks)
Lorenz 2013 ACT6011 QD	Full paper	Placebo	Sulfonulureas ± metformin	4
Ratner 2010 DRI6012 QD	Full paper	Placebo	Metformin	24
Pan 2012 GetGoal-M-Asia	Abstract	Placebo	Sulfonulureas ± metformin	24
Riddle 2013 GetGoal-Duo1	Full paper	Placebo	Insulin	24
Riddle 2013 GetGoal-L	Full paper	Placebo	Insulin	24
Seino 2012 GetGoal-L-Asia	Full paper	Placebo	Insulin	24
Ahren 2013 GetGoal-M	Full paper	Placebo	Metformin	24
Fonseca 2012 GetGoal-Mono	Full paper	Placebo	Diet	24
Ratner 2012 GetGoal-S	Abstract	Placebo	Sulfonulureas ± metformin	76
PDY6797 QD	Unpublished	Placebo	Sulfonulureas ± metformin	76
Rosenstock 2013 GetGoal-X	Full paper	Exenatide	Metformin	24
Kapitza 2013 PDY10931	Full paper	Liraglutide	Metformin	4
Seino 2012 EFC10780	Full paper	Sitagliptin	Metformin	24
Pinget 2013 GetGoal-P	Full paper	Placebo	Pioglitazone ±metformin	24
Bolli 2013 GetGoal-F1	Full paper	Placebo	Metformin	24

*Collateral interventions were administered equally to the lixisenatide and control groups

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Table 2 Characteristics of included patients (mean and standard deviation)

Trial	Body Mass Index (kg/m2)				Weight (kg)				Glycated haemoglobin (%)			
	Lixisenatide		Controls		Lixisenatide		Controls		Lixisenatide		Controls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lorenz 2013 ACT6011 QD	31.38	4.05	29.77	3.76	89.40	14.66	83.80	14.68	8.54	1.07	8.87	1.07
Ratner 2010 DRI6012 QD	32.01	4.28	31.74	4.15	89.37	17.00	87.68	13.63	7.58	0.65	7.53	0.63
Bolli 2013 GetGoal-F1	32.53	5.36	32.37	5.45	88.81	17.98	87.87	17.37	8.05	0.88	8.03	0.82
Riddle 2013 GetGoal-Duo1	31.99	6.63	31.65	6.01	87.31	21.76	86.75	20.41	7.56	0.55	7.60	0.54
Seino 2012 GetGoal-L-Asia	25.36	3.69	25.15	3.94	65.93	13.00	65.60	12.47	8.54	0.73	8.52	0.78
Pan 2012 GetGoal-M-Asia	26.75	3.86	27.08	3.75	73.18	13.93	72.74	13.64	7.95	0.81	7.85	0.71
Ahren 2013 GetGoal-M	32.84	6.34	33.12	6.45	89.57	20.91	90.15	20.14	8.06	0.89	8.06	0.90
Ratner 2012 GetGoal-S	30.13	6.62	30.42	6.64	82.30	21.76	84.42	22.83	8.28	0.86	8.21	0.84
Riddle 2013 GetGoal-L	31.91	6.17	32.56	6.32	87.10	20.01	88.94	20.84	8.42	0.88	8.37	0.84
Pinget 2013 GetGoal-P	33.66	6.71	34.44	7.04	92.93	22.90	96.74	25.58	8.08	0.90	8.06	0.79

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Fonseca 2012 GetGoal-Mono	31.99	6.66	31.76	6.69	87.77	21.58	86.08	22.21	8.03	0.89	8.07	0.91
PDY6797	25.09	3.65	26.39	3.50	71.54	16.77	76.92	16.07	8.19	0.82	8.38	0.75
Rosenstock 2013 GetGoal-X	33.68	6.27	33.51	6.54	94.01	19.63	96.09	22.52	7.95	0.81	7.97	0.78
Seino 2012 EFC10780	36.76	7.25	36.76	6.34	98.51	23.48	100.56	23.77	8.16	0.89	8.09	0.96
Kapitza 2013 PDY10931	31.23	3.93	31.33	4.08	91.16	15.28	92.88	16.59	7.20	0.63	7.41	0.81

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Table 3 Baseline liver blood tests expressed as units/litre (mean and standard deviation, SD)

