Universidade Federal do Rio Grande do Sul

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Safety and Efficacy of new antihyperglycemic agents in type 2 diabetes treatment: systematic reviews and meta-analyzes

Tese de Doutorado

Lana Catani Ferreira Pinto

Porto Alegre, 2019.

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Orientadora: Profa. Dra. Cristiane B. Leitão

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“Conhece-te,

Aceita-te,

Supera-te.”

(Santo Agostinho)

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ABREVIATION'S LIST

UGDP - University Group Diabetes Program

FDA – Food and Drug Administration

EMA – European Medicines Agency

DPP-4 – Dipeptidyl Peptidase-4

GLP-1 – Glucagon-like peptide 1

SGLT2 – Sodium Glucose Transporter-2 inhibitors

CANVAS - Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes

EMPAREG - Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes

NNH – number needed to harm

CI – confidence interval

TSA – trial sequential analysis

PRISMA - Preferred Reporting Items for Systematic Reviews and Meta-analyses

PROSPERO - International Prospective Register of Systematic Reviews

RCT – randomised clinical trial

MEDLINE

EMBASE

MeSH - Medical Subject Heading

GRADE - Grading of Recommendations Assessment, Development and Evaluation

OAD – oral antidiabetics

NR – not reported

OR – odds ratio

HbA1c – glycated hemoglobin

RR – relative risk

RESUMO

A segurança dos novos agentes anti-hiperglicemiantes é uma causa de preocupação maior na prática clínica. Existem perguntas com relação à segurança pancreática das incretinas, tanto inibidores da DPP-4 quanto análogos do GLP-1. Por outro lado, os inibidores da SGLT-2 foram associados com efeitos adversos menores como infecções genitais micóticas e infecções do trato urinário, mas também existem raros relatos de efeitos adversos mais graves. Como tanto análogos do GLP-1 quanto inibidores da SGLT-2 estão associados com redução da mortalidade em pacientes com diabetes melito tipo 2, o que provavelmente provocará aumento no seu uso no futuro, é muito importante definir seu perfil de segurança.

Outra pergunta não respondida com relação aos inibidores da SGLT-2 diz respeito aos benefícios clínicos das diferentes doses disponíveis dos agentes e a redução da HbA1c e do peso proporcionada por estas doses. Dado o exposto, os objetivos desta tese são: avaliar a segurança pancreática dos inibidores da DPP-4 com relação à pancreatite aguda e à neoplasia maligna de pâncreas; avaliar a segurança pancreática dos análogos do GLP-1 com relação ao câncer de pâncreas; avaliar os efeitos adversos associados aos inibidores da SGLT-2; e avaliar a eficácia das diferentes doses de inibidores da SGLT-2.

O primeiro estudo não achou associação entre inibidores da DPP-4 e câncer de pâncreas, no entanto, um pequeno risco para pancreatite aguda foi encontrado, apesar desse achado não ser definitivo.

O segundo estudo analisou a associação entre análogos do GLP-1 e câncer pancreático. Nesse estudo, o TSA confirmou que número suficiente de pacientes foi randomizado e que não há associação desse medicamento e câncer de pâncreas, considerando um NNH de 1000 e o tempo limitado de seguimento dos estudos incluídos (1,7 anos).

O último estudo explorou as diferenças entre os inibidores da SGLT-2 em doses diferentes e comparados um com o outro. Nessa análise, canagliflozina 300 mg pareceu o mais potente dos inibidores da SLGT-2 em reduzir a HbA1c e o peso, entretanto as diferenças não parecem ser clinicamente relevantes. Os demais inibidores da SGLT-2 em doses diferentes levaram a reduções similares em ambos os desfechos. Com relação aos efeitos adversos, os inibidores da SGLT-2 foram associados com aumento no risco para infecções genitais.

Essa tese reafirma a segurança dos novos agentes anti-hiperglicemiantes. Os resultados também enfatizam a importância de prescrever os medicamentos anti-hiperglicemiantes considerando não apenas efeitos metabólicos e segurança, mas também eventos cardiovasculares e mortalidade.

ABSTRACT

The safety of new antihyperglycemic agents is a major source of concern in clinical practice. There are questions regarding pancreatic safety of incretins, either for DPP-4 inhibitor and GLP-1 agonists. On the other hand, SGLT-2 inhibitors have been associated with minor side effects as genital and urinary infections but reports on rare and more serious outcomes have been published. As GLP-1 agonists and SGLT-2 inhibitors are associated with reduction in the mortality of type 2 diabetic patients, reason why its clinical use is expected to increase in the future, it is very important to clarify their safety profile.

Another unsolved question in SGLT-2 inhibitors is the clinical benefits of different commercially available agents and dosages on reduction of HbA1c and body weight. Given that, the objectives of these thesis were: to assess the pancreatic safety of DPP-4 inhibitors regarding acute pancreatitis and pancreatic cancer; to assess the pancreatic safety of GLP-1 inhibitors regarding pancreatic cancer; to assess the adverse events associated with SGLT-2 inhibitors; and to assess the efficacy of different doses of SGLT-2 inhibitors.

The first study didn't find an association between DPP-4 inhibitors and pancreatic cancer, however found a small risk for acute pancreatitis with DPP-4 inhibitors use, even though the latter finding is not definitive.

The second study analyzed the relationship between GLP-1 analogues and pancreatic cancer. In this study, TSA confirmed that enough patients were randomized and again no association of the medications and pancreatic cancer was observed considering a NNH of 1000 and the short mean follow-up of the included trials (1.7 years).

The last study explored the differences among SGLT-2 inhibitors in different doses and compared one to each other to one. In this analysis, canagliflozin 300 mg seemed to be the most potent SGLT-2 inhibitors in reducing HbA1c and body weight, however the differences don't look clinically relevant. The remaining SGLT2 inhibitors in different doses lead to statistically similar effects for both outcomes. Regarding side effects, SGLT-2 inhibitors were associated with increased risk for genital infections.

This thesis reinforces the safety of the newest antihyperglycemic agents. The results also emphasize the importance of prescribing antihyperglycemic agents after considering not only metabolic effects and safety, but also cardiovascular events and mortality.

INTRODUCTION

Safety regarding new therapeutics has been a major concern in all areas of Medicine. In diabetes treatment, worries regarding medications' safety started in the very first randomised trial (1). In the University Group Diabetes Program (UGDP) study, patients randomised to tolbutamide were early discontinued due to excess of cardiovascular mortality (1). Moreover, patients randomised to phenformin experienced also greater cardiovascular mortality than insulin group. Importantly, more cases of lactic acidosis were reported with phenformin, including a fatal case (1). Later on, this adverse event lead to discontinuation of phenformin production and selling.

Decades later, troglitazone was approved by the Food and Drug Administration (FDA) in 1997, even though the medical officer assigned to evaluate the medication recommended against its approval(2). Soon after, reports of acute liver failure started showing up, and the manufacturer added warnings to the label of troglitazone, requiring monthly evaluation of liver enzymes (3). In the same year, it was removed from the market in England, later in the U.S.A and finally in Japan. It was never approved in the rest of Europe.

Other antihyperglycemic medications from the same class as troglitazone continued to be used for type 2 diabetes treatment for several years, albeit there was a concern of fluid retention with both rosiglitazone and pioglitazone. Rosiglitazone was commercialized in Europe until 2010, when a meta-analysis of randomised clinical trials suggested a higher risk of heart failure with rosiglitazone and two meta-analyses of cohort studies found a higher risk of heart failure compared

to pioglitazone (4-6). In September 2010, the European Medicines Agency (EMA) recommended its suspension from the European market considering the benefits no longer outweighed the risks.

