Medicine

Efficacy and Safety of Lixisenatide for Inadequately Controlled Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

--Manuscript Draft--

Manuscript Number:	MD-D-16-00071R1
Article Type:	OA: Systematic Review and Meta-Analysis (PRISMA Compliant)
Section/Category:	4300 Endocrinology
Keywords:	Lixisenatide; Diabetes mellitus; meta-analysis; review
Manuscript Region of Origin:	CHINA
Abstract:	Objective: We aimed to systematically evaluate the efficacy and safety of lixisenatide in patients with type 2 diabetes mellitus. Methods: PubMed, EMBASE, Cochrane Library, ClinicalTrials.gov, and the Chinese Science Citation Database were searched up to May 2015. Randomized controlled trials estimating the efficacy and safety of lixisenatide in patients with type 2 diabetes mellitus were eligible for inclusion. Two authors independently extracted data in a prespecified Microsoft Excel spreadsheet. Review Manager 5.3 software was used to make meta-analysis. Weighted mean difference (WMD) and relative risk (RR) together with their corresponding 95% confidence intervals were estimated. Results: Ten multicenter randomized controlled trials involving 5,142 patients were eligible for inclusion finally. Lixisenatide could more significantly reduce the level of HbA1c (WMD=-0.38; 95% CI [-0.47, -0.28]), and had a higher proportion of patients who achieved the HbA1c targets of <7.0% (RR=1.84, 95% CI [1.68, 2.02]) and <6.5% (RR=3.00, 95% CI [2.49, 3.61]) compared with placebo. It was also associated with significant reduction in fasting plasma glucose and 2-hour postprandial plasma glucose versus placebo. The any adverse events, gastrointestinal adverse events and symptomatic hypoglycemia were significantly increased in lixisenatide-treatment group, but it did not increase the risks of serious adverse events, death, or severe hypoglycemia. Conclusions: Lixisenatide was more effective than placebo for patients with inadequately controlled type 2 diabetes mellitus, and the mild-to-moderate adverse events were tolerated in current short-term follow-up.

This manuscript has been respectively revised by two authors (You Cheng Zhang, Man Cai Wang), and disagreement was resolved by discussion. Specific content as follows:

COMMENTS TO AUTHOR

This paper very adequately evaluated the efficacy and safety of Lixisenatide for patients with inadquately treated type 2 diabetes. From a monumental 598 database, only 10 finally was selected for the use for meta-analysis. It is still a big paper, with many tables ahd figures. These are not easy to read. I suggest that more information are added to the figures, particularly figure 2 (incomplehasibal as it stands) and also figues 3-5.

Response to Reviewers:
Figures in this paper were made by the software RevMan 5.3, and they included all information. Figures in this paper were same with these in paper "Efficacy and Safety of Vedolizumab for Inflammatory Bowel Diseases: A Systematic Review and Meta-Analysis of Randomized Controlled Trials", which was published in Medicine.

Editorial Formatting Comments

1. Title page: Be sure the title page lists all author names, degrees, and affiliations.

Response to Reviewers:

Man-cai Wang[1,2,3], MD, Xiang-wen Wang[1,2,3], MD, Yu-jing Ma[1,2,3], MD, Peng-cheng Liu[1,2,3], MD, Xiao-xiang Hou[1,2,3], MD, Tian-long Ma[1,2,3], MD, Zhen-gang Wei[1,2,3], MD, Ya-wu Zhang[1,2,3], MD, PhD, Xiao-dong Xu[1,2,3], MD, PhD, You-cheng Zhang[1,2,3], MD, PhD

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2. Title: Be sure the title includes any specific terms as directed in the reporting guidelines for your type of article (for example, "case report" should be in the title of a CARE-compliant article). The following guidelines specify terms that should be in the title: CARE, CHEERS, CONSORT, PRISMA.

Response to Reviewers:

PRISMA-Efficacy and Safety of Lixisenatide for Inadequately Controlled Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized

Controlled Trials

3. Abstract: Be sure to use a structured abstract, with headings. Use the specific headings listed in the guidelines checklist if your report is based on the CARE, CHEERS, CONSORT, or PRISMA guidelines.

Response to Reviewers:

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Conclusions: Lixisenatide was more effective than placebo for patients with inadequately controlled type 2 diabetes mellitus, and the mild-to-moderate adverse events were tolerated in current short-term follow-up.

4. List of abbreviations: If not already included, please include a list of abbreviations used in the text as part of the manuscript text file following the title page. Use abbreviations sparingly in the text, and spell them out the first time you use them. Abbreviations used in tables should be spelled out at the bottom of the table.

Response to Reviewers:

List of abbreviations:

T2DM=type 2 diabetes mellitus

IDF=International Diabetes Federation

TCM=Traditional Chinese Medicine

GLP-1=glucagon-like peptide-1

RAs=receptor agonists

DPP-4=dipeptidyl peptidase-4

CSCD=Chinese Science Citation Database

OAD=oral antidiabetic agents

FPG=fasting plasma glucose

RevMan=Review Manager

PPG=postprandial plasma glucose

AE=adverse events

RCT= randomized controlled trial

WMD=Weighted mean difference

RR=relative risk

CI= confidence intervals

5. Ethical review, Methods section: If not already included, please state in the Methods section that an ethics committee or institutional review board approved the study, and list the board's name. If ethical approval was waived or not necessary, please state the reason. If the study involves patient consent, state explicitly that informed consent was or was not given, and state the reason if not given.

Response to Reviewers:

The ethical approval was not necessary in this meta-analysis, all of the data were directly extracted from the published RCTs about lixisenatide for patients with T2DM.

6. Funding/Conflict of Interest information: List any source of funding or anything that could be perceived as a conflict of interest in the Acknowledgments section.

Response to Reviewers:

The work was supported by Gansu province Science and Technology Support Program (1204FKCA138). No potential conflict of interest relevant to this article was

reported.

7. Acknowledgments: If you list anyone by name in the Acknowledgments section, please confirm that the person gives permission to be named.

Response to Reviewers:

Thanks for the support of Gansu province Science and Technology Support Program.

The sponsors had no involvement in the study design, data collection and analysis, the writing or the decision to submit the manuscript for publication.

8. License to Publish: If not already submitted, complete and submit a copy of the Open Access-License to Publish (LTP). The LTP is available to download from the home page of our website, under Forms. Be sure to select the kind of license you would like to use if the paper is accepted. (The different kinds of licenses determine how others can use your work after publication, varying from not restrictive at all to very restrictive.) Fill out all schedules (parts of the form). The corresponding author can sign the form on behalf of all authors; we need only one copy of the form. The form can be filled out and signed electronically, then uploaded as a submission item.

Response to Reviewers:

We have finished it.

PRISMA-Efficacy and Safety of Lixisenatide for Inadequately Controlled Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

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Conflicts of Interest and Source of Funding: The work was supported by Gansu province Science and Technology Support Program (1204FKCA138). No potential conflict of interest relevant to this article was reported.

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Abstract

Objective: We aimed to systematically evaluate the efficacy and safety of lixisenatide in patients with type 2 diabetes mellitus.

Methods: PubMed, EMBASE, Cochrane Library, ClinicalTrials.gov, and the Chinese Science Citation Database were searched up to May 2015. Randomized controlled trials estimating the efficacy and safety of lixisenatide in patients with type 2 diabetes mellitus were eligible for inclusion. Two authors independently extracted data in a prespecified Microsoft Excel spreadsheet. Review Manager 5.3 software was used to make meta-analysis. Weighted mean difference (WMD) and relative risk (RR) together with their corresponding 95% confidence intervals were estimated.

Results: Ten multicenter randomized controlled trials involving 5,142 patients were eligible for inclusion finally. Lixisenatide could more significantly reduce the level of HbA1c (WMD=-0.38; 95% CI [-0.47, -0.28]), and had a higher proportion of patients who achieved the HbA1c targets of <7.0% (RR=1.84, 95% CI [1.68, 2.02]) and <6.5% (RR=3.00, 95% CI [2.49, 3.61]) compared with placebo. It was also associated with significant reduction in fasting plasma glucose and 2-hour postprandial plasma glucose versus placebo. The any adverse events, gastrointestinal adverse events and symptomatic hypoglycemia were significantly increased in lixisenatide-treatment group, but it did not increase the risks of serious adverse events, death, or severe hypoglycemia.