Trial	Alanine aminotransferase				Aspartate aminotransferase				Alkaline phosphatase			
	Lixisenatide		Controls		Lixisenatide		Controls		Lixisenatide		Controls	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lorenz 2013 ACT6011 QD	25.48	8.18	25.91	12.50	19.43	4.95	19.23	4.62	83.52	27.00	83.55	19.43
Ratner 2010 DRI6012 QD	28.31	19.42	25.24	13.94	24.65	15.38	23.49	11.35	73.60	28.28	75.47	41.00
Bolli 2013 GetGoal-F1	30.69	16.34	29.34	15.41	23.93	10.30	23.62	10.68	72.83	21.58	74.54	21.23
Riddle 2013 GetGoal-Duo1	23.93	17.09	24.71	12.90	21.16	8.81	21.67	8.64	71.18	21.65	70.95	23.29
Seino 2012 GetGoal-L-Asia	25.19	12.85	23.03	10.34	22.78	7.56	21.43	7.79	71.90	17.59	71.01	20.11
Pan 2012 GetGoal-M-Asia	31.36	19.32	32.82	20.69	24.13	11.07	25.14	11.08	78.89	21.60	80.48	26.75
Ahren 2013 GetGoal-M	31.08	16.58	33.48	46.58	24.38	11.74	26.12	22.07	78.88	24.24	74.65	25.15
Ratner 2012 GetGoal-S	26.93	12.56	27.72	12.64	21.45	7.09	21.54	7.20	73.40	21.41	72.69	22.69
Riddle 2013 GetGoal-L	26.57	17.61	25.92	12.57	22.88	12.13	22.31	7.76	81.38	25.84	78.56	25.43

Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on liver blood tests: systematic review with individual patient data meta-analysis of randomised controlled trials. Gluud et al

Pinget 2013 GetGoal-P	24.66	12.01	24.59	10.86	21.46	8.93	21.48	8.27	75.24	29.68	73.13	22.49
Fonseca 2012 GetGoal-Mono	29.74	16.05	25.43	11.40	23.71	11.78	21.91	7.40	78.95	21.10	80.07	26.42
PDY6797	23.72	11.15	27.00	14.67	23.97	15.33	23.73	9.52	61.59	14.64	70.73	18.19
Seino 2012 EFC10780	35.70	20.63	39.83	23.64	25.50	13.76	28.74	20.54	81.97	25.11	83.61	28.13
Rosenstock 2013 GetGoal-X	28.50	13.06	30.65	15.83	22.17	7.92	23.28	9.27	72.43	22.13	72.15	19.86
Kapitza 2013 PDY10931	33.88	16.36	34.23	16.99	27.31	13.47	26.45	9.07	67.56	16.62	67.58	20.07

Effects of the glucagon-like peptide-1 receptor agonist lixisenatide on liver blood tests: systematic review with individual patient data meta-analysis of randomised controlled trials. Gluud et al

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

Lise L. Gluud[1,2], Filip K. Knop[1], Tina Vilsbøll[1]

[1]Diabetes Research Centre, Department of Medicine, Gentofte Hospital, University of Copenhagen, Denmark

[2]Department of Gastroenterology, Hvidovre Hospital, University of Copenhagen, Denmark

L.L. Gluud, Consultant, DMSc

F.K. Knop, Associate Professor, Specialist Registrar, PhD

T. Vilsbøll, Professor, Consultant, DMSc

Running Head: Lixisenatide for type 2 diabetes and elevated liver blood tests

Correspondence to: L.L. Gluud, Department of Gastroenterology, Copenhagen University Hospital Hvidovre, Kettegaards Alle 30, DK-2650 Hvidovre, Denmark; liselottegluud@yahoo.dk

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What we already know

Patients with type 2 diabetes (especially those who are overweight) have a high risk of developing non-alcoholic fatty liver disease (NAFLD). Alanine aminotransferase (ALT) is associated with NAFLD, especially in the early stages. Interventions that improve glycaemic control may have beneficial effects on NAFLD in type 2 diabetes. The glucagon-like peptide-1 receptor agonist lixisenatide improves glycaemic control in type 2 diabetes.

What this study adds

Lixisenatide increases the proportion of patients with normalisation of ALT compared with placebo or active comparators.

The effect of lixisenatide on normalisation of ALT was confirmed in subgroup analyses on patients who were obese or overweight, but not for patients with a normal weight.

No beneficial or detrimental effects of lixisenatide on aspartate aminotransferase, alkaline phosphatase or bilirubin were identified.

Abstract

Objective To evaluate the effects of the glucagon-like peptide-1 receptor agonist lixisenatide on elevated liver blood tests in patients with type 2 diabetes.

Design Systematic review.

Data sources Electronic and manual searches were combined.

Study selection Randomised controlled trials (RCTs) on lixisenatide versus placebo or active comparators for type 2 diabetes were included.

Participants Individual patient data were retrieved to calculate outcomes for patients with elevated liver blood tests.

Main outcome measures Normalisation of alanine aminotransferase (ALT) and aspartate aminotransferase.

Data Synthesis The results of included trials were combined in meta-analyses. Sequential, subgroup and regression analyses were performed to evaluate heterogeneity and bias.

Results We included 12 RCTs on lixisenatide versus placebo and three RCTs with the active comparators liraglutide, exenatide, or sitagliptin. The mean treatment duration was 29 weeks. Lixisenatide increased the proportion of patients with normalisation of ALT (risk difference: 0.07; 95% confidence interval: 0.01 to 0.14; number needed to treat: 14 patients, p=0.042). The effect was not confirmed in sequential analysis. No effects of lixisenatide were identified on AST, alkaline phosphatase or bilirubin. No evidence of bias

was identified. Mixed effect multilevel meta-regression analyses suggested that the benefit of lixisenatide on ALT was limited to patients who were overweight or obese.

