

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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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 (AST).

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 3 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% CI 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 suggest that the benefit of lixisenatide on ALT was limited to patients who were overweight or obese.

Conclusions: 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.

Trial registration number: PROSPERO; CRD42013005779.

INTRODUCTION

The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing and the costs

Strengths and limitations of this study

- This systematic review of randomised controlled trials evaluates if lixisenatide has a beneficial effect on liver blood tests associated with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic 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 classified 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.

are considerable.^{1–3} About 10% of patients with NAFLD develop non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma. Obesity and decreased insulin sensitivity are associated with NAFLD and NASH, which are 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 of

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 errors. Biopsy-related complications including bleeding still occur in ultrasonically guided techniques.^{10 11}

Treatment of NAFLD and NASH is important. Antidiabetic 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 sensitisers 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 GLP-1RA 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 (<http://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 diabetes 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 the normalisation of the composite outcome measures 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 to February 2014), Cochrane Library (Issue 2, 2014), EMBASE (1974 to February 2014) and Web of Science (1900 to February 2014). The search strategy in the Cochrane Library was lixisenatide, ti,ab,kw (Word variations have been searched). 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 (<http://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 classified 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 judgement 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 risk 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 risk 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 (eg, 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 (eg, 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 from 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 prespecified outcomes (primary and secondary), that are of interest for the review, have been reported in the prespecified way; The study protocol is not available, but it is clear that the published reports include all expected outcomes, including those that were prespecified (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 prespecified primary outcomes have been reported; one or more primary outcomes is reported using measurements, analysis methods or subsets of the data that were not prespecified; one or more reported primary outcomes were not prespecified (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 V.13 (STATA Corp, Texas, USA). Random effects meta-analyses were

performed due to the expected heterogeneity between studies. The results were expressed as risk differences with 95% CIs; p values and I^2 as measures 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 (glycated haemoglobin (HbA1c) $\leq 8.5\%$ (69 mmol/mol)), duration of diabetes (≥ 5 years) and body mass index (BMI) (normal weight ≤ 25 , overweight >25 or obese >30 kg/m²). Post hoc analyses were performed to evaluate the effect of the change in body weight 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 α 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 HbA1c 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