Conclusions: Lixisenatide was more effective than placebo for patients with inadequately controlled type 2 diabetes mellitus, and the mild-to-moderate adverse events were tolerated in current short-term follow-up.

Keywords: Lixisenatide; diabetes mellitus; Meta-analysis; Review

Introduction

Diabetes mellitus is one of the leading causes of death and disability all over the world (1). Today, there are 382 million people living with diabetes, and a further 316 million with impaired glucose tolerance are at high risk from it (2). According to the International Diabetes Federation (IDF), 80% of people with diabetes live in low- and middle-income countries, of which China is on the verge of being overwhelmed by diabetes. It has the highest number of people with diabetes in the world, with a prevalence of 8.3% (2).

Although a variety of lifestyle and pathophysiological approaches are now available, because of the progressive nature of the disease, current managements often fails to achieve the glycaemic targets (i.e.<6.5% or <7.0%) in the long term (3, 4, 5). In order to maintain glycaemic control, multiple glucose-lowering agents and/or insulin are required for patients with type 2 diabetes mellitus (T2DM). However, the combined therapies are associated with significant adverse effects such as hypoglycaemia, weight gain and gastrointestinal (GI) intolerance (3, 6). Traditional Chinese Medicine (TCM) is a special treatment in China. It has a long history of more than 2,000 years for the treatment of diabetes mellitus (7). Unfortunately, the role of TCM and other herbal medicines in the management of type 2 diabetes is still not sufficiently established now (8). In this regard, developing more effective and better-tolerated glucose-lowering therapies is an urgent need.

The incretin system plays a significant role in the maintenance of glucose homeostasis. Some evidence suggests that glucagon-like peptide-1 (GLP-1) secretion is reduced in subjects with impaired glucose tolerance or T2DM, whereas the responsiveness to GLP-1 is preserved (9). In recent years, glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have shown the promise as an important therapeutic option in treatment of T2DM (5, 10), which are due to the combination of both the promotion of insulin secretion and inhibition of glucagon secretion (11, 12). Studies of in vitro and animal models suggested that GLP-1 RAs have the potential to preserve pancreatic islet β-cells by enhancing proliferation and inhibiting apoptosis

(4, 5). In addition, they can reduce the energy intake and therefore may result in weight loss (4). The benefits of cardioprotection and neuroprotection are also noticed in some studies (13, 14, 15).

Native GLP-1 is not suitable for treatment of T2DM. It can be rapidly resolved by dipeptidyl peptidase-4 (DPP-4), its half-life is less than 2 minutes (5, 15). Exenatide and liraglutide are two representatives of the DDP-4-resistent GLP-1 receptor agonists (16). Their advantages included a low propensity to cause hypoglycaemia, the ability to promote weight loss and provide extra cardiovascular benefits (6, 17). Lixisenatide (AVE0010) is the third synthetic GLP-1 receptor agonists, it is a 44 amino acid exendin-4-like GLP-1 receptor agonist, which is modified at C-terminally with six Lys residues and one Pro deleted (5, 9, 18). Recent clinical studies have shown that lixisenatide was highly possibly effective in patients with T2DM. However, various GLP-1 receptor agonists may have substantial differences in their duration of action and clinical profile. What's more, the uncertainty of some adverse events was presented during those studies. Thus, the objective of the meta-analysis is to systematically evaluate the efficacy and safety of lixisenatide in patients with T2DM.

Methods

The ethical approval was not necessary in this meta-analysis, all of the data were directly extracted from the published RCTs about lixisenatide for patients with T2DM.

Search Strategy

MEDLINE, EMBASE, Cochrane library, ClinicalTrials.gov, and the Chinese Science Citation Database (CSCD) were searched up to May 2015. The search strategy was not limited by language and region. We performed this search using both free text and medical subject headline, search terms included lixisenatide, AVE0010, and diabetes mellitus.

Inclusion Criteria

Eligibility criteria included: 1) Study design: randomized controlled trials (RCTs), single-blind or double-blind, without language limitation; 2) Population: patients aged >18 years, with inadequately controlled type 2 diabetes, with a glycated hemoglobin (HbA1c) level of 7-10%. 3) Intervention: lixisenatide or placebo was given subcutaneously, with or without oral antidiabetic agents (OADs)/insulin, the dose of lixisenatide was not limited. 4) Outcomes: reported at least one of the HbA1c, HbA1c< 7.0%, HbA1c< 6.5%, fasting plasma glucose (FPG), body weight, rescue therapy, and adverse events. Studies were excluded if the raw data could not be extracted.

Study Selection and Data Extraction

Studies were assessed independently by two authors according to the inclusion criteria above. Two authors independently extracted data in a prespecified Microsoft Excel spreadsheet. Disagreement was resolved by discussion with a third author. The follow data were collected: 1) Study characteristics (e.g. author, year of publication, sample size, age, sex ratio, duration of diabetes, baseline HbA1c). 2) Intervention measures (e.g. does of lixisenatide, once-daily or twice-daily, one-step or two-step, morning or

evening). 3) Efficacy: (e.g. HbA1c, HbA1c< 7.0%, HbA1c< 6.5%, fasting plasma glucose (FPG), body weight, rescue therapy). 4) Safety: (e.g. serious adverse events, death, gastrointestinal disorders, symptomatic hypoglycemia, severe hypoglycemia). 5) Risk of bias: (e.g. random sequence generation, allocation concealment, blinding).

Risk of Bias Assessment

Two authors independently evaluated the methodological quality of included studies according to the Cochrane Risk of Bias tool for RCTs (19), and differences were also resolved by discussion with a third author.

Statistical Analysis

Data were pooled using Review Manager 5.3 software (RevMan 5.3). Weighted mean difference (WMD) and relative risk (RR) together with their corresponding 95% confidence intervals (CI) were estimated. We analyzed all of the outcomes with a random effects model in order to give a more conservative estimate of the efficacy and safety. The statistical heterogeneity was estimated using the I^2 statistic with a cutoff of 50%, and the $\chi 2$ test with a P value <0.10. If the I^2 >50% and P <0.10, we would remove the studies with obvious heterogeneity and re-analysis the data. Finally, a fixed effects model was used to estimate the stability of this meta-analysis, and publication bias was assessed by the funnel plot.

Results

Study Characteristics

Ten eligible multicenter RCTs (5, 6, 16, 20-26) were included finally, with a total sample size of 5,142. The flow chart of trial selection was shown in Figure 1. Three RCTs (27-29) were finally excluded because of a short-term follow-up (<12 weeks), one open-label RCT (18) was also removed in this meta-analysis. Table 1 represented the characteristics of included studies. The baseline HbA1c was 7-10% in all studies, duration of follow-up respectively were 24 weeks (6, 20-26), 13 weeks (5), and 12 weeks (16). Lixisenatide was subcutaneously given 20μg once daily in most of the

included studies (6, 16, 20-26), except for Ratner et al. (5). Metformin (5, 20-26), sulfonylurea (6, 20-23), thiazolidinedione (26), pioglitazone (24), or insulin (6, 25, 26) were used in different studies for the glycemic control.

Risk of Bias

Overall, the risk of bias was low (Figure 2), risk of bias of each outcome was presented in the forest plots below. Patients were randomly divided into two groups, and allocation concealment was performed by using a centralized interactive voice response system in 7 studies (5, 6, 16, 24-26). All of the eligible studies were double-blind trials (5, 6, 16, 20-26). No incomplete outcome data and selective reporting existed among them.

Efficacy of lixisenatide for T2DM

HbA1c level

Five RCTs (20, 21, 24-26) reported the HbA1c level at the end of follow-up. Great heterogeneity existed among these studies (I^2=71%, p=0.004). Meta-analysis shown that there was statistically significant difference between lixisenatide and placebo groups (WMD=-0.44; 95% CI [-0.59, -0.29]) (Table S1). Rosenstock et al. (20) had an obvious heterogeneity with others according to the forest plot. After removing it, we got a consistent result compared with that before (WMD=-0.38; 95% CI [-0.47, -0.28]) (Figure 3).