Conclusion This review suggests that lixisenatide increases the proportion of obese or overweight patients with type 2 diabetes who achieve normalisation of ALT. Additional research is needed to determine if the findings translate to clinical outcome measures.

Strengths and limitations of this study

- This systematic review of randomised controlled trials evaluates if lixisenatide has a beneficial effect of liver blood tests associated with non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH).
- Based on analyses of individual patient data, lixisenatide increases the proportion of patients with normalisation of alanine aminotransferase (ALT) compared with placebo or active comparators. In subgroup analyses, the effect was verified for patients who were obese or overweight, but not for normal weight patients.
- The analyses include data from published and unpublished trials with intention-to-treat analyses of all patients included irrespective of compliance or follow up. The bias control was classed as adequate in all trials based on four or five of the five components included in the Cochrane bias assessment tool.
- Although ALT is the most sensitive biochemical marker of NAFLD and NASH, important effects may be overlooked because patients with severe liver disease were excluded from the trials.
- The available data did not allow for assessment of clinical outcome measures such as development of cirrhosis or hepatocellular carcinoma.

Introduction

The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing and the costs are considerable.[1-3] About ten per cent of patients with NAFLD develop nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. Obesity and decreased insulin sensitivity are associated with NAFLD and NASH, which common in patients with type 2 diabetes.[4-6] NAFLD is generally an asymptomatic disease. Elevated transaminases are independent predictors of NAFLD although the sensitivity is low.[7] A large proportion of patients with type 2 diabetes and elevated transaminases have NAFLD or NASH. A systematic review on observational studies found that routinely available biochemical markers may be used in the assessment of NAFLD.[8] Elevation of alanine aminotransferase (ALT) is more common than aspartate aminotransferase (AST).[9] The gold standard for the assessment of patients with NAFLD is to perform a liver biopsy, but the procedure is associated with risks and potential sampling error. Biopsy-related complications including bleeding still occur in ultrasonically guided techniques.[10 11]

Treatment of NAFLD and NASH is important. Anti-diabetic interventions have been assessed as a potential treatment option. A systematic review from 2007 found three randomised controlled trials (RCTs) on metformin and pioglitazone in patients with NAFLD or NASH.[12] The review found that the interventions increased the proportion of patients with normalisation of ALT. A subsequent health technology assessment on insulin sensitizers for NAFLD reached a similar conclusion.[13] However, pioglitazone is associated with a considerable risk of serious adverse events including cardiovascular disease and bladder cancer.[14 15] Alternative treatment options are therefore needed. Recent studies suggest that glucagon-like peptide-1 receptor agonists (GLP-1RA) improve

insulin sensitivity and resistance - most likely via their body weight-lowering effect.[16] In addition, GLP-1RAs may have a direct effect on hepatocytes reducing hepatic steatosis via glucagon-like peptide-1 receptors in the liver.[17] A review on the GLP1-RA liraglutide and exenatide found that the interventions reduce ALT and AST in patients with type 2 diabetes or obesity.[18] Unfortunately, analyses of patients with elevated liver blood tests were not available. Lixisenatide is a GLP-1RA that improves glycaemic control and reduces body weight in patients with type 2 diabetes.[19 20] There are no RCTs on lixisenatide for patients with NAFLD. However, several trials on lixisenatide included patients who were overweight and allowed inclusion of patients with mildly elevated liver blood tests. The trials were therefore likely to include a relatively large proportion of patients with NAFLD. We therefore conducted a systematic review with outcomes recalculated based on individual patient data from RCTs to determine the effect of lixisenatide on elevated liver blood tests in patients with type 2 diabetes.

Methods

This review is based on a registered protocol (CRD42013005779). The review methods follow the recommendations described in the Cochrane Handbook for Reviews on Interventions (www.cochrane.org). The main objective was to compare the effect of lixisenatide versus placebo or other active interventions on normalisation of liver transaminases. RCTs were included irrespective of blinding, language or publication status. Adult patients with type 2 and elevated liver blood tests were included irrespective of gender or body weight.

Based on previous evidence,[9] the primary outcome measures were normalisation of ALT and AST. Secondary outcome measures included normalisation of alkaline phosphatase and bilirubin as well as a normalisation of the composite outcome measure combining all liver blood tests. The pharmaceutical company producing lixisenatide (Sanofi-Aventis) provided data and additional information on the design of included trials. All outcomes were recalculated based on individual patient data.

All authors participated in the identification and selection of trials. Excluded trials were listed with the reason for exclusion. Eligible trials were identified through electronic and manual searches. Electronic searches were performed without language restrictions in MEDLINE (1946-Feb 2014), Cochrane Library (Issue 2, 2014), Embase (1974 to Feb 2014) and Web of science (1900-Feb 2014). The search strategy in the Cochrane library was lixisenatide, ti,ab,kw (Word variations have been searched) in the Cochrane Library. In Medline, the search strategy was (lixisenatide AND (("randomized controlled trial"[Publication Type]) OR ("controlled clinical trial"[Publication Type])). Additional manual searches were performed in reference lists of relevant papers, correspondence with experts, the pharmaceutical company producing lixisenatide and the World Health Organisation Trial Search Database (http://apps.who.int/trialsearch/).