In reaction to the cardiovascular adverse events observed with glitazones, the FDA released a guideline containing rules for approval of new antihyperglycemic agents(7). It stated that the trials should last longer than the typical 3 to 6 months and should contain the results of cardiovascular outcomes. In this way, the cardiovascular profile as well as adverse events would be further analyzed and described.

More recently, the focus of concerns with safety looked directly to incretins, both Dipeptidyl Peptidade-4 (DPP-4) inhibitors and Glucagon-LikePeptide-1 (GLP-1) agonists (8). In 2007, in response to reports of acute pancreatitis in patients using exenatide, the FDA added a warning on the medication labeling. In addition, concerns regarding thyroid safety were raised, as studies showed a small but increased risk of medullary thyroid cancer in rodents using liraglutide, one of the GLP-1 agonists (9; 10). Sitagliptin, a DPP-4 inhibitor, was also implicated with increased risk of acute pancreatitis (11). Several reports of pancreatitis, including fatal cases, have been described in people treated with sitagliptin and other DPP-4 inhibitors and some studies have assessed this association (12-14). Moreover, in 2016 the FDA released a new warning on DPP-4 inhibitors, regarding its association with heart failure (15).

Finally, the argument on side effects reached the youngest class of antihyperglycemic agents, the Sodium-Glucose Transporter 2 (SGLT2) inhibitors. Since its introduction, concerns on genital tract infections were raised (16). After that, some reports on the occurrence of ketoacidosis caused more rumors on the prescription of SGLT-2 inhibitors(17). Later, the results of the Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes (CANVAS) trial put canagliflozin on the spot leading diabetologists to ask if canagliflozin is related to an increase in

limbs amputations (18). Finally, in September 2018 the rare occurrence of Fournier's gangrene was also reported in association with the use of this antihyperglycemic agent (19).

Yet on the SGLT2 inhibitors, some fase 2 and fase 3 studies had shown that different doses of SGLT-2 inhibitors produced similar changes in both HbA1c and body weight (20; 21), but these studies had limited number of patients randomised and probably were overlooked. Nonetheless, the results of Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes (EMPAREG) outcomes trial were reassuring and deserve a second look. In this trial, the lower dose of empagliflozin (10 mg) produced the same reduction in A1c and cardiovascular outcomes as the higher dose (25 mg)(22). If lower doses produce lower incidence of adverse events is not clear with this trial.

All of these evidences show the importance of assessing the adverse effects of new medications and to counterpoise the potential risks and benefits when prescribing them .

Given the exposed above, the objectives of this thesis are:

- Assess the pancreatic safety of DPP-4 inhibitors regarding acute pancreatitis and pancreatic cancer.
- Assess the pancreatic safety of GLP-1 inhibitors regarding pancreatic cancer.
- Assess the adverse events associated with SGLT-2 inhibitors.
- Assess efficacy of different doses of SGLT-2 inhibitors.

REFERENCES

1. Knatterud GL, Meinert CL, Klimt CR, Osborne RK, Martin DB. Effects of hypoglycemic agents on vascular complications in patients with adult-onset diabetes. IV. A preliminary report on phenoformin results. JAMA. 1971;217(6):777-84.
2. Funk C, Ponelle C, Scheuermann G, Pantze M. Cholestatic potential of troglitazone as a possible factor contributing to troglitazone-induced hepatotoxicity: in vivo and in vitro interaction at the canalicular bile salt export pump (Bsep) in the rat. Mol Pharmacol. 2001;59(3):627-35.
3. Aljada A, Garg R, Ghanim H, Mohanty P, Hamouda W, Assian E, et al. Nuclear factor-kappaB suppressive and inhibitor-kappaB stimulatory effects of troglitazone in obese patients with type 2 diabetes: evidence of an antiinflammatory action? J Clin Endocrinol Metab. 2001;86(7):3250-6.
4. Masubuchi Y. Metabolic and non-metabolic factors determining troglitazone hepatotoxicity: a review. Drug Metab Pharmacokinet. 2006;21(5):347-56.
5. Chen X, Yang L, Zhai SD. Risk of cardiovascular disease and all-cause mortality among diabetic patients prescribed rosiglitazone or pioglitazone: a meta-analysis of retrospective cohort studies. Chin Med J (Engl). 2012;125(23):4301-6.
6. Blind E, Dunder K, de Graeff PA, Abadie E. Rosiglitazone: a European regulatory perspective. Diabetologia. 2011;54(2):213-8.
7. Loke YK, Kwok CS, Singh S. Comparative cardiovascular effects of thiazolidinediones: systematic review and meta-analysis of observational studies. BMJ. 2011;342:d1309.
8. Food and Drug Administration. FDA investigating reports of possible increased risk of pancreatitis and pre-cancerous findings of the pancreas from incretin mimetic drugs for type 2 diabetes 2013. Available from: https://www.fda.gov/Drugs/DrugSafety/ucm343187.htm. Acessed on January 16th, 2019.
9. Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology. 2000;141(12):4600-5.
10. Vrang N, Jelsing J, Simonsen L, Jensen AE, Thorup I, Søeborg H, et al. The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis. Am J Physiol Endocrinol Metab. 2012;303(2):E253-64.

11. Olansky L. Do incretin-based therapies cause acute pancreatitis? J Diabetes Sci Technol. 2010;4(1):228-9.
12. Butler PC, Elashoff M, Elashoff R, Gale EA. A critical analysis of the clinical use of incretin-based therapies: Are the GLP-1 therapies safe? Diabetes Care. 2013;36(7):2118-25.
13. Li L, Shen J, Bala MM, Busse JW, Ebrahim S, Vandvik PO, et al. Incretin treatment and risk of pancreatitis in patients with type 2 diabetes mellitus: systematic review and meta-analysis of randomised and non-randomised studies. BMJ. 2014;348:g2366.
14. Roshanov PS, Dennis BB. Incretin-based therapies are associated with acute pancreatitis: Meta-analysis of large randomised controlled trials. Diabetes Res Clin Pract. 2015;110(3):e13-7.
15. Food and Drug Administration. FDA Drug Safety Communication: FDA adds warnings about heart failure risk to labels of type 2 diabetes medicines containing saxagliptin and alogliptin. Available from: https://www.fda.gov/Drugs/DrugSafety/ucm486096.htm. Acessed on January 16th, 2019.
16. Shyangdan DS, Uthman OA, Waugh N. SGLT-2 receptor inhibitors for treating patients with type 2 diabetes mellitus: a systematic review and network meta-analysis. BMJ Open. 2016;6(2):e009417.
17. Peters AL, Buschur EO, Buse JB, Cohan P, Diner JC, Hirsch IB. Euglycemic Diabetic Ketoacidosis: A Potential Complication of Treatment With Sodium-Glucose Cotransporter 2 Inhibition. Diabetes Care. 2015;38(9):1687-93.
18. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med. 2017;377(7):644-57.
19. Food and Drug Administration. FDA warns about rare occurrences of a serious infection of the genital area with SGLT2 inhibitors for diabetes 2018. Available from: https://www.fda.gov/Drugs/DrugSafety/ucm617360.htm. Acessed on January 16th, 2019.
20. Wilding JPH, Norwood P, T'joen C, Bastien A, List JF, Fiedorek FT. A study of dapagliflozin in patients with type 2 diabetes receiving high doses of insulin plus insulin sensitizers: applicability of a novel insulin-independent treatment. Diabetes Care. 2009; 32 (9): 1656-62.
21. Inagaki N, Kondo K, Yoshinari T, Maruyama N, Susuta Y, Kuki H. Efficacy and safety of canagliflozin alone or as add-on to other oral antihyperglycemic drugs in Japanese patients with type 2 diabetes: A 52-week open-label study. Diab Obes Metab. 2013; 15: 1136-45.
22. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med. 2015; 373: 2117-2128.