HbA1c< 7.0%

All of the included RCTs (5, 6, 16, 20-26) reported the patients number of HbA1c< 7.0%. Moderate heterogeneity existed among these studies (I^2=56%, p=0.001). There was statistically significant difference compared lixisenatide with placebo for patients with T2DM (RR=1.90, 95% CI [1.70, 2.12]) (Table S1). After removing the obviously heterogeneous study Seino et al. (6), the result was similar with that before (RR=1.84, 95% CI [1.68, 2.02]) (Figure 4).

HbA1c< 6.5%

All of the included RCTs (5, 6, 16, 20-26) also reported the patients number of HbA1c < 6.5%. No significant heterogeneity existed among them (I^2=43%, p=0.02). Meta-analysis shown that there was statistically significant difference compared lixisenatide with placebo (RR=3.00, 95% CI [2.49, 3.61]) (Figure 5).

Fasting plasma glucose

Six RCTs (20-22, 24-26) reported the level of fasting plasma glucose (FPG), the raw data of one study could not be obtained (22). No significant heterogeneity existed among these studies (I^2=45%, p=0.10). It was shown that there was statistically significant difference between lixisenatide and placebo groups (WMD=-0.47, 95% CI [-0.71, -0.23]) (Table S1).

Body weight

Body weight was reported in four (20, 21, 25, 26) RCTs. No heterogeneity existed among these studies (I^2=0%, p=0.53). There was no statistical difference for body weight when compared lixisenatide with placebo groups (WMD=-1.04, 95% CI [-3.17, 0.37]) (Table S1).

Rescue therapy

Seven RCTs (6, 16, 20, 21, 23-25) reported the proportion of rescue therapy during follow-up. No heterogeneity existed among these studies (I^2=0%, p=0.63). Our meta-analysis shown that there was statistically significant difference between lixisenatide and placebo groups (RR=0.40, 95% CI [0.31, 0.53]) (Table S1).

Others

Three RCTs (20, 25, 26) reported the glucose excursion and 2-hour postprandial plasma glucose (PPG) after a standardized breakfast. Meta-analysis shown that there was statistically significant differences between lixisenatide and placebo groups (WMD=-3.31, 95% CI [-3.90, -2.72]; WMD=-3.41, 95% CI [-4.00, -2.82]).

Safety of lixisenatide for T2DM

Any adverse events and discontinuation

All of the included RCTs (5, 6, 16, 20-26) reported the outcomes of any adverse events (AE) and discontinuation due to AE. No significant heterogeneity existed among these studies (I^2=27%, p=0.13; I^2=0%, p=0.78). It was shown that lixisenatide may increase the risk of any adverse events and the rate of discontinuation due to AE, there were statistically significant differences versus placebo (RR=1.13, 95% CI [1.08, 1.18]; RR=2.42, 95% CI [1.87, 3.14]) (Table S1).

Serious adverse events and death

Ten RCTs (5, 6, 16, 20-26) reported the serious adverse events, death occurred in five included studies (6, 20, 21, 25, 26). Both of them had no heterogeneity (I^2=0%, p=0.69; I^2=0%, p=0.82). Lixisenatide was not associated with serious adverse events or death for patients with T2DM, no statistically significant difference existed when compared with placebo (RR=1.00, 95% CI [0.75, 1.34]; RR=0.73, 95% CI [0.18, 2.98]) (Table S1).

Gastrointestinal adverse events

Gastrointestinal adverse events included gastrointestinal disorders, nausea, vomiting, and diarrhea. They were respectively reported in nine (6, 16, 20-26), ten (5, 6, 16, 20-26), ten (5, 6, 16, 20-26), and nine (5, 6, 20-26) RCTs. Meta-analysis shown that, compared with placebo in patients with T2DM, lixisenatide could increase the risks of gastrointestinal disorders (I^2=69%, p=0.0002; RR=2.06, 95% CI [1.72, 2.47]), nausea (I^2=36%, p=0.06; RR=4.17, 95% CI [3.36, 5.17]), vomiting (I^2=18%, p=0.23; RR=5.23, 95% CI [3.59, 7.62]), and diarrhea (I^2=11%, p=0.32; RR=1.27, 95% CI [1.03, 1.56]), there were statistically significant differences in these events (Table S1).

Hypoglycemia

Ten RCTs (5, 6, 16, 20-26) reported the patients of symptomatic hypoglycemia, of which severe hypoglycemia occurred in three studies (20, 25, 26). No significant heterogeneity existed among them (I^2=0%, p=0.77; I^2=0%, p=0.88). Meta-analysis shown that, compared with placebo in patients with T2DM, lixisenatide was associated with the risk of symptomatic hypoglycemia (RR=1.59, 95% CI [1.35,

1.89]), but there was no statistically significant difference in severe hypoglycemia (RR=2.83, 95% CI [0.47, 16.90]) (Table S1).

Skin reaction

Injection-site reactions and allergic reaction were respectively reported in nine (6, 16, 20-26) and five (6, 16, 24-26) RCTs. Our meta-analysis shown that lixisenatide could increase the risk of injection-site reactions (I^2=0%, p=0.74; RR=2.05, 95% CI [1.43, 2.95]), but no statistical difference in allergic reaction between lixisenatide and placebo groups (I^2=0%, p=0.97; RR=2.11, 95% CI [0.68, 6.54]) (Table S1).

Publication Bias

Publication bias was estimated by funnel plots of HbA1c< 7.0% and HbA1c< 6.5%. They were relatively symmetrical, suggesting that there is no publication bias in this meta-analysis (Figure S1-2).

Sensitivity analysis

Sensitivity analysis was performed by using a fixed effects model to estimate the stability of this meta-analysis. All of the results were consistent with these above (Table S1).

Discussion

Incretin based therapies, such as the glucagon-like peptide-1 (GLP-1) receptor agonists, represent a major advance in T2DM treatment (29, 30). Several GLP-1 receptor agonists are available and are increasingly used as monotherapy or as “add-on” to OADs/insulin (18, 31). As a novel agent in this class, lixisenatide is one of the centers of attention for T2DM now. Our meta-analysis shown that, compared with placebo group, lixisenatide could significantly reduce the level of HbA1c, and a significantly higher proportion of patients achieved HbA1c targets of <7.0% and <6.5%. Meanwhile, lixisenatide was associated with a significant reduction in fasting plasma glucose (FPG) and 2-hour postprandial plasma glucose (PPG) after a

standardized breakfast versus placebo. All these conclusions above are consistent with those current studies (6, 16, 20-26). Horowitz et al. (30) hold the view that GLP-1 participates in the regulation of appetite and energy intake. However, compared lixisenatide with placebo in this meta-analysis, there was no statistically significant difference in body weight at the end of follow-up. It is inconsistent with the dominant idea in some studies (20, 21, 25, 26, 30, 32).

The mechanisms of lixisenatide glucose-lowering effect have been investigated in several studies (30). It was shown that lixisenatide could restore the first and second-phase insulin responses and accelerate glucose disposition (30, 33). At the same time, lixisenatide could reduce the postprandial glycemic excursions by producing sustained slowing of gastric emptying, which is of fundamental significance for patients with T2DM (34). In addition, it is thought that GLP-1 could reduce appetite and energy intake (35), although no statistically significant difference was found in body weight in this meta-analysis. The most noteworthy is that various GLP-1 receptor agonists may exhibit different effects on pre and postprandial glucose. Buse et al. (36) performed a direct comparison between liraglutide and exenatide, two different kinds of GLP-1 receptor agonists, in patients with inadequately controlled T2DM. Results shown that liraglutide more significantly reduced the FPG, while exenatide had a greater effect on PPG excursions. Similar results were demonstrated between lixisenatide and liraglutide in diabetics (37).

The safety profile of lixisenatide is another focus in this meta-analysis. The adverse events were experienced by 69% (2289/3302) of participants administered lixisenatide compared with 62% (1086/1748) receiving placebo. Lixisenatide was associated with the potential risk of any adverse events ($p<0.01$), and more large proportion of patients with lixisenatide discontinued treatment due to the adverse events as well ($p<0.01$). Even so, there were no statistical differences in both serious adverse events and death compared lixisenatide with placebo ($p>0.05$, both).