Two authors extracted data in an independent manner (LG and TV). Disagreements were resolved through discussion. The bias risk assessment was based on the Cochrane Collaboration Risk of Bias Assessment tool (www.cochrane.org). The assessment included the separate domains random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective bias and other biases. Each domain was classed as having a high, uncertain or low risk of bias.

Random sequence generation: Low risk' of bias: The investigators describe a random component in the sequence generation process such as: referring to a random number table or using a computer random number generator. Unclear risk of bias: Insufficient information about the sequence generation process to permit judgment of 'low risk' or 'high risk'. High risk of bias: The investigators describe a non-random component in the sequence generation process.

Allocation concealment: Low risk of bias: Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: Central allocation (including telephone, web-based and pharmacy-controlled). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: Participants or investigators enrolling participants could possibly foresee assignments.

Blinding of participants and personnel: Low risk of bias: No blinding or incomplete blinding, but the outcome is not likely to be influenced by lack of blinding (e.g., objective outcome measures such as blood tests). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: No blinding or incomplete blinding, and the outcome is likely to be influenced by lack of blinding.

Blinding of outcome assessment: Low risk of bias: No blinding of outcome assessment, but the outcome measurement is not likely to be influenced by lack of blinding (e.g., blood tests). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: No blinding of outcome assessment and the outcome measurement is likely to be influenced by lack of blinding.

Incomplete outcome data: Low risk of bias: No missing outcome data; reasons for missing outcome data unlikely to be related to true outcome. Missing outcome data

balanced in numbers across intervention groups, with similar reasons for missing data across groups, The proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; missing data have been imputed using appropriate methods. Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: Reason for missing outcome data likely to be related to true outcome; the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; 'as-treated' analysis done with substantial departure of the assigned intervention; potentially inappropriate application of simple imputation.

Selective reporting: Low risk of bias: The study protocol is available and all of the study's pre-specified outcomes (primary and secondary) that are of interest in the review have been reported in the pre-specified way; The study protocol is not available, but it is clear that the published reports include all expected outcomes, including those that were pre-specified (convincing text of this nature may be uncommon). Unclear risk of bias: Insufficient information to permit judgement of 'low risk' or 'high risk'. High risk of bias: Not all of the study's pre-specified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data that were not pre-specified; one or more reported primary outcomes were not pre-specified (unless clear justification for their reporting is provided, such as an unexpected adverse effect); one or more outcomes of interest in the review are reported incompletely so that they cannot be entered in a meta-analysis; the study report fails to include results for a key outcome that would be expected to have been reported for such a study.

Other bias: Low risk: The study appears to be free of other sources of bias. Unclear risk of bias: Insufficient information to assess whether an important risk of bias exists. High risk:

The study had a potential source of bias related to the specific study design used, or has been claimed to have been fraudulent, or had some other problem.

Statistical analysis

The analyses were performed in Stata version 13 (STATA Corp, Texas, USA). Random effects meta-analyses were performed due to an expected between study heterogeneity. The results were expressed as risk differences with 95% confidence intervals (CI) and p values and I^2 as a measure of heterogeneity and with the number needed to treat for statistically significant outcome measures. We defined I^2 values below 30% as unimportant, 30-50% as moderate heterogeneity, 50-75% as substantial heterogeneity and >75% as considerable heterogeneity. All patients were included in the analysis irrespective of compliance or follow-up and with imputation of outcomes for patients with missing outcome data (intention to treat). Mixed effect multilevel meta-regression and subgroup analyses were performed to evaluate heterogeneity. The meta-regression analysis evaluated the influence of the metabolic regulation (HbA_{1c} ≤8.5% (69 mmol/mol)), duration of diabetes (≥5 years), and body mass index (BMI) (normal weight ≤25 kg/m^2, overweight >25 kg/m^2 or obese >30 kg/m^2). Post hoc analyses were performed to evaluate the effect of the change in bodyweight and ALT. The subgroup analyses evaluated the influence of publication status (full paper articles compared with abstracts and unpublished trials), control groups and collateral interventions. Since all trials had a low risk of bias, we did not perform subgroup analyses on bias control. Publication bias and other small study effects were estimated in regression analyses (Harbord's test) and funnel plots. We performed sequential analyses to evaluate the robustness of results from meta-analyses with a statistically significant result. The analysis was performed with alpha 5%, power 80%,

model-based diversity correction 12%, relative risk reduction 8% and control group incidence rate 51%.

Results

The initial searches identified 531 potentially eligible records (figure 1). After reading the titles and abstracts, duplicates and records that clearly did not describe RCTs on lixisenatide were excluded. One ongoing trial was excluded because data were not yet available. The remaining records referred to 15 multicentre RCTs that were included in the qualitative and quantitative analyses (table 1). Eleven trials were published as full paper articles,[21-31] three were published as abstracts[32-34] and one was unpublished.