Chapter 2

Dipeptidyl Peptidase-4 inhibitors, pancreatic cancer and acute pancreatitis: A meta-analysis with trial sequential analysis

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ABSTRACT

The use of dipeptidyl peptidase-4 (DPP-4) inhibitors may be associated with pancreatic cancer and acute pancreatitis. Recent meta-analyses have reported conflicting findings. Therefore, we performed a meta-analysis to assess the risk of both pancreatic cancer and acute pancreatitis associated with the use of DPP-4 inhibitors. We also analysed whether the number of patients included is enough to reach conclusions, by means of trial sequential analysis. We included randomised controlled trials, lasting 24 weeks or more, that compared DPP-4 inhibitors versus placebo or other antihyperglycemic agents.

A total of 59,404 patients were included. There was no relationship between the use of DPP-4 inhibitors and pancreatic cancer (Peto odds ratio 0.65; 95% CI 0.35-1.21), and the optimal sample size was reached to determine a number needed to harm (NNH) of 1000 patients. DPP-4 inhibitors were associated with increased risk for acute pancreatitis (Peto odds ratio 1.72; 95% CI 1.18-2.53), with a NNH of 1066 patients, but the optimal sample size for this outcome was not reached.

In conclusion, there is no association between DPP-4 inhibitors and pancreatic cancer, and a small risk for acute pancreatitis was observed with DPP-4 inhibitors use, although the latter finding is not definitive.

INTRODUCTION

Dipeptidyl peptidase-4 (DPP-4) inhibitors or gliptins are incretin mimetic oral antihyperglycemic agents whose clinical use has steadily increased over the past ten years(1) . These medications are not associated with severe hypoglycemia and have a neutral effect on weight. However, there are concerns that the use of DPP-4 inhibitors may be associated with increased risk for pancreatic cancer and acute pancreatitis(2; 3) .

An early study analysed the FDA reports of pancreatic cancer and concluded that there was a 2.7 fold increase in the risk for pancreatic cancer with DPP-4 inhibitor use(2) . Another study suggested that DPP-4 inhibitor use was associated with the occurrence of α-cell hyperplasia, that is, increased proliferation and dysplasia, with potential evolution into neuroendocrine tumors(4). Later, a pooled analysis of clinical trials with sitagliptin suggested no association between use of this medication and pancreatic cancer(5) . The lack of association between DPP-4 inhibitor use and pancreatic cancer was evaluated in a polled analysis including only two large randomised trials and no association was found(6) . Recently, three meta-analyses assessed the risk for acute pancreatitis among patients using gliptins. Li et. al. analysed the results of 60 randomised and non-randomised trials and found no increased risk of pancreatitis in patients treated with gliptins, compared to controls(7) . Despite this reassuring finding, the inclusion of observational studies might have influenced the results due to selection bias. Conversely, two other meta-analyses analysed the results of three large studies assessing the cardiovascular risk of sitagliptin, saxaglitin and alogliptin, and found contradictory results(1;3) In these studies, the use of DPP-4 inhibitors increased the risk of pancreatitis. Importantly, the potential cases of acute pancreatitis were adjudicated in these three trials.

Considering the potential association between DPP-4 inhibitor use and both pancreatic cancer and acute pancreatitis, we performed a meta-analysis including all randomised trials with DPP-4 inhibitor use of at least 24 weeks duration, in order to analyse whether there is an increased risk for pancreatic cancer and/or acute pancreatitis. We also assessed if the number of patients randomised in these trials is sufficient to reach definitive conclusions by means of trial sequential analysis (TSA).

METHODS

Protocol and registration

This systematic review and meta-analysis follows recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) protocol(8) and it was registered at the International Prospective Register of Systematic Reviews (PROSPERO) under the number CRD42016953346.

Patient Involvement

No patients were involved in the study.

Information source and search strategy

We performed a systematic literature search for all randomised clinical trials (RCTs) that compared DPP-4 inhibitor use with either placebo or other antihyperglycemic medications. We searched MEDLINE, EMBASE, Cochrane Central and Clinicaltrials.gov from database inception to May 2016. We also searched abstracts from the most recent meetings of the American Diabetes Association and the European Association for the Study of Diabetes. The search strategy combined the Medical Subject Heading (MeSH) terms “sitagliptin” OR “saxagliptin” OR “linagliptin” OR “alogliptin” OR “vildagliptin” AND “diabetes mellitus, type

2" AND a validated filter to identify RCTs.(9) All eligible trials were considered for review, regardless of language. A manual search of reference lists of key articles was also performed.

Eligibility criteria

The inclusion criteria were: (1) RCTs, (2) DPP-4 inhibitor use versus any comparator, (3) treatment for at least 24 weeks, (4) definition of events of acute pancreatitis and/or pancreatic cancer, (5) inclusion of patients ≥18 y old, and (6) diagnosis of type 2 diabetes according to the American Diabetes Association criteria.(10)

Study selection and data collection

Two independent investigators (L.C.P. and S.S.B.) selected studies based on titles and abstracts. Studies satisfying inclusion criteria, or those with abstracts that lacked crucial information to decide upon their exclusion, were retrieved for full-text evaluation. Both investigators also analyzed the selected trials and extracted data; disagreements were resolved by a third reviewer (D.V.R.). The following information was extracted: first author's name, year of publication, sample size and dropouts, age, gender, trial duration, treatment in use prior to randomization, acute pancreatitis and pancreatic cancer events.

Risk of bias in individual studies and the quality of meta-analysis

The quality of studies was assessed according to the Cochrane Collaboration tool for risk of bias, including the six domains: random sequence generation; allocation concealment; blinding; incomplete outcome data; selective reporting; and other biases such as adjudication of events(11; 12). In adjudicated trails, the diagnosis was confirmed by the following criteria: symptoms of abdominal pain or vomiting and evidence of pancreatic inflammation (eg. elevated pancreatic enzymes, amylase or lipase > 3x the upper limit normal; in patients with chronic pancreatitis, enzyme elevations >2x the upper limit normal) or evidence of acute pancreatitis documented by

imaging abdominal computerized tomography, magnetic resonance image or ultrasound showing focal, diffuse and inhomogeneous gland enlargement. The quality of the metanalysis for each outcome (pancreatic cancer and acute pancreatitis) was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (13). Each meta-analysis was rated as high, moderate, low or very low quality.

Synthesis of results

We compared the events of interest in patients randomised to use of DPP-4 inhibitors versus the events in patients randomised to the control strategy (placebo or other antihyperglycemic medications). The outcomes of interest were pancreatic cancer and acute pancreatitis.

Data was summarised with direct meta-analysis to compare DPP-4 inhibitors with placebo and other antihyperglicemic agents. We performed analysis through Peto odds ratio and Mantel-Haeszel. We used Peto odds ratio in the primary analyses as it is more conservative (can identify smaller associations), and is superior when dealing with rare events. Heterogeneity was assessed by the Cochran Q test (*p*-value of 0.1 was considered as statistically significant) and the I^2 test (values greater than 50% were considered to indicate elevated statistical heterogeneity). For studies with zero events in both arms, continuity correction was performed to include this data on TSA analyses. To access if the length of the trials was related to the outcome, we performed meta-regression, using study duration as a covariate.

Furthermore, to address whether current information is sufficient for firm conclusions, we performed TSA of the identified studies. This analysis is analogous to sample size estimation or interim analysis of a single study(14;15), and is associated with a cumulative meta-analysis which is represented by the Z-curve. Therefore, we calculated the sample size required to detect or reject a minimal relevant difference between DPP-4 inhibitors and control(1; 4). We set this

minimal relevant difference as an absolute difference of 0.1% in the incidence of both outcomes (pancreatic cancer and acute pancreatitis) between groups based on results of previous trials (1). We conducted the TSA with an overall 5% risk of type I error and 20% risk of type II error (power of 80%).