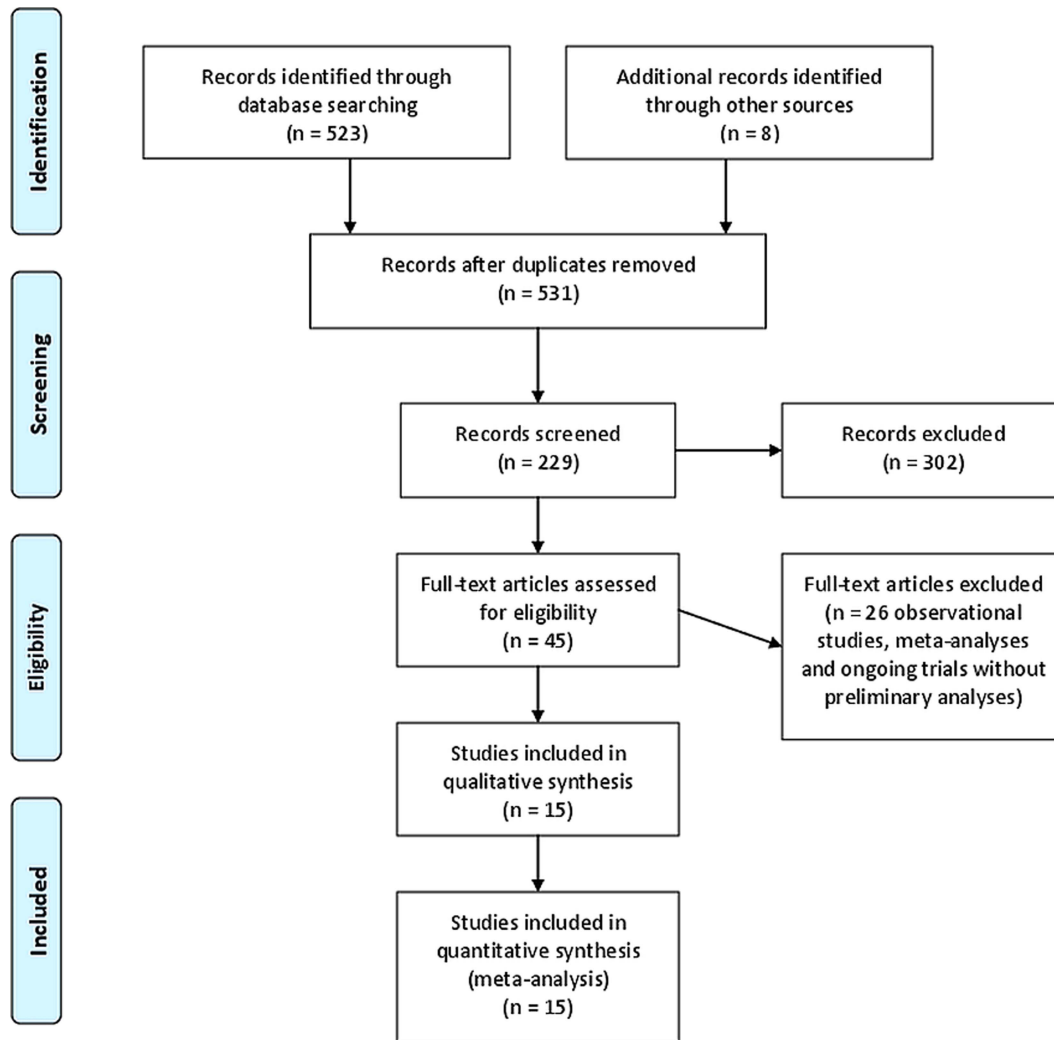


Figure 1 Trial flow chart.

Table 1 Characteristics of included trials

Trial	Publication status	Control	Collateral interventions*	Duration (weeks)
Lorenz 2013 ACT6011 QD	Full paper	Placebo	Sulfonureas±metformin	4
Ratner 2010 DRI6012 QD	Full paper	Placebo	Metformin	24
Pan 2012 GetGoal-M-Asia	Abstract	Placebo	Sulfonureas±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	Sulfonureas±metformin	76
PDY6797 QD	Unpublished	Placebo	Sulfonureas±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.

group ranged from 43 to 61 years (table 2). For the lixisenatide group, mean BMI ranged from 25.1 to 36.8 kg/m² and the mean HbA1c from 7.2% to 8.5% (53–69 mmol/mol). For the control group, the mean BMI ranged from 25.2 to 36.8 kg/m² and the HbA1c 7.4% to 8.9% (56–74 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, classified 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 µg 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 sulfonylurea (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, 1070 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; I²=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 BMI<25 or ≥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 BMI≥25 (0.07; 0.02 to 0.12;

Table 2 Characteristics of included patients (mean and SD)

Trial	Body mass index (kg/m ²)				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
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

Table 3 Baseline liver blood tests expressed as units/litre (mean and 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 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 PDY6797	29.74	16.05	25.43	11.40	23.71	11.78	21.91	7.40	78.95	21.10	80.07	26.42
Seino 2012 EFC10780	23.72	11.15	27.00	14.67	23.97	15.33	23.73	9.52	61.59	14.64	70.73	18.19
Rosenstock 2013 GetGoal-X	35.70	20.63	39.83	23.64	25.50	13.76	28.74	20.54	81.97	25.11	83.61	28.13
Kapitza 2013 PDY10931	28.50	13.06	30.65	15.83	22.17	7.92	23.28	9.27	72.43	22.13	72.15	19.86
	33.88	16.36	34.23	16.99	27.31	13.47	26.45	9.07	67.56	16.62	67.58	20.07

$p=0.004$), but not in patients with $BMI < 25$ (-0.04 ; -0.36 to 0.27 ; $p=0.28$). There was a moderate correlation between change in body weight 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.