The trials included patients with type 2 diabetes diagnosed based on glycated haemoglobin or fasting glucose with inadequate metabolic control on current intervention regimens. The exclusion criteria varied, but overall, none of the trials included patients with an ongoing drug or alcohol abuse, or patients with pancreatitis, gastric surgery, inflammatory bowel disease or other severe systemic illnesses such as alcoholic liver disease. All trials were designed to evaluate the effect of lixisenatide on metabolic regulation.

None of the included trials found statistically significant differences between patient characteristics in the lixisenatide and control groups. The proportion of men was 50% and the mean age in the lixisenatide and control group ranged from 43 to 61 years (table 2). For the lixisenatide group, mean BMI ranged from 25.1 to 36.8 kg/m^2 and the mean HbA_{1c} from 7.2 to 8.5% (53 to 69 mmol/mol). For the control group, the mean BMI ranged from 25.2 to 36.8 kg/m^2 and the HbA_{1c} 7.4 to 8.9% (56 to 74 mmol/mol). Table 3 shows the mean baseline liver blood tests in the lixisenatide and control groups. The proportion of

patients with elevated ALT ranged from 20 to 77% for the lixisenatide group and from 19 to 75% for the control groups. All trials randomised patients based on computer-generated random numbers with central randomisation. RCTs with a placebo control group were double blind with blinding of participants and personnel. RCTs with an active control group were open. All outcomes were objective (blood tests). We estimated that the outcomes were not likely to be influenced by lack of blinding and therefore classed all trials as having a low risk of bias in the domains blinding of participants and personnel and blinding of outcome assessment. All patients were accounted for and included in the analyses and all clinically relevant outcomes were defined and reported. No discrepancies were detected between the protocol and reported outcomes. No other biases were detected. We therefore classed all RCTs as having a low risk of bias.

The duration of therapy ranged from 4 to 76 weeks (mean 29 weeks). Two trials (one unpublished)[21] were designed to evaluate dose titration. The dose of lixisenatide was 20 microgram once-daily in the remaining trials. Twelve trials compared lixisenatide versus placebo and three trials compared lixisenatide versus active controls administered once-daily (liraglutide and sitagliptin) or twice daily (exenatide). The collateral interventions (background therapy) were metformin (five trials), metformin and sulphonylurea (four trials), metformin and pioglitazone (one trial), insulin (three trials) or diet (one trial). The collateral interventions were administered equally to the lixisenatide and control groups.

In total, 1,070 patients had elevated ALT at baseline (figure 2). Lixisenatide had a beneficial effect on normalisation of ALT (risk difference 0.07; 95% CI 0.01 to 0.14; I^2=23%; number needed to treat 14 patients, p=0.042). The sequential analysis confirmed the primary meta-analysis when using the traditional 5% level of statistical significance (figure 3), but not after adjusting for multiple testing (the trial monitoring boundary was not

crossed). Mixed effect multilevel meta-regression analyses of double blind trials (figure 4) found no effect of the metabolic regulation, duration of diabetes or BMI on the overall result (p>0.05 for all analyses). There was no difference between patients with a BMI<25 or a BMI≥25. When the analyses were repeated for RCTs with an active control group, lixisenatide had a beneficial effect on normalisation of ALT among patients who were obese (p=0.01) or overweight (p=0.004), but not among normal weight patients (p=0.98). Random effects subgroup meta-analyses of RCTs with an active comparator found a beneficial effect of lixisenatide in patients with a BMI≥25 (0.07; 0.02 to 0.12; p=0.004), but not in patients with a BMI<25 (-0.04; -0.36 to 0.27; p=0.28). There was a moderate correlation between change in bodyweight and change in ALT (regression coefficient=0.38). The baseline metabolic regulation and duration of diabetes did not predict the intervention effect. No evidence of small study effects was seen in regression analysis (Harbord's test, p=0.26) or funnel plots (figure 5). Subgroup analyses showed no differences between trials stratified by the publication status, control groups or collateral interventions.

In total, 191 of 303 (37%) patients randomised to lixisenatide and 128 of 216 (41%) controls achieved normalisation of AST after treatment (figure 5). Random effects meta-analysis found no effect of lixisenatide on AST (0.06; -0.04 to 0.17), alkaline phosphatase (-0.10; -0.23 to 0.03), bilirubin (-0.12, -0.30 to 0.07) or normalisation of all liver blood tests (0.01, -0.01 to 0.03). No differences between subgroups were identified.