We evaluated publication bias with visual inspection of funnel plots and with Begg and Egger's tests. If a small study bias was identified, we would perform the trim and fill computation to explore the effect of missing studies on the outcomes.

The analyses were performed using RevMan software version 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and STATA 12.0 (Stata Inc., College Station, Texas, USA). The TSA was performed with TSA software (Centre for Clinical Intervention Research Department, Copenhagen, Denmark).

RESULTS

Our search retrieved 763 articles. After scanning through titles and abstracts, as well as removing duplicates, 186 articles remained for full-text evaluation. Afterwards, 38 trials were included for analysis (**Figure 1 – Study Flowchart**).

Selected studies were published between 2009 and 2015. Mean trial duration was 63.5 weeks (range, 24-260). The analysis included 59,404 patients, 39,970 (62.1%) were men and the mean age was 57.39 ± 5.12 years. The main characteristics of included trials are presented in **Table 1**. Results regarding the individual quality of included trials are presented in Supplemental Material.

The analysis of the funnel plots and Beggs and Egger's tests suggested no publication bias for either outcome (pancreatic cancer and acute pancreatitis).

DPP-4 inhibitors and pancreatic cancer

There were 16 events of pancreatic cancer in the DPP-4 inhibitor group and 24 events in the control group. DPP-4 inhibitors were not associated with increased risk for pancreatic cancer in the direct meta-analysis (Peto odds ratio 0.65; 95% CI 0.35-1.21) (**Figure 2A - Forest Plot for association between DPP-4 inhibitors and pancreatic cancer**). Similar results were observed with Mantel-Haenszel analysis (0.65; 95% CI 0.35-1.19). When we performed TSA, DPP-4 inhibitors were still not associated with pancreatic cancer (Peto odds ratio 0.66; 95% CI 0.36-1.19) and the number of randomised patients for this outcome surpassed the futility boundary (**Figure 2B - TSA for pancreatic cancer**). Meta-regression did not show an interference of study duration on the outcome (p = 0.867; 8 studies included) (**Supplemental Material**).

DPP-4 inhibitors and acute pancreatitis

There were 64 events of acute pancreatitis in the DPP-4 inhibitor group and 39 events in the control group. DPP-4 inhibitors were associated with an increased risk for acute pancreatitis in direct meta-analysis (Peto odds ratio 1.72; 95% CI 1.18-2.53; **Supplemental Material**) or with an absolute risk difference of 0.1% (representing a number needed to harm (NNH) of 1066). Mantel-Haenszel analysis showed comparable results (1.52; 95% CI 1.05-2.18). As we aimed to be conservative, TSA was performed to assess whether there was enough information to reach a definite conclusion regarding the association between DPP-4 inhibitors and acute pancreatitis. For this outcome, the number of patients evaluated (n = 59,404) did not reach the optimal sample size (n = 140,665) and the boundaries of benefit, harm or futility were not crossed, (Peto odds ratio 1.34; 95% CI 1.00-1.79). When performing meta-regression, no interference of study duration on acute pancreatitis was seen (p = 0.252; 25 studies included).

DISCUSSION

The results of the present review indicate that the use of DPP4 inhibitors is not associated with increased risk for pancreatic cancer. Furthermore, the TSA meta-analysis confirmed that the number of patients available was enough to reach this conclusion. There seems to be an association between the use of DPP-4 inhibitors and acute pancreatitis, even though the number of randomised patients for this conclusion was not enough, and the estimated risk for acute pancreatitis is small (one patient in 1066 patients treated with DPP-4 inhibitors).

Concern regarding the association between DPP-4 inhibitor use and pancreatic cancer was raised after a review of cases reported by the FDA(2). Other studies have suggested an association between DPP-4 inhibitor use and pancreatic cancer(4; 5) but there is still an ongoing debate on this topic. On the other hand, several observational studies have explored the association between DPP-4 inhibitors and pancreatitis(16; 17) However, due to study design characteristics, the results may be affected by selection and confounding biases. As there is a great number of randomised trials evaluating these medications, a systematic review with meta-analysis of these studies is recommended to properly address this clinical question.

Before this review, three other meta-analyses evaluated the association between clinical use of DPP-4 inhibitors and acute pancreatitis. The first one(7) did not find an association between use of DPP-4 inhibitors and acute pancreatitis; however, this review included not only randomised trials but also prospective and retrospective observational cohort studies. Most importantly, the events were not adjudicated. The other two(1; 3) found an increased risk of acute pancreatitis in patients treated with DPP-4 inhibitors; however, they only included the three large cardiovascular randomised trials, EXAMINE, SAVOR-TIMI 53 and TECOS(18-20) In these trials, a specialised committee adjudicated the diagnosis of acute pancreatitis. None of these

reviews performed TSA to evaluate whether the results were definitive and, more importantly, none of them evaluated the risk for pancreatic cancer associated with use of DPP-4 inhibitors. Our study adds new information regarding this point. It included all randomised trials with DPP-4 inhibitor use that lasted for at least 24 weeks, and through TSA meta-analysis, evaluated whether the number of cases are enough to support the conclusions. There was a small risk for acute pancreatitis, so that it would be necessary to treat 1066 patients to have one case of acute pancreatitis, but the number of patients included in the meta-analysis was not sufficient to support this conclusion. Of note, due to the large number of diabetic patients using DPP-4 inhibitor worldwide, a great number of cases of acute pancreatitis might be prevented by taking into account pre-existing risk factors for acute pancreatitis, such as gallstones and hypertriglyceridemia, when considering prescription of this medication.

On the other hand, GLP1 agonist use is not associated with higher risk for acute pancreatitis, as recently pointed by a meta-analysis from Storgaard et al (21). Receptors for GLP-1 are largely found in the pancreatic ducts as well as in the pancreatic islets. Acinar and duct cells respond to GLP-1 therapy with proliferation(22; 23). A previous study in rats exposed to sitagliptin, reported hemorrhagic pancreatitis in one rat and acinar to ductal metaplasia in others(24). So, the association between incretins and acute pancreatitis has a biological plausibility. However, the explanation on why DPP-4 inhibitors are associated with pancreatitis and GLP-1 agonists are not, remains unclear(21).

When it comes to pancreatic cancer, no association between use of gliptins and pancreatic cancer was observed, and TSA meta-analysis showed that there were enough patients randomised for this observation.

The main limitation of our meta-analysis was the duration of the trials (mean of 63.5 minimum and maximum of 24 and 260 weeks) that may be insufficient to evaluate the development of pancreatic cancer. We tried to overcome this limitation including study duration as a covariate in the meta-regression and it did not have an influence on the outcome. However, we must consider that this analysis might have lack of power due to the number of included trials. The criteria used for diagnosis of acute pancreatitis in trials is a limitation. In adjudicated trails, the diagnosis was confirmed by an adjudication committee and the criteria used were clearly described. However, in non-adjudicated trials, the criteria used are not so straightforward. Nonetheless the analysis restricted to only studies with adjudication did not change the results. Furthermore, due to the design of the present study, we were not able to explore whether there is a specific sub-group of diabetic patients with increased susceptibility to this complication. The included trials did not describe the acute pancreatic risk factors, such as hypertriglyceridemia, alcohol consumption, and previous history of cholelithiasis. The only factor classically associated with acute pancreatitis that was mentioned was smoking status, and it was similar in intervention and controls groups.

Finally, there is enough information to suggest lack of association between the use of DPP-4 inhibitors and pancreatic cancer, but not of acute pancreatitis. The last one seems to be a continued concern and data of additional studies are needed. Despite this uncertainty, the implicated risk is small.