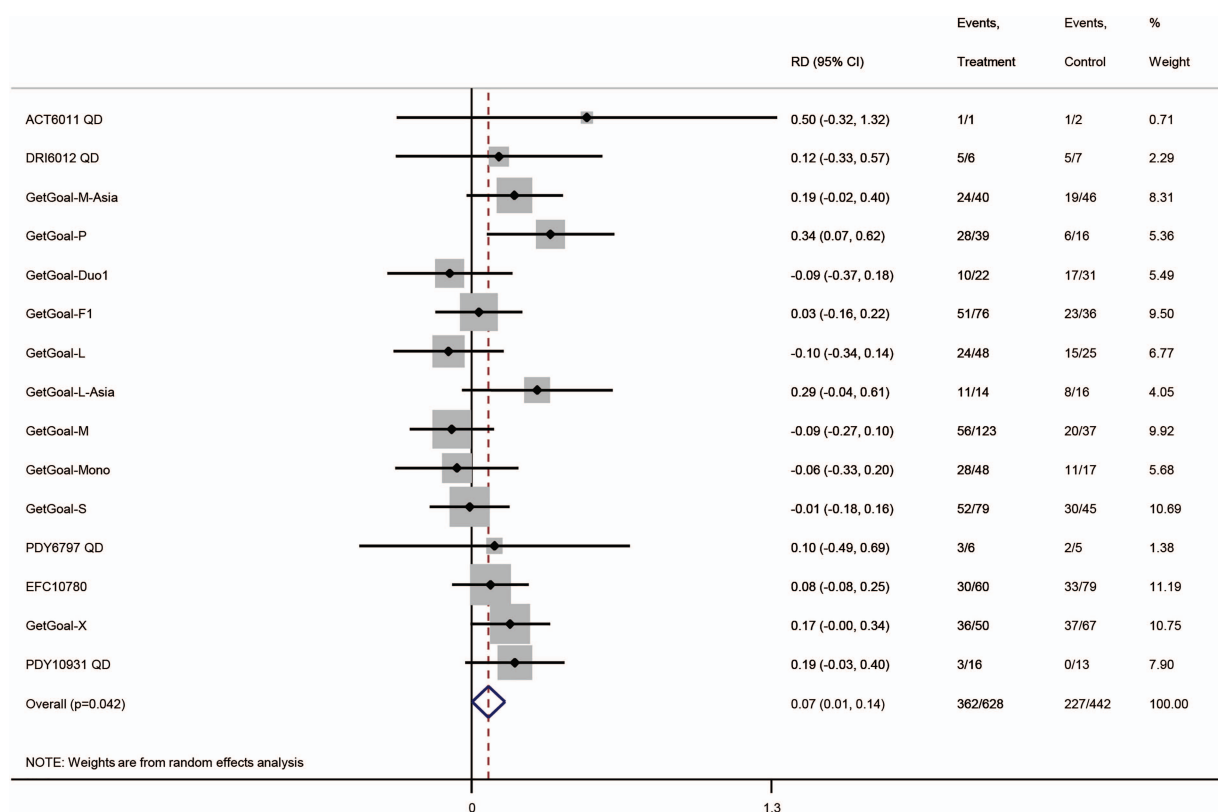
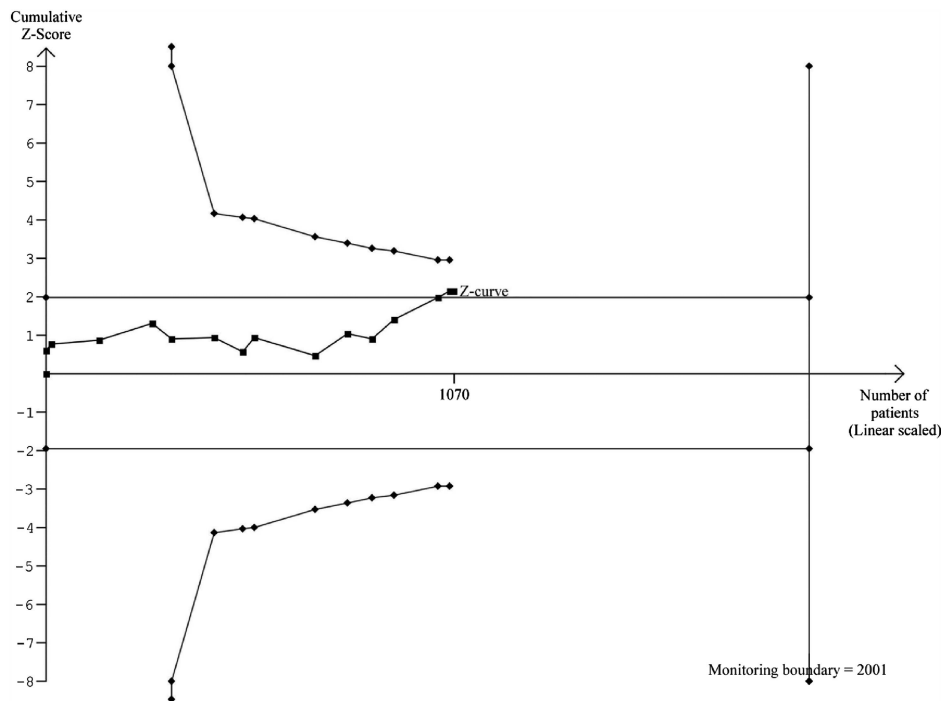


Figure 2 Random effects meta-analysis of randomised controlled trials on normalisation of alanine aminotransferase (ALT). The result of the analysis is expressed as the risk difference (RD) with 95% CIs and level of significance (p value). The intervention comparisons are lixisenatide versus placebo 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 α 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).