Discussion

This systematic review evaluated the effects of lixisenatide on elevated transaminases among patients with type 2 diabetes and a high risk of NAFLD. Analyses of outcome

measures recalculated based on individual patient data showed that lixisenatide increased the proportion of patients who achieved normal ALT levels compared with placebo or other glucose lowering agents. The number needed to treat was 14 patients suggesting that the size of the potential benefit is clinically relevant. Our subgroup analyses suggested that lixisenatide was more effective in obese patients. We also found that lixisenatide was more effective than other active controls, which included liraglutide, sitagliptin and exenatide. However, the number of patients in the subgroup analyses was small and the findings therefore uncertain. Furthermore, ALT is used in the diagnostic evaluations and follow-up of patients with NAFLD and NASH in clinical practice, but previous evidence suggests that the sensitivity is low. The low sensitivity suggests that we may overlook intervention benefits. Additional information about outcomes such as histological changes is needed.

Individual patient data meta-analyses are based on the original research data instead of data extracted from published reports. The benefits of this approach include a reduced risk of errors as well as the ability to perform the relevant subgroup and sensitivity analyses. The quality of such analyses is high and individual patient data meta-analyses are considered as a 'gold standard' of systematic reviews.[35 36] The main limitation of our review is related to the number of events and patients. As demonstrated in our sequential analyses, the available evidence cannot support or refute clinically relevant intervention effects. None the included trials were specifically designed to evaluate the effect of lixisenatide on patients with NAFLD or NASH. However, one of the specific strengths of the meta-analytic approach is that it allows an assessment of questions not posed by the individual studies. In general, analyses of specific subgroups may be difficult in systematic reviews of RCT that are based on published data. By contrast, such subsets of participants can be analysed when individual patient data are collected.[37] Lixisenatide only appeared

to have an effect on ALT, which is the most sensitive biochemical marker of NAFLD. The objective nature of the outcome measure strengthens the validity of our findings. Theoretically, our analyses would have been more sensitive if we had analysed the change in ALT as a continuous outcome. However, there is no clear evidence between quantitative changes in ALT and intervention effects. We found no beneficial or detrimental effects when analysing the remaining liver blood tests.

Incretin-based therapies such as lixisenatide and other GLP-1RAs are an important part of the pharmacological treatment of patients with type 2 diabetes. Experimental studies suggest that activation of GLP-1 receptors may prevent diabetes-related comorbidity including obesity and NASH[38] and that GLP-1RAs may improve hepatic steatosis.[39] The beneficial effects of GLP-1RA include improved glycaemic control as well as beneficial effects on bodyweight, blood pressure, cholesterol and cardiovascular biomarkers. Lixisenatide is a once-daily GLP-1RA. The included RCTs found beneficial effects of lixisenatide used as monotherapy or in combination with metformin, sulphonylureas, thiazolidinediones or basal insulin glargine. The improved glycaemic control was mainly demonstrated in RCTs with a placebo control. Conversely, we found that the benefit of lixisenatide on liver blood tests was more pronounced in RCTs with an active control group. This result suggests that improved metabolic regulation and reductions in body weight may not be the only reason for the potentially beneficial effect on lixisenatide on NAFLD. Some experimental studies suggest that hepatocytes have specific GLP-1 receptors.[17 40] The findings are controversial. In theory, different GLP-1RA may vary in their receptor affinity. The differences between the intervention effects in RCTs with an active comparator and placebo-controlled trials could also reflect the proportion of patients who were overweight. However, at present there is no clear evidence to support or refute

this theory. Although we found a potential difference between lixisenatide and other GLP-1RA, the number of trials and the number of patients was too small to make any definite conclusions.

It may be argued that the beneficial effect of lixisenatide reflects changes to the daily intake of alcohol due to gastrointestinal adverse effects. However, none of the primary RCTs included patients with an ongoing alcohol abuse or alcoholic liver disease. None of the RCTs collected data on the exact daily intake of alcohol during the trial. We were therefore unable to determine the potential influence of alcohol.

In conclusion, the risk of bias in this systematic review was small supporting the validity of our findings. The use of lixisenatide seems to have beneficial effects on elevated levels of ALT in patients with type 2 diabetes and could possibly have a role in the treatment of patients with type 2 diabetes and NAFLD. We found potential differences between patients who were obese or normal weight and trials with a placebo control or active comparator. However, additional trials are clearly needed to assess our findings. The evidence does not allow definite treatment recommendations.

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Competing interests None of the authors have competing interests specifically related to the present work. L.L. Gluud has participated as an investigator in a trial funded by MSD.F.K. Knop has received research funding from Sanofi-Aventis and lecture fees from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Gilead Sciences, Merck Sharp & Dohme, Novo Nordisk, Ono Pharmaceuticals, Sanofi-Aventis, and Zealand Pharma, is part of the Advisory Boards of Eli Lilly, Bristol-Myers Squibb/AstraZeneca, Novo Nordisk and Zealand Pharma, and has consulted for AstraZeneca, Gilead Sciences, Ono Pharmaceuticals, Novo Nordisk, Sanofi-Aventis and Zealand Pharma. T. Vilsbøll has received fees for being part of an advisory board from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, GI Dynamics, Merck Sharp & Dohme, Novo Nordisk, Sanofi-Aventis and Takeda, and received fees for speaking from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Novartis, Sanofi-Aventis, Takeda and Zealand Pharma, and received research support from Novo Nordisk.

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Legends to figures

Figure 1 Trial flow chart.