Author Contribution Statement. Author contributions: L.C.P. retrieved the full texts, abstracted the data, performed statistical analysis, wrote the first draft of the manuscript and

revised the final version; D.V.R. retrieved the full texts, abstracted the data, and revised the final version of the manuscript; S.S.B. retrieved the full texts, abstracted the data and revised the final version of the manuscript; C.B.L. revised the final version of the manuscript; J.L.G. conceived the study idea and revised the final version of the manuscript. L.C.P. is the guarantor for the contents of the article, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1 Tkáč, I. & Raz, I. Combined Analysis of Three Large Interventional Trials With Gliptins Indicates Increased Incidence of Acute Pancreatitis in Patients With Type 2 Diabetes. *Diabetes Care*, doi:10.2337/dc15-1707 (2016).

2 Elashoff, M., Matveyenko, A. V., Gier, B., Elashoff, R. & Butler, P. C. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. *Gastroenterology* **141**, 150-156, doi:10.1053/j.gastro.2011.02.018 (2011).

3 Roshanov, P. S. & Dennis, B. B. Incretin-based therapies are associated with acute pancreatitis: Meta-analysis of large randomised controlled trials. *Diabetes Res Clin Pract* **110**, e13-17, doi:10.1016/j.diabres.2015.10.014 (2015).

4 Butler, A. E. *et al.* Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes* **62**, 2595-2604, doi:10.2337/db12-1686 (2013).

5 Engel, S. S., Round, E., Golm, G. T., Kaufman, K. D. & Goldstein, B. J. Safety and tolerability of sitagliptin in type 2 diabetes: pooled analysis of 25 clinical studies. *Diabetes Ther* **4**, 119-145, doi:10.1007/s13300-013-0024-0 (2013).

6 Buse, J. B. *et al.* Pancreatic Safety of Sitagliptin in the TECOS Study. *Diabetes Care* **40**, 164-170, doi:10.2337/dc15-2780 (2017).

7 Li, L. *et al.* Incretin treatment and risk of pancreatitis in patients with type 2 diabetes mellitus: systematic review and meta-analysis of randomised and non-randomised studies. *BMJ* **348**, g2366 (2014).

8 Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G. & Group, P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* **339**, b2535 (2009).

9 Robinson, K. A. & Dickersin, K. Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. *Int J Epidemiol* **31**, 150-153 (2002).

10 Standards of Medical Care in Diabetes-2016: Summary of Revisions. *Diabetes Care* **39 Suppl 1**, S4-5, doi:10.2337/dc16-S003 (2016).

11 Higgins, J. P. T. & Green, S. Cochrane Handbook for Systematic Reviews of Interventions. *The Cochrane Collaboration* (2011).

12 Higgins, J. P. *et al.* The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* **343**, d5928 (2011).

13 Guyatt, G. H. *et al.* Incorporating considerations of resources use into grading recommendations. *Bmj* **336**, 1170-1173, doi:10.1136/bmj.39504.506319.80 (2008).
14 Wetterslev, J., Thorlund, K., Brok, J. & Gluud, C. Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis. *J Clin Epidemiol* **61**, 64-75, doi:10.1016/j.jclinepi.2007.03.013 (2008).
15 Thorlund, K. *et al.* User manual for Trial Sequential Analysis (TSA). *Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, Denmark*, 1-115 (2011).
16 Garg, R., Chen, W. & Pendergrass, M. Acute pancreatitis in type 2 diabetes treated with exenatide or sitagliptin: a retrospective observational pharmacy claims analysis. *Diabetes Care* **33**, 2349-2354, doi:10.2337/dc10-0482 (2010).
17 Sudhakaran, C., Kishore, U., Anjana, R. M., Unnikrishnan, R. & Mohan, V. Effectiveness of sitagliptin in asian Indian patients with type 2 diabetes-an Indian tertiary diabetes care center experience. *Diabetes Technol Ther* **13**, 27-32, doi:10.1089/dia.2010.0120 (2011).
18 White, W. B. *et al.* Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* **369**, 1327-1335, doi:10.1056/NEJMoa1305889 (2013).
19 Scirica, B. M. *et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* **369**, 1317-1326, doi:10.1056/NEJMoa1307684 (2013).
20 Green, J. B. *et al.* Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* **373**, 232-242, doi:10.1056/NEJMoa1501352 (2015).
21 Storgaard, H., Cold, F., Gluud, L. L., Vilsbøll, T. & Knop, F. K. Glucagon-like peptide-1 receptor agonists and risk of acute pancreatitis in patients with type 2 diabetes. *Diabetes Obes Metab* **19**, 906-908, doi:10.1111/dom.12885 (2017).
22 Butler, P. C., Elashoff, M., Elashoff, R. & Gale, E. A. A critical analysis of the clinical use of incretin-based therapies: Are the GLP-1 therapies safe? *Diabetes Care* **36**, 2118-2125, doi:10.2337/dc12-2713 (2013).
23 Perfetti, R., Zhou, J., Doyle, M. E. & Egan, J. M. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. *Endocrinology* **141**, 4600-4605, doi:10.1210/endo.141.12.7806 (2000).
24 Matveyenko, A. V. *et al.* Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin. *Diabetes* **58**, 1604-1615, doi:10.2337/db09-0058 (2009).

Table 1. Characteristics of included trials

Author Year	*n*	Follow-up (weeks)	Men (%)	Mean age (y)	Background treatment
Ahren 2014	1012	104	47.6	54.4	Metformin
Araki 2013	561	26	70.4	60.0	Naïve or OADs
Arechevaleta 2011	1035	30	54.4	56.3	Metformin
Arjona-Ferreira 2013	426	54	59.8	64.2	Diet, Exercise or OAD

Bajaj 2014	272	24	48.5	53.8	Metformin + Pioglitazone
Barnett 2012	455	52	41.3	58.0	Insulin or Insulin + Metformin
Bergenstal 2010	514	26	51.7	52.5	Metformin
TECOS 2015	14671	260	70.7	66.0	Metformin, Pioglitazone, Sulphonylurea or Insulin
DeFronzo 2015	674	24	53.7	56.2	Metformin
DeFronzo 2012	743	26	46.4	54.1	Metformin
Del Prato 2014	2639	104	49.7	55.4	Metformin
Fredrich 2012	366	24	45.9	54.9	Naïve
Gallwitz 2012	1552	104	60.2	59.8	Metformin
Henry 2014	1615	54	56.5	NR	Diet, Exercise, Metformin or Sulphonylurea

Hollander 2009	565	24	49.6	54.0	Tiazolidinedione
Inagaki 2013	574	52	69.9	60.9	OADs
Jadzinsky 2009	1309	24	49.2	52.0	Naïve
SAVOR TIMI 53 2013	16492	140	66.9	65.0	Non-incretin therapies
Leiter 2014	507	52	53.7	63.3	OADs
Lewin 2015	667	24	53.8	54.6	Naïve
Mintz 2014	858	104	51.7	57.6	Metformin
Nauck 2007	1172	52	59.2	56.7	Metformin
Nauck 2014	1098	104	46.5	54.1	Metformin
Nowicki 2011	170	52	42.9	66.5	OADs or Insulin

Olansky 2011	1250	44	56.8	49.7	Diet + Exercise
Pfutzner 2011	1306	76	49.2	52.0	Naïve
Pratley 2012	665	52	52.9	55.3	Metformin
Rosenstock 2009	401	24	50.9	53.5	Naïve
Rosenstock 2009	390	26	41.3	NR	Insulin
Rosenstock 2010	655	26	48.9	52.6	Naïve
Schernthaner 2013	756	52	55.9	56.7	Metformin + Sulphonylurea
Schernthaner 2015	720	52	61.8	72.6	Metformin
Seck 2010	1172	104	59.2	56.7	Metformin
Sheu 2015	1261	52	52.2	60.0	Insulin