The incidence of gastrointestinal adverse events was higher in lixisenatide group than placebo group. The most common gastrointestinal adverse events included gastrointestinal disorders, nausea, vomiting, and diarrhea. Most of them were mild to moderate in intensity, dissipated with ongoing use and resolved within 6-8 weeks (23, 30, 36). In our meta-analysis, the percentage of symptomatic hypoglycemia was 10.9% (360/3302) for lixisenatide and 8.60% (151/1748) for placebo ($p<0.01$). However, the incidence of severe hypoglycemia was comparable between lixisenatide and placebo ($p=0.25$). In addition, lixisenatide could significantly reduce the frequency of rescue therapy ($p<0.01$). What`s more, lixisenatide provided lower incidence of hypoglycemia and better gastrointestinal tolerability compared with other GLP-1 receptor agonists, such as exenatide and liraglutide (18, 37).

There were several strengths in our meta-analysis. First, all of the eligible studies were randomized controlled trials, with a larger sample size of 5,142. Meanwhile, double-blind was performed in both patients and investigators. Second, an interactive voice response or web-based system was used in most of these studies to ensure the sufficiently concealment of random allocation. Third, all of the patients aged >18 years, with HbA1c 7-10%. However, one limitation that could not be ignored was the short-term follow up (≤ 24 weeks). As a new drug, longer-term studies are required to estimate the efficacy and safety of lixisenatide in patients with T2DM.

Compared with placebo, lixisenatide could significantly reduce the levels of HbA1c, FPG and PPG, and higher proportion of patients achieved the HbA1c targets of <7.0% and <6.5% in lixisenatide-treatment group. It increased the incidence of mild-to-moderate gastrointestinal adverse events and symptomatic hypoglycemia, but it was not associated with serious adverse events, death, or severe hypoglycemia. In conclusion, lixisenatide was effective and relatively tolerated in patients with inadequately controlled T2DM.

Acknowledgements:

Thanks for the support of Gansu province Science and Technology Support Program. The sponsors had no involvement in the study design, data collection and analysis, the writing or the decision to submit the manuscript for publication.

Reference

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Figures legends:

Figure 1. The flow chart of trial selection

Figure 2. The risk of bias in this meta-analysis

Figure 3. The meta-analysis of HbA1c level at the end of follow-up

Figure 4. The meta-analysis of HbA1c < 7%

Figure 5. The meta-analysis of HbA1c < 6.5%

Figure S1. Funnel plot of HbA1c <7.0%

Figure S2. Funnel plot of HbA1c< 6.5%

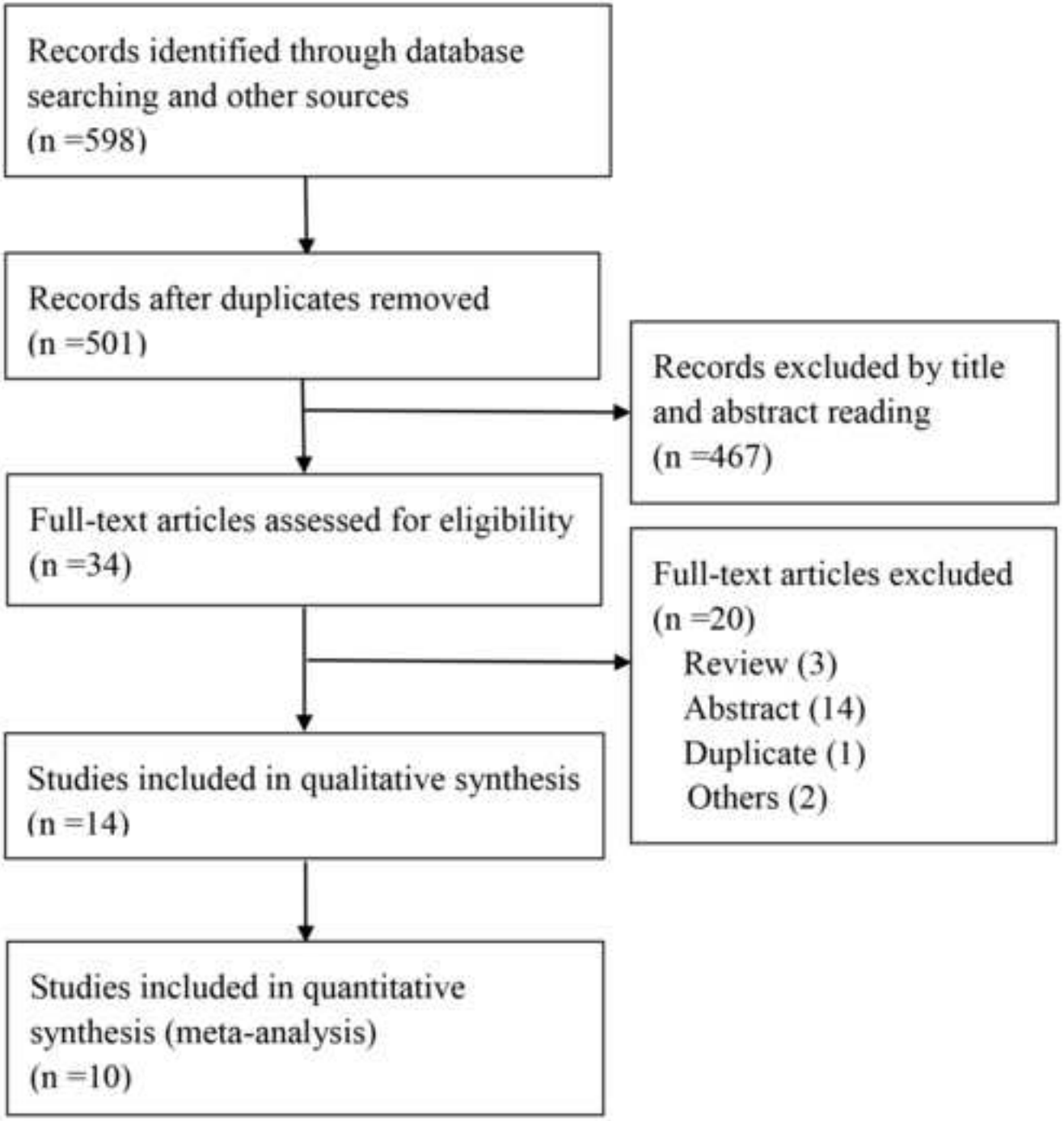
OA_Table

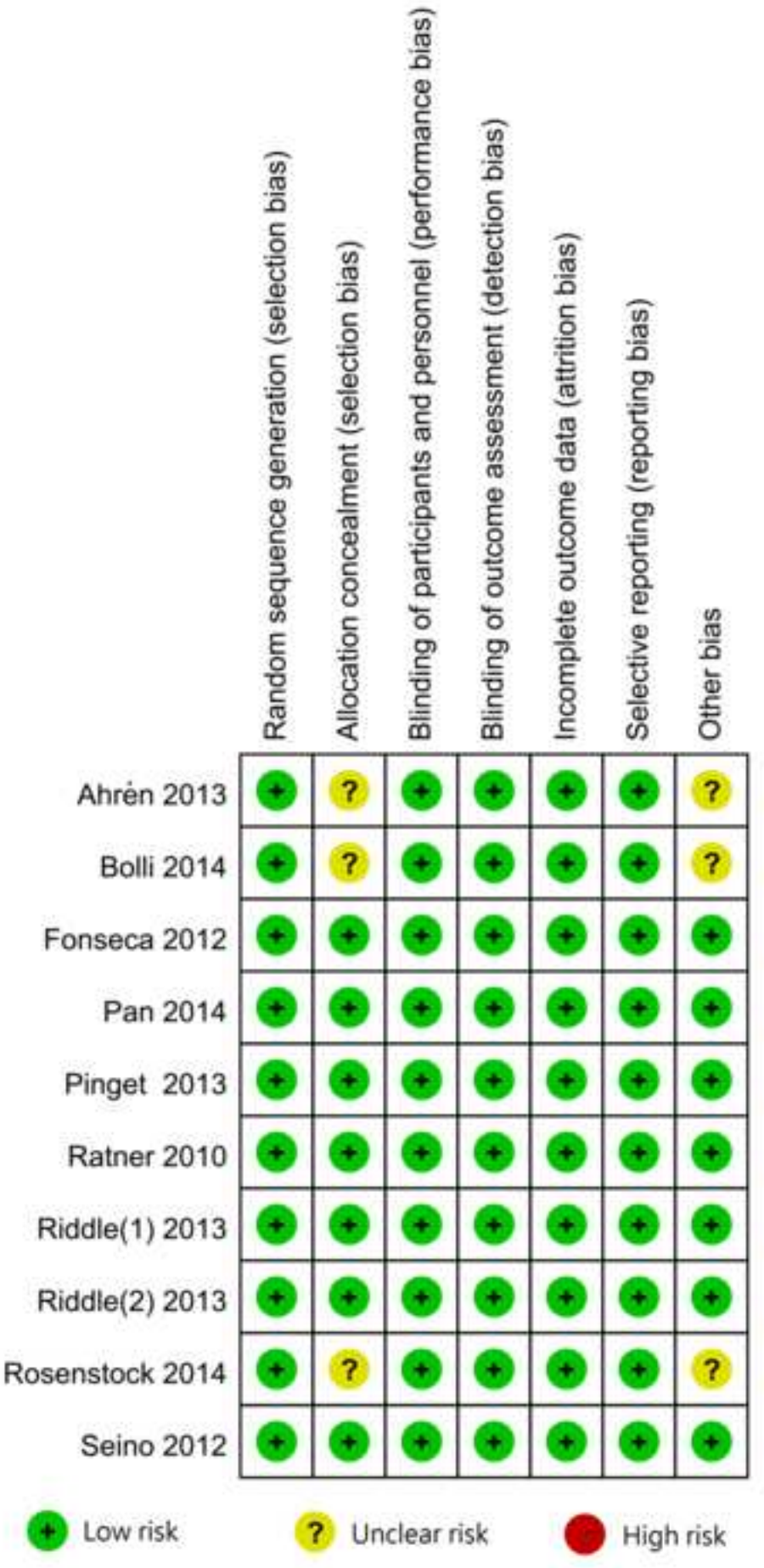
Table 1. The characteristics of included studies in this meta-analysis

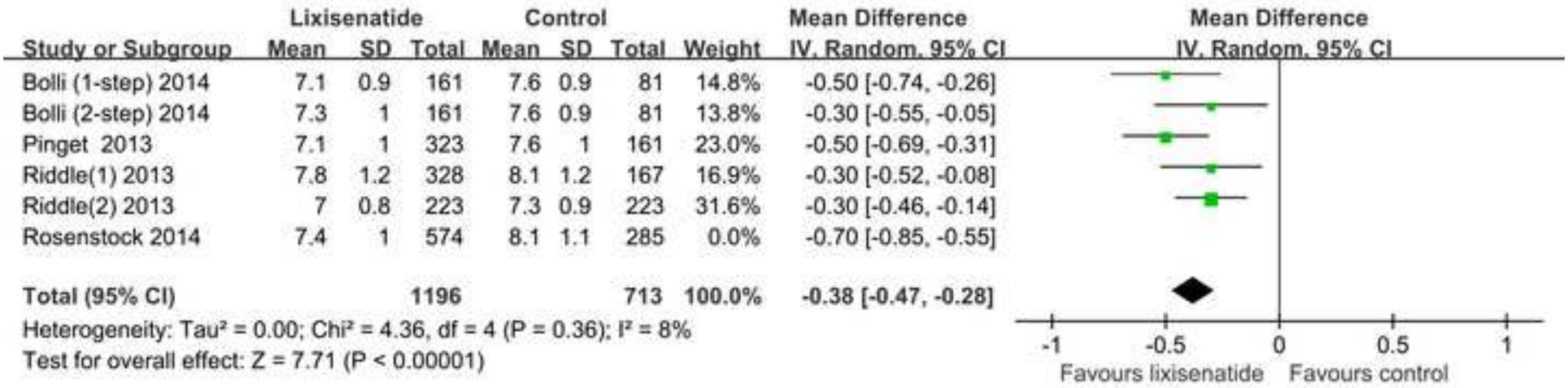
Num.	Author, et al.	Sample	Sex (Male, %)		Age		BMI		HbA1c (%)		Duration（years）		follow up
			LIXI	Placebo	LIXI	Placebo	LIXI	Placebo	LIXI	Placebo	LIXI	Placebo	
1	Rosenstock	574/285	284(49.5)	150(52.6)	57.0±9.8	57.8±10.1	30.1±6.6	30.4±6.6	8.3±0.9	8.2±0.8	9.1±6.0	9.8±6.2	24w
2	Bolli (1-step)	161/81	44(27.3)	45(28.1)	55.4±8.9	58.2±9.8	33.0±5.8	32.4±5.5	8.0±0.9	8.0±0.8	5.8±3.9	6.2±4.7	24w
	Bolli (2-step)	161/81	45(28.0)	45(28.1)	54.6±8.9	58.2±9.8	32.1±4.8	32.4±5.5	8.1±0.9	8.0±0.8	6.0±4.6	6.2±4.7	24w
3	Pan	196/194	101(51.5)	91(46.9)	54.5±10.3	55.1±10.5	26.8±3.9	27.1±3.8	7.95±0.81	7.85±0.71	6.5±4.6	6.8±4.8	24w
4	Ahrén (morning)	255/170	98(38.4)	81(47.6)	54.5±9.2	55.0±9.4	33.2±6.9	33.1±6.5	8.0±0.9	8.1±0.9	6.2±5.3	5.9±4.7	24w
	Ahrén (evening)	255/170	114(44.7)	81(47.6)	54.8±10.4	55.0±9.4	32.5±5.8	33.1±6.5	8.1±0.9	8.1±0.9	6.2±5.4	5.9±4.7	24w
5	Pinget	323/161	171(53)	82(51)	56.0±9.5	55.3±9.5	33.7±6.7	34.4±7.0	8.1±0.9	8.1±0.8	8.1±5.4	8.1±5.6	24w
6	Riddle	328/167	146(45)	82(49)	57±10	57±10	31.9±6.2	32.6±6.3	8.4±0.9	8.4±0.8	12.5±7.0	12.4±6.3	24w
7	Riddle	223/223	109(49)	113(51)	56±10	56±10	32.0±6.6	31.7±6.0	7.6±0.5	7.6±0.5	9.6±6.0	8.7±5.8	24w
8	Seino	154/157	69(44.8)	80(51.0)	58.7±10.2	58.0±10.1	25.4±3.7	25.2±3.9	8.54±0.73	8.52±0.78	13.7±7.7	14.1±7.7	24w
9	Fonseca (1-step)	119/122	63(52.9)	60(49.2)	53.8±10.9	54.1±11.0	31.7±6.6	31.8±6.7	8.07±0.9	8.07±0.9	0.2-23.9*	0.2-12.5	12w
	Fonseca (2-step)	120/122	63(52.5)	60(49.2)	53.3±9.7	54.1±11.0	32.3±6 .7	31.8±6.7	7.98±0.9	8.07±0.9	0.2-21.5*	0.2-12.5	12w
10	Ratner (5μg QD)	55/109	26(47.3)	61(56.0)	56.8±7.8	56.3±9.2	30.7±4.6	31.7±4.2	7.58±0.7	7.53±0.6	7.2±4.9	7.1±5.4	13w
	Ratner (10μg QD)	52/109	31(59.6)	61(56.0)	55.4±9.2	56.3±9.2	31.9±4.0	31.7±4.2	7.52±0.6	7.53±0.6	6.2±4.1	7.1±5.4	13w
	Ratner (20μg QD)	55/109	28(50.9)	61(56.0)	55.4±9.9	56.3±9.2	32.0±4.3	31.7±4.2	7.58±0.7	7.53±0.6	6.4±6.8	7.1±5.4	13w
	Ratner (30μg QD)	54/109	27(50.0)	61(56.0)	56.5±8.7	56.3±9.2	31.6±3.6	31.7±4.2	7.52±0.7	7.53±0.6	6.0±4.8	7.1±5.4	13w
	Ratner (5μg BID)	53/109	25(47.2)	61(56.0)	57.1±8.2	56.3±9.2	31.6±4.2	31.7±4.2	7.60±0.6	7.53±0.6	6.2±6.0	7.1±5.4	13w
	Ratner (10μg BID)	56/109	29(51.8)	61(56.0)	56.0±7.9	56.3±9.2	32.8±4.4	31.7±4.2	7.54±0.6	7.53±0.6	6.4±5.0	7.1±5.4	13w
	Ratner (20μg BID)	54/109	20(37.0)	61(56.0)	56.7±8.3	56.3±9.2	32.7±4.4	31.7±4.2	7.61±0.7	7.53±0.6	6.6±5.1	7.1±5.4	13w
	Ratner (30μg BID)	54/109	23(42.6)	61(56.0)	55.3±9.1	56.3±9.2	32.3±4.5	31.7±4.2	7.46±0.5	7.53±0.6	7.0±5.4	7.1±5.4	13w