Figure 2 Random effects meta-analysis of randomised controlled trials on normalisation of alanine aminotransferase (ALT). The result of the analysis is expressed as the risk difference (RD) with 95% confidence intervals (CI) and level of significance (p value). The intervention comparisons are lixisenatide versus placebo or active interventions. The included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT.

Figure 3 Sequential analysis of risk ratios (random effects) in randomised controlled trials on lixisenatide versus placebo or active interventions for patients with type 2 diabetes and elevated alanine aminotransferase (ALT) at baseline. The analysis was performed with alpha 5%, power 80%, model-based diversity correction 12%, relative risk reduction 8% and control group incidence rate 51%. The outcome measure is normalisation of ALT. The analysis shows that lixisenatide has a beneficial effect on normalisation of ALT when assessed using the traditional 5% level of significance (the horizontal line), but not after adjusting for cumulative assessment (the trial monitoring boundary).

Figure 4 Mixed model meta-regression analysis of the effect of lixisenatide versus placebo on normalisation of alanine aminotransferase (ALT). Included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT. The figure shows the estimated intervention effect (normalisation of ALT) on the log-odds ratio scale in relation to the baseline body weight of included patients from 12 randomised placebo-controlled trials. The size (area) of each circle is inversely proportional to the variance of the log-odds ratio (the larger the circle the less the variance).

Figure 5 Random effects meta-analysis of randomised controlled trials on normalisation of aspartate aminotransferase (AST). The result of the analysis is expressed as the risk difference (RD) with 95% confidence intervals (CI) and level of significance (p value). The intervention comparisons are lixisenatide versus placebo or active interventions. The included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT.