Wainstein 2012	521	32	53.6	52.3	Diet + Exercise
EXAMINE 2013	5380	208	67.9	60.9	OADs
Weistock 2015	1098	26	47.4	54	Metformin
Williams-Herman 2012	306	24	52.0	53.7	Diet + Exercise

OADs oral antidiabetics; NR not reported

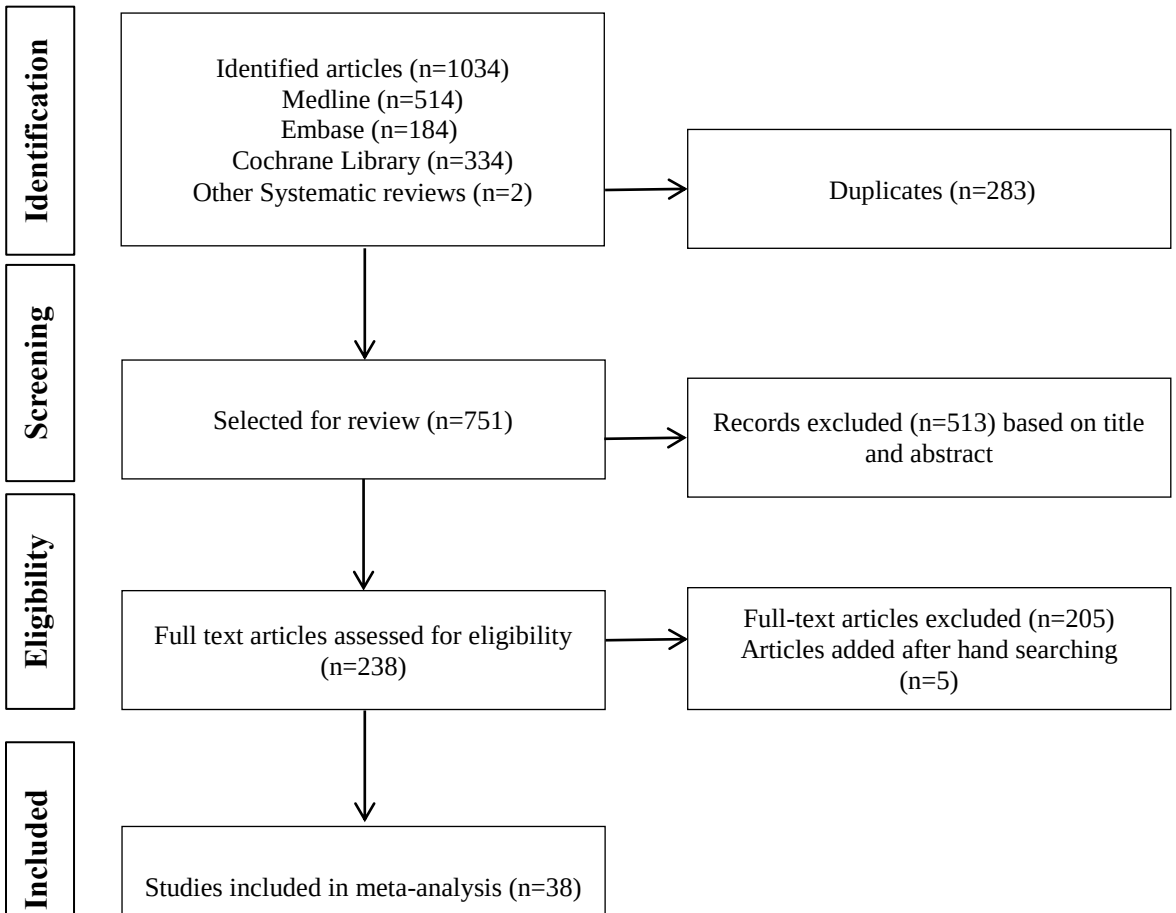


Figure 1 – Study Flowchart

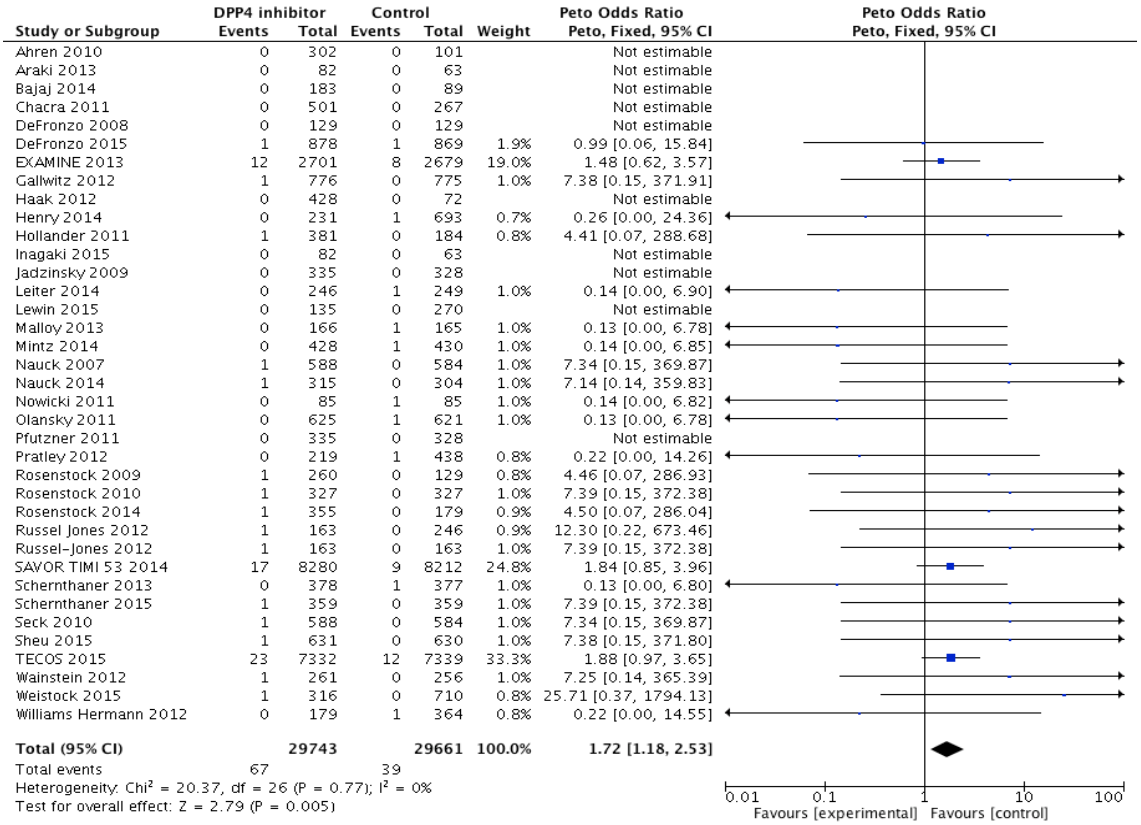


Figure 2A. Forest Plot for association between DPP-4 inhibitors and acute pancreatitis