*duration of diabetes mellitus was not all ≥1 year since diagnosis.

LIXI, Lixisenatide; BMI, body mass index.



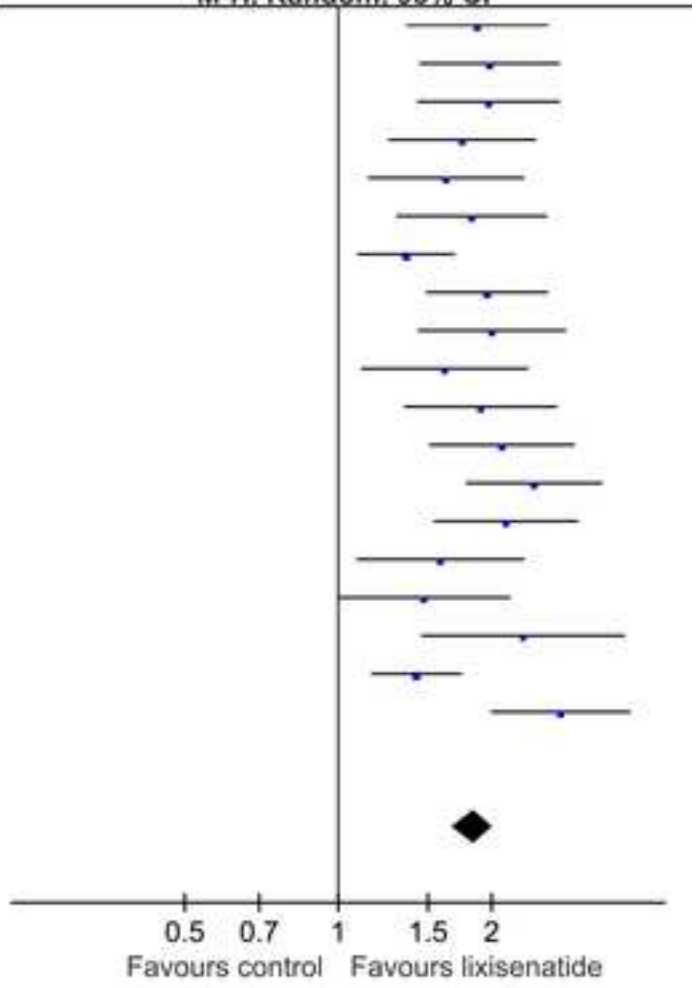




Study or Subgroup	Lixisenatide Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Ahrén (evening) 2013	104	255	37	170	5.3%	1.87 [1.36, 2.58]
Ahrén (morning) 2013	110	255	37	170	5.4%	1.98 [1.44, 2.72]
Bolli (1-step) 2014	74	156	38	158	5.3%	1.97 [1.43, 2.72]
Bolli (2-step) 2014	64	152	38	158	5.0%	1.75 [1.25, 2.44]
Fonseca (1-step) 2012	53	114	32	112	4.7%	1.63 [1.14, 2.32]
Fonseca (2-step) 2012	59	113	32	112	4.9%	1.83 [1.30, 2.57]
Pan 2014	103	195	75	193	8.0%	1.36 [1.09, 1.70]
Pinget 2013	169	323	43	161	6.3%	1.96 [1.49, 2.58]
Ratner (10µg BID) 2010	36	56	35	109	5.0%	2.00 [1.43, 2.80]
Ratner (10µg QD) 2010	27	52	35	109	4.2%	1.62 [1.11, 2.36]
Ratner (20µg BID) 2010	33	54	35	109	4.8%	1.90 [1.35, 2.69]
Ratner (20µg QD) 2010	37	55	35	109	5.1%	2.10 [1.51, 2.91]
Ratner (30µg BID) 2010	42	54	35	109	5.6%	2.42 [1.78, 3.30]
Ratner (30µg QD) 2010	37	54	35	109	5.2%	2.13 [1.54, 2.96]
Ratner (5µg BID) 2010	27	53	35	109	4.2%	1.59 [1.09, 2.32]
Ratner (5µg QD) 2010	26	55	35	109	4.0%	1.47 [1.00, 2.18]
Riddle(1) 2013	86	328	19	167	3.1%	2.30 [1.45, 3.65]
Riddle(2) 2013	121	223	85	223	8.5%	1.42 [1.16, 1.75]
Rosenstock 2014	209	574	38	285	5.4%	2.73 [1.99, 3.74]
Seino 2012	55	154	8	157	0.0%	7.01 [3.45, 14.22]
Total (95% CI)		**3121**		**2781**	**100.0%**	**1.84 [1.68, 2.02]**
Total events	1417		754			

Heterogeneity: $Tau^2 = 0.01$; $Chi^2 = 28.41$, df = 18 (P = 0.06); $I^2 = 37\%$

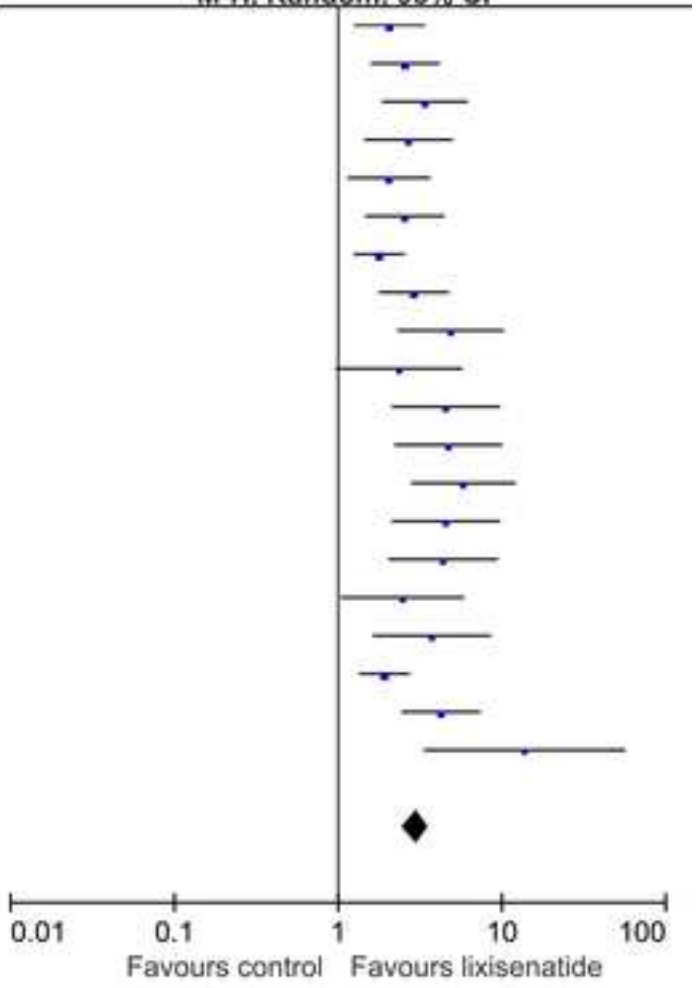
Test for overall effect: Z = 13.00 (P < 0.00001)