In total, 191 of 303 (37%) patients randomised for lixisenatide and 128 of 216 (41%) for 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

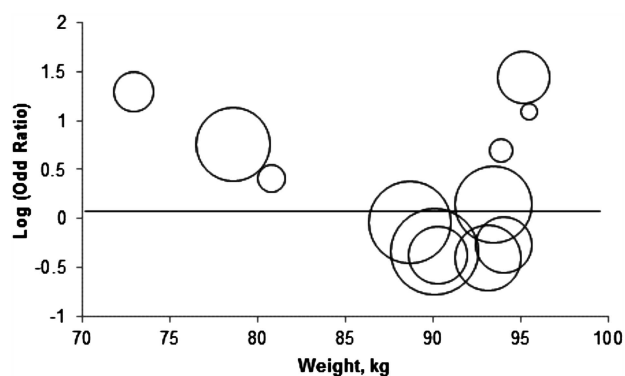


Figure 4 Mixed model meta-regression analysis of the effect of lixisenatide versus placebo on normalisation of alanine aminotransferase (ALT). Included patients have type 2 diabetes and elevated ALT at baseline. The outcome measure is normalisation of ALT. The figure shows the estimated intervention effect (normalisation of ALT) on the log-OR 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 OR (the larger the circle the lesser the variance).

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. Further, 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 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 of 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

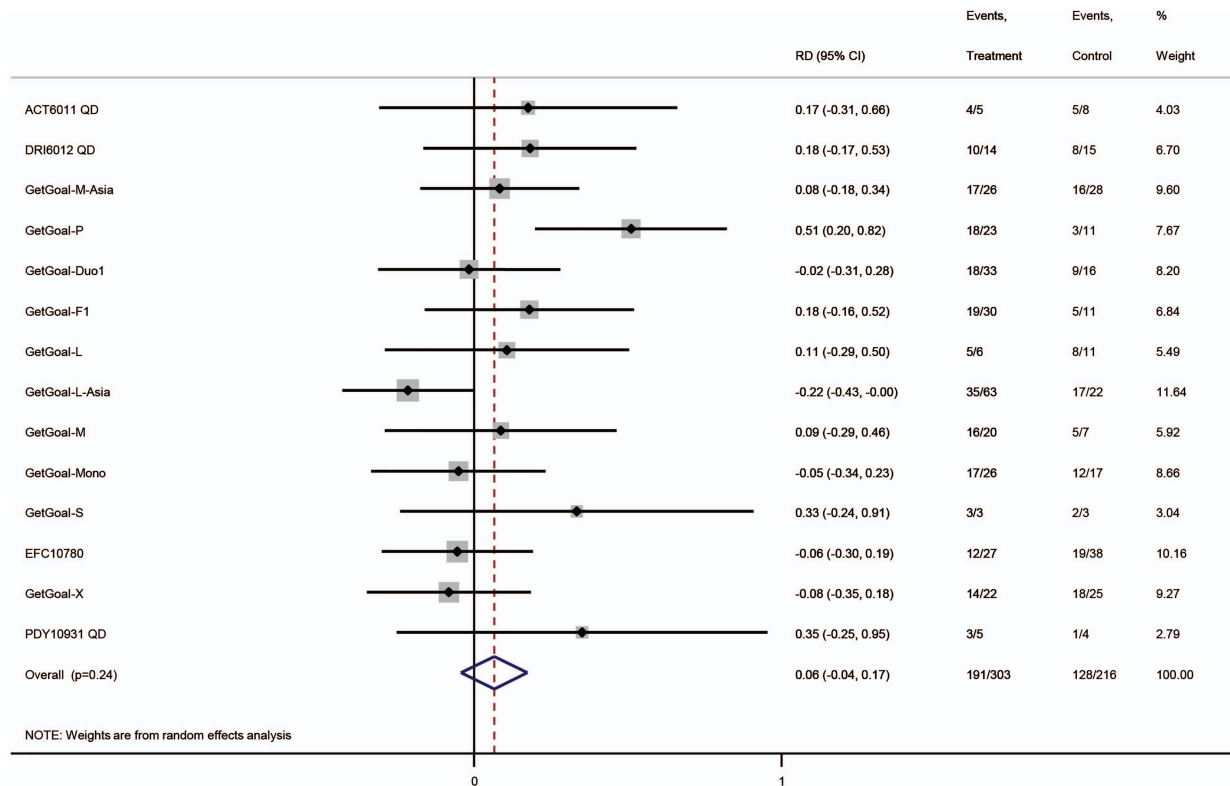


Figure 5 Random effects meta-analysis of randomised controlled trials on normalisation of aspartate aminotransferase (AST). The result of the analysis is expressed as the risk difference (RD) with 95% CIs and level of significance (p value). The intervention comparisons are lixisenatide versus placebo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

individual studies. In general, analyses of specific subgroups may be difficult in systematic reviews of RCTs 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 this 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-IRAs 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-IRAs may improve hepatic steatosis.³⁹ The beneficial effects of GLP-IRA include improved glycaemic control as well as beneficial effects on body weight, blood pressure, cholesterol and cardiovascular biomarkers. Lixisenatide is a once-daily GLP-IRA. The included RCTs found beneficial effects of lixisenatide used as monotherapy or in combination with metformin, sulfonylureas, 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-IRA 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-IRA, the number of trials and the number of patients were 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 in 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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