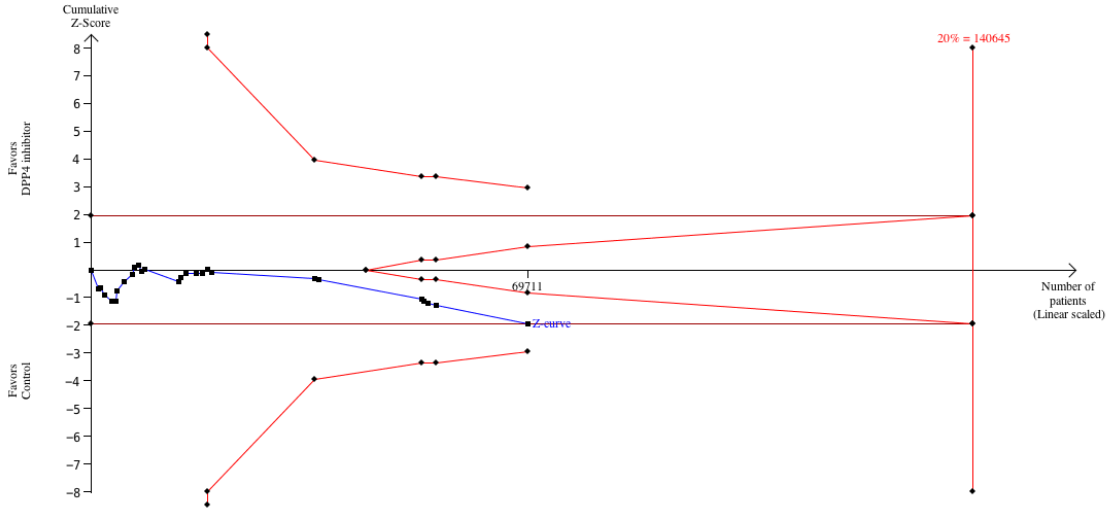
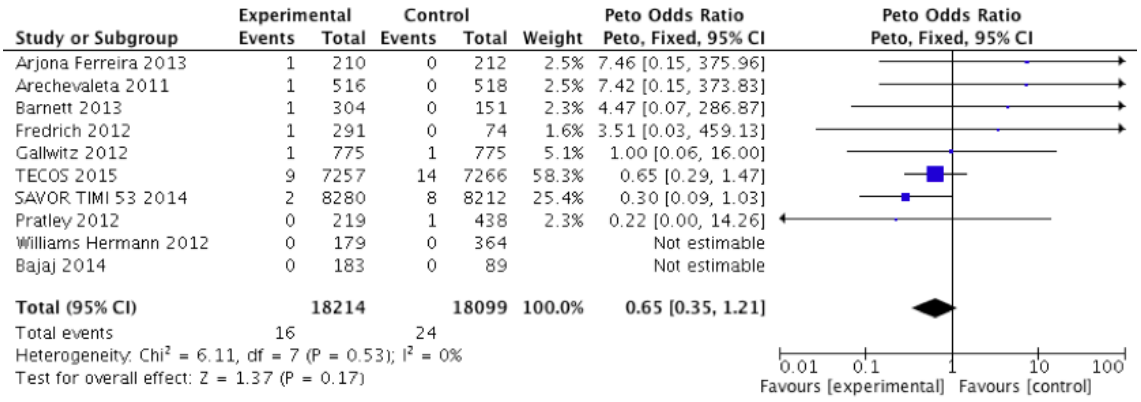
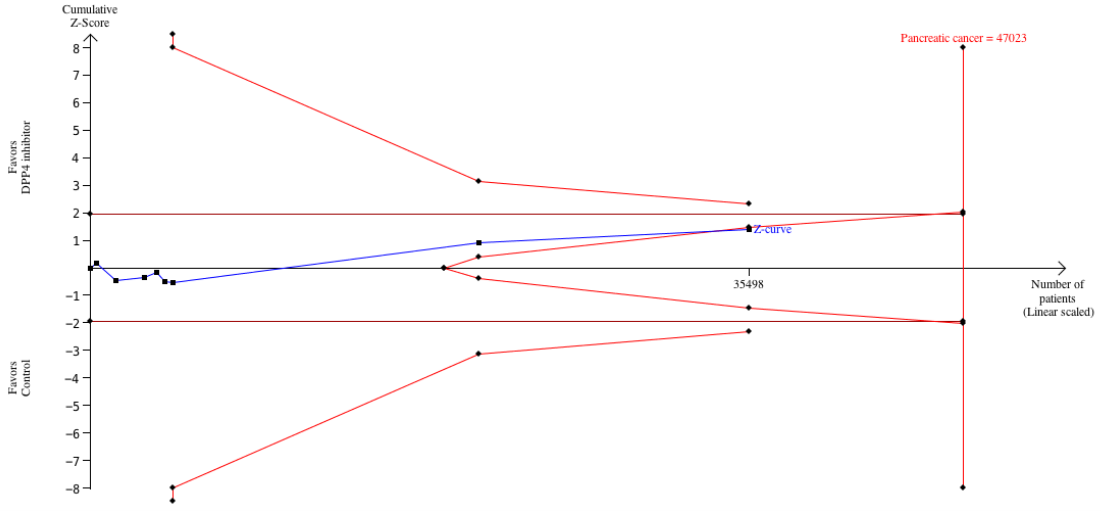


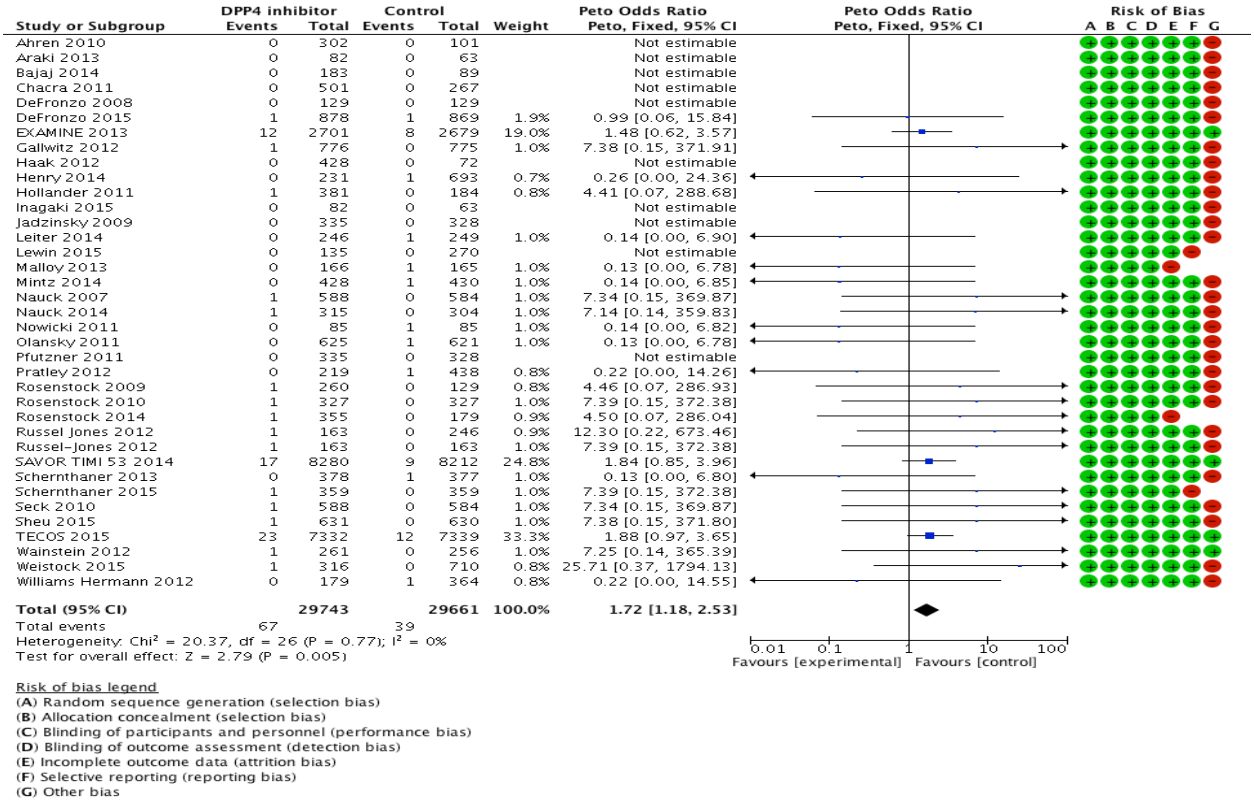
Figure 2B. TSA for acute pancreatitis



Supplemental figure 1. Forest Plot for association between DPP-4 inhibitors and pancreatic cancer