Study or Subgroup	Lixisenatide Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Ahrén (evening) 2013	49	225	18	170	6.5%	2.06 [1.24, 3.40]
Ahrén (morning) 2013	61	225	18	170	6.7%	2.56 [1.57, 4.17]
Bolli (1-step) 2014	40	156	12	158	5.3%	3.38 [1.84, 6.19]
Bolli (2-step) 2014	31	152	12	158	5.1%	2.69 [1.43, 5.03]
Fonseca (1-step) 2012	29	114	14	112	5.6%	2.04 [1.14, 3.64]
Fonseca (2-step) 2012	36	113	14	112	5.8%	2.55 [1.46, 4.46]
Pan 2014	63	195	35	193	8.4%	1.78 [1.24, 2.56]
Pinget 2013	93	323	16	161	6.6%	2.90 [1.76, 4.76]
Ratner (10µg BID) 2010	20	56	8	109	4.1%	4.87 [2.29, 10.34]
Ratner (10µg QD) 2010	9	52	8	109	3.2%	2.36 [0.97, 5.76]
Ratner (20µg BID) 2010	18	54	8	109	4.0%	4.54 [2.11, 9.77]
Ratner (20µg QD) 2010	19	55	8	109	4.0%	4.71 [2.20, 10.06]
Ratner (30µg BID) 2010	23	54	8	109	4.2%	5.80 [2.78, 12.11]
Ratner (30µg QD) 2010	18	54	8	109	4.0%	4.54 [2.11, 9.77]
Ratner (5µg BID) 2010	17	53	8	109	3.9%	4.37 [2.02, 9.47]
Ratner (5µg QD) 2010	10	55	8	109	3.3%	2.48 [1.04, 5.92]
Riddle(1) 2013	44	328	6	167	3.5%	3.73 [1.62, 8.58]
Riddle(2) 2013	69	223	36	223	8.5%	1.92 [1.34, 2.74]
Rosenstock 2014	111	574	13	285	5.9%	4.24 [2.43, 7.40]
Seino 2012	27	154	2	157	1.5%	13.76 [3.33, 56.88]
Total (95% CI)		**3215**		**2938**	**100.0%**	**3.00 [2.49, 3.61]**
Total events	787		260			

Heterogeneity: $Tau^2 = 0.07$; $Chi^2 = 33.52$, df = 19 (P = 0.02); $I^2 = 43\%$

Test for overall effect: Z = 11.65 (P < 0.00001)



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Efficacy and Safety of Lixisenatide for Inadequately Controlled Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomized Controlled Trials	1
ABSTRACT			
Structured summary	2	**Objective**: We aimed to systematically evaluate the efficacy and safety of lixisenatide in patients with type 2 diabetes mellitus. **Methods**: PubMed, EMBASE, Cochrane Library, ClinicalTrials.gov, and the Chinese Science Citation Database were searched up to May 2015. Randomized controlled trials estimating the efficacy and safety of lixisenatide in patients with type 2 diabetes mellitus were eligible for inclusion. Two authors independently extracted data in a prespecified Microsoft Excel spreadsheet. Review Manager 5.3 software was used to make meta-analysis. Weighted mean difference (WMD) and relative risk (RR) together with their corresponding 95% confidence intervals were estimated. **Results**: Ten multicenter randomized controlled trials involving 5,142 patients were eligible for inclusion finally. Lixisenatide could more significantly reduce the level of HbA1c (WMD=-0.38; 95% CI [-0.47, -0.28]), and had a higher proportion of patients who achieved the HbA1c targets of <7% (RR=1.84, 95% CI [1.68, 2.02]) and <6.5% (RR=3.00, 95% CI [2.49, 3.61]) compared with placebo. It was also associated with significant reduction in fasting plasma glucose and 2-hour postprandial plasma glucose versus placebo. The any adverse events, gastrointestinal adverse events and symptomatic hypoglycemia were significantly increased in lixisenatide-treatment group, but it did not increase the risks of serious adverse events, death, or severe hypoglycemia. **Conclusions**: Lixisenatide was more effective than placebo for patients with inadequately controlled type 2 diabetes mellitus, and the mild-to-moderate adverse events were tolerated in current short-term follow-up.	3
INTRODUCTION			
Rationale	3	The incretin system plays a significant role in the maintenance of glucose homeostasis. Some evidence suggests that glucagon-like peptide-1 (GLP-1) secretion is reduced in subjects with impaired glucose tolerance or T2DM, whereas the responsiveness to GLP-1 is preserved. In recent years, glucagon-like peptide-1 receptor agonists (GLP-1 RAs) have shown the promise as an important therapeutic option in treatment of T2DM, which are due to the combination of both the promotion of insulin secretion and inhibition of glucagon secretion. Studies of in vitro and animal models suggested that GLP-1 RAs have the potential to preserve pancreatic islet β-cells by enhancing proliferation and	4-5

		inhibiting apoptosis. In addition, they can reduce the energy intake and therefore may result in weight loss. The benefits of cardioprotection and neuroprotection are also noticed in some studies. Native GLP-1 is not suitable for treatment of T2DM. It can be rapidly resolved by dipeptidyl peptidase-4 (DPP-4), its half-life is less than 2 minutes. Exenatide and liraglutide are two representatives of the DDP-4-resistent GLP-1 receptor agonists. Their advantages included a low propensity to cause hypoglycaemia, the ability to promote weight loss and provide extra cardiovascular benefits. Lixisenatide (AVE0010) is the third synthetic GLP-1 receptor agonists, it is a 44 amino acid exendin-4-like GLP-1 receptor agonist, which is modified at C-terminally with six Lys residues and one Pro deleted. Recent clinical studies have shown that lixisenatide was highly possibly effective in patients with T2DM. However, various GLP-1 receptor agonists may have substantial differences in their duration of action and clinical profile.	
Objectives	4	1) Study design: randomized controlled trials (RCTs), single-blind or double-blind, without language limitation; 2) Population: patients aged >18 years, with inadequately controlled type 2 diabetes, with a glycated hemoglobin (HbA1c) level of 7-10%. 3) Intervention: lixisenatide or placebo was given subcutaneously, with or without oral antidiabetic agents (OADs)/insulin, the dose of lixisenatide was not limited. 4) Outcomes: reported at least one of the HbA1c, HbA1c< 7.0%, HbA1c< 6.5%, fasting plasma glucose (FPG), body weight, rescue therapy, and adverse events.	6
METHODS			
Protocol and registration	5	No	
Eligibility criteria	6	1) Study design: randomized controlled trials (RCTs), single-blind or double-blind, without language limitation; 2) Population: patients aged >18 years, with inadequately controlled type 2 diabetes, with a glycated hemoglobin (HbA1c) level of 7-10%. 3) Intervention: lixisenatide or placebo was given subcutaneously, with or without oral antidiabetic agents (OADs)/insulin, the dose of lixisenatide was not limited. 4) Outcomes: reported at least one of the HbA1c, HbA1c< 7.0%, HbA1c< 6.5%, fasting plasma glucose (FPG), body weight, rescue therapy, and adverse events.	6
Information sources	7	PubMed, EMBASE, the Cochrane Library, etc.	6