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Figure 1

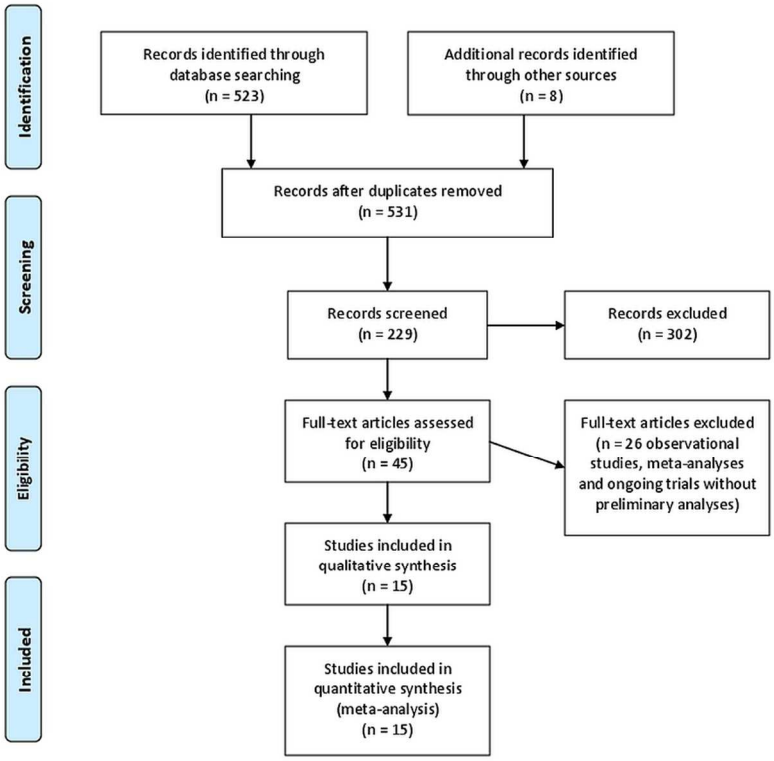

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Figure 2

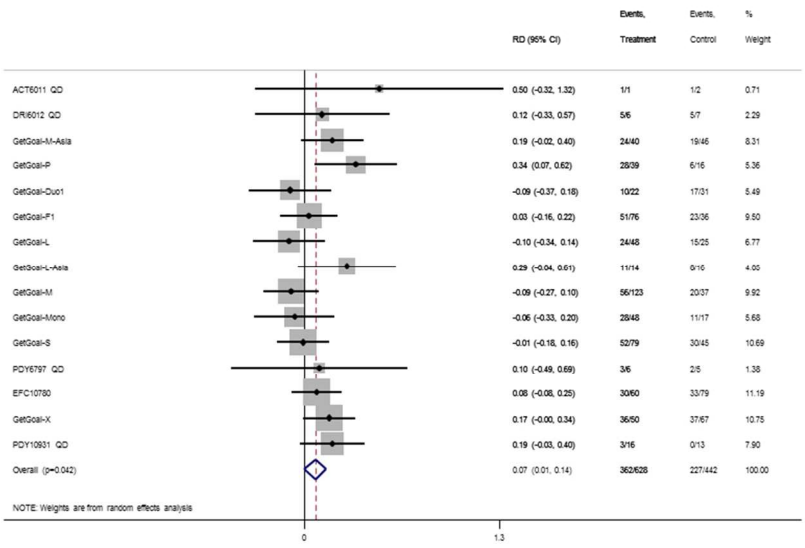

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Figure 3

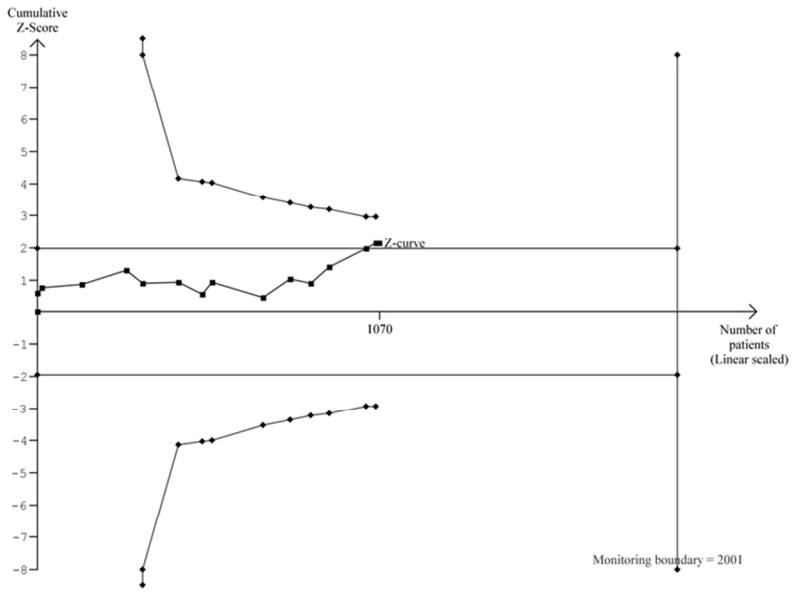

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Figure 4

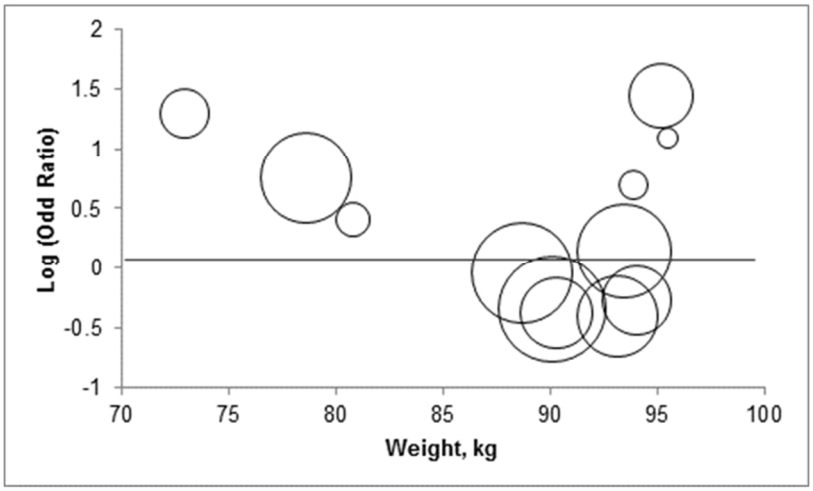

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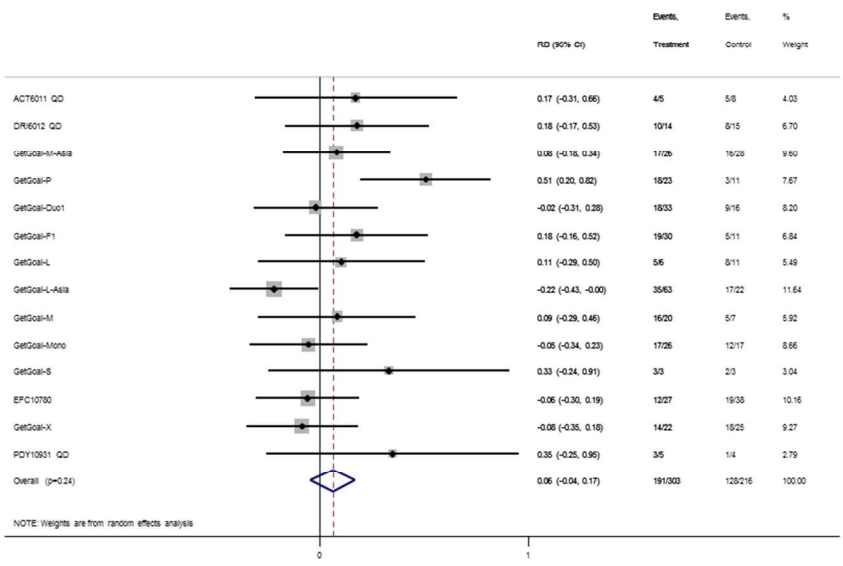
Figure 5



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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5-6
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6-7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	8-9
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	8-9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	8-9
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	8-9
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	9
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	9-10 and tables
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10-11 and figures
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	10-11 and figures
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	10-11
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	11-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11-13
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11-13
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	14

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