PRISMA 2009 Checklist

Search	8	MEDLINE, EMBASE, Cochrane library, ClinicalTrials.gov, and the Chinese Science Citation Database (CSCD) were searched up to May 2015. The search strategy was not limited by language and region. We performed this search using both free text and medical subject headline, search terms included lixisenatide, AVE0010, and diabetes mellitus.	6
Study selection	9	Studies were assessed independently by two investigators, disagreement was resolved by discussion with a third reviewer.	6
Data collection process	10	Two authors independently extracted data in a prespecified Microsoft Excel spreadsheet. Disagreement was resolved by discussion with a third author.	6
Data items	11	The follow data were collected: 1) Study characteristics (e.g. author, year of publication, sample size, age, sex ratio, duration of diabetes, baseline HbA1c). 2) Intervention measures (e.g. does of lixisenatide, once-daily or twice-daily, one-step or two-step, morning or evening). 3) Efficacy: (e.g. HbA1c, HbA1c< 7.0%, HbA1c< 6.5%, fasting plasma glucose (FPG), body weight, rescue therapy). 4) Safety: (e.g. serious adverse events, death, gastrointestinal disorders, symptomatic hypoglycemia, severe hypoglycemia). 5) Risk of bias: (e.g. random sequence generation, allocation concealment, blinding).	6-7
Risk of bias in individual studies	12	Two authors independently evaluated the methodological quality of included studies according to the Cochrane Risk of Bias tool for RCTs , and differences were also resolved by discussion with a third author.	7
Summary measures	13	Data were pooled using Review Manager 5.3 software (RevMan 5.3). Weighted mean difference (WMD) and relative risk (RR) together with their corresponding 95% confidence intervals (CI) were estimated.	7
Synthesis of results	14	We analyzed all of the outcomes with a random effects model in order to give a more conservative estimate of the efficacy and safety. The statistical heterogeneity was estimated using the I2 statistic with a cutoff of 50%, and the $\chi 2$ test with a P value <0.10. If the I2 >50% and P <0.10, we would remove the studies with obvious heterogeneity and re-analysis the data.	7

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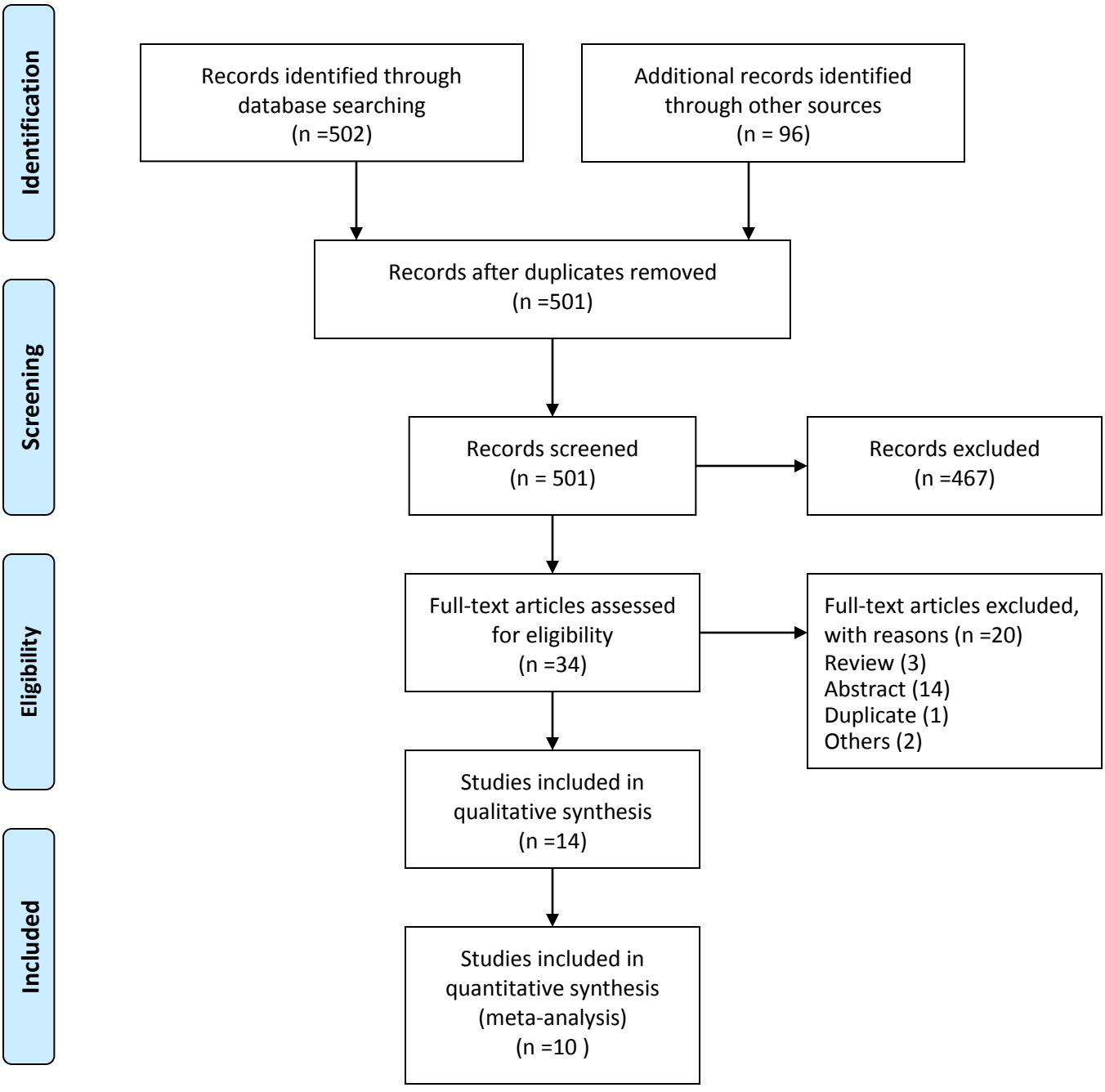
Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Overall, the risk of bias was low (Figure 2), risk of bias of each outcome was presented in the forest plots below. Patients were randomly divided into two groups, and allocation concealment was performed by using a centralized interactive voice response system in 7 studies. All of the eligible studies were double-blind trials. No incomplete outcome data and selective reporting existed among them.	8
Additional analyses	16	A fixed effects model was used to estimate the stability of this meta-analysis, and publication bias was assessed by the funnel plot.	7
RESULTS			
Study selection	17	Ten eligible multicenter RCTs (5, 6, 16, 20-26) were included finally, with a total sample size of 5,142.	7
Study characteristics	18	Three RCTs were finally excluded because of a short-term follow-up (<12 weeks), one open-label RCT was also removed in this meta-analysis. Table 1 represented the characteristics of included studies. The baseline HbA1c was 7-10% in all studies, duration of follow-up respectively were 24 weeks, 13 weeks, and 12 weeks. Lixisenatide was subcutaneously given 20μg once daily in most of the included studies, except for Ratner et al. Metformin, sulfonylurea, thiazolidinedione, pioglitazone, or insulin were used in different studies for the glycemic control.	7-8
Risk of bias within studies	19	Overall, the risk of bias was low, risk of bias of each outcome was presented in the forest plots below. Patients were randomly divided into two groups, and allocation concealment was performed by using a centralized interactive voice response system in 7 studies. All of the eligible studies were double-blind trials. No incomplete outcome data and selective reporting existed among them. Figure 2.	8
Results of individual studies	20	Figure 3, 4, Table S1.	8-11
Synthesis of results	21	Figure 3, 4, Table S1.	8-11
Risk of bias across studies	22	Figure 2.	7
Additional analysis	23	Publication bias was estimated by funnel plots of HbA1c< 7.0% and HbA1c< 6.5%. They were relatively symmetrical,	11

		suggesting that there is no publication bias in this meta-analysis (Figure S1-2). Sensitivity analysis was performed by using a fixed effects model to estimate the stability of this meta-analysis. All of the results were consistent with these above (Table S1).	
DISCUSSION			
Summary of evidence	24	Compared with placebo, lixisenatide could significantly reduce the levels of HbA1c, FPG and PPG, and higher proportion of patients achieved the HbA1c targets of <7.0% and <6.5% in lixisenatide-treatment group. It increased the incidence of mild-to-moderate gastrointestinal adverse events and symptomatic hypoglycemia, but it was not associated with serious adverse events, death, or severe hypoglycemia.	13
Limitations	25	One limitation that could not be ignored was the short-term follow up (≤ 24 weeks). As a new drug, longer-term studies are required to estimate the efficacy and safety of lixisenatide in patients with T2DM.	13
Conclusions	26	Lixisenatide was effective and relatively tolerated in patients with inadequately controlled T2DM.	13
FUNDING			
Funding	27	The work was supported by Gansu province Science and Technology Support Program (1204FKCA138).	1,14

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. doi:10.1371/journal.pmed1000097

PRISMA 2009 Flow Diagram



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