Supplemental figure 2. TSA for pancreatic cancer



Supplemental figure 3. Forest Plot for association between DPP-4 inhibitors and pancreatitis and risk of bias

Chapter 3

Glucagon-like peptide-1 receptor agonists and pancreatic cancer: a meta-analysis with trial sequential analysis

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ABSTRACT

We aimed to assess if GLP-1 agonists are associated with pancreatic cancer. Systematic review and meta-analysis of randomised trials with GLP-1 agonists as an intervention was performed. Trial sequential analysis (TSA) was performed to assess if the available information is sufficient to reject this association. Twelve trials met the study criteria, with a total of 36,397 patients. GLP-1 analogues did not increase the risk for pancreatic cancer when compared to other treatments (OR 1.06; 95% CI 0.67 to 1.67; I^2 14%). TSA confirmed that enough patients were randomised and again no association of the medications and pancreatic cancer was observed considering a NNH of 1000 and the short mean follow-up of the included trials (1.7 years). Larger studies with longer duration would be required to exclude a greater NNH and to aside concerns regarding possible influence of study duration and the outcome.

Keywords: GLP-1 agonist, meta-analysis, systematic review

INTRODUCTION

Glucagon-like peptide-1 (GLP-1) agonists bind and activate the GLP-1 receptor, which results in lower glucose plasma values in diabetic subjects, increased satiety and reduced body weight. GLP-1 agonists promote the release of insulin in response to hyperglycaemia, inhibit the secretion of glucagon, slow gastric emptying, and augment satiety by directly affecting the central nervous system.(1) Receptors for GLP-1 are found in pancreatic islets and in pancreatic acini and ducts; basic research shows that GLP-1 therapy may lead to acinar and duct cell proliferation.(2; 3)

Based on observational data, a 2011 report identified an increased risk for pancreatitis and pancreatic cancer in patients on incretin therapy, (4) which led to a Food and Drug Administration (FDA) warning on the pancreatic safety of GLP-1 agonists.(5) Two short-term studies were performed at the FDA's request. These studies were carried out with exenatide and liraglutide in a rat model of diabetes, and they increased concerns with respect to the possible adverse effects of GLP-1 mimetic therapy on exocrine pancreas. Both drugs led to an elevation in pancreatic enzymes. One rat treated with exenatide died of pancreatic necrosis, and other animals had findings of acinar-to-ductal metaplasia and foci of ductal hyperplasia, which were interpreted as premalignant changes.(6; 7)

Later, a systematic review of case reports suggested that liraglutide therapy was associated with acute pancreatitis. (8) Nonetheless, a recent meta-analysis by Storgaard et al,(9) which included only trials with adjudicated pancreatitis events, did not show an association of GLP-1 agonists and acute pancreatitis.(9)

Notably, there still remains a controversy regarding pancreatic cancer. This topic was evaluated in two large cohort studies: in the first study, an increased risk for pancreatic cancer was observed in "new users",(10) whereas no relation was observed in the second study.(11) Another recent meta-analysis reported no association between GLP-1 agonist use and pancreatic cancer.(12) However, no attempt was made to ascertain if the available number of patients on GLP-1 agonist use or the number of events were enough for definitive conclusions. In the case of a meta-analysis with a negative result, it is crucial to establish if the pooled information is sufficiently powered to exclude the association between the factor being studied (GLP-1 agonist use) and the outcome (pancreatic cancer). Since the relation between GLP-1 agonists and pancreatic cancer is still unclear, the aim of this systematic review and meta-analysis is to assess if these anti-hyperglycaemic medications have an association with pancreatic cancer. We also aimed to determine if there is sufficient evidence to exclude this association by means of trial sequential analysis (TSA).

METHODS

Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) protocol recommendations' were followed (13) and was registered at the International Prospective Register of Systematic Reviews (PROSPERO) under the number CRD42016953346.
We searched MEDLINE, EMBASE, Cochrane Central and Clinicaltrials.gov from database inception to September 2017. The search strategy combined the Medical Subject Heading (MeSH) terms "exenatide" OR "liraglutide" OR "semaglutide" OR "dulaglutide" OR "albiglutide" OR "lixizenatide"AND "diabetes mellitus, type 2" AND a filter to identify RCTs.(14) Regardless of language, all eligible trials were considered for review.

The inclusion criteria were as follows: (1) RCTs, (2) GLP-1 agonist use versus any comparator, (3) treatment for at least 48 weeks, (4) definition of events of pancreatic cancer, (5) inclusion of patients ≥18 y old, and (6) diagnosis of type 2 diabetes according to the American Diabetes Association criteria.(15)

For trials that fulfilled all inclusion criteria but did not mention pancreatic cancer events, an e-mail was sent to the corresponding author asking for the data. Of 17 e-mails sent, four e-mails were returned to sender (the e-mail of the author did not exist or had changed) and 4 e-mails received replies, two of them containing pancreatic cancer data.

Two independent investigators (L.C.P. and M.R.F.) selected studies based on titles and abstracts. Studies that met the inclusion criteria, or those with abstracts that lacked information to decide upon their exclusion, were included in full-text evaluation. Both investigators also analysed full texts and extracted data.

Risk of bias in individual studies and the quality of meta-analysis

In order to assess the quality of studies, the Cochrane Collaboration tool for risk of bias was used.(16) Regarding risk of bias, we considered the non-adjudication of events to be "other bias". Quality of meta-analysis was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.(17)

Data Analysis

We compared the events of interest in patients randomised to use of GLP-1 agonists versus the events in patients randomised to the control strategy (placebo or other antihyperglycemic medications). The outcome of interest was pancreatic cancer.

Data were summarized with Mantel-Haenszel odds ratio (OR) with direct meta-analysis to compare the GLP-1 agonist group with the control group. Heterogeneity was assessed by the

Cochran Q test (*p*-value of 0.1 was considered statistically significant) and the I^2 test (values greater than 50% were considered to indicate elevated statistical heterogeneity).

We performed a TSA on the identified studies to address whether the current evidence might be sufficient for firm conclusions. This analysis is associated with a cumulative meta-analysis represented by the Z-curve. Therefore, we were able to estimate the sample size required to accept or reject a minimal difference between GLP-1 agonists and control.(18; 19) This difference is arbitrary and must be clinically relevant. We set it as an absolute difference of 0.1% between groups, which is more conservative than the difference found in previous trials.(20) We conducted the TSA with an overall 5% risk of type I error and 20% risk of type II error (power of 80%). In this way, the analysis is able to reach a number needed to harm (NNH) of at least 1000. For studies with zero events in both arms, continuity correction was performed, and their data were included in TSA analyses.

Publication bias was evaluated with a visual inspection of funnel plots and with Begg's and Egger's tests. If a small study bias was identified, we then performed the trim and fill computation to explore the effect of missing studies on the outcomes.

The analyses were performed using RevMan software version 5.3 (Cochrane Collaboration, Copenhagen, Denmark) and STATA 12.0 (Stata Inc., College Station, Texas, USA). The TSA was performed with TSA software (Centre for Clinical Intervention Research Department, Copenhagen, Denmark).

RESULTS

Our search retrieved 2099 articles. After running through titles and abstracts and removing duplicates, 48 articles remained for full-text evaluation. Finally, 12 trials were included for analysis (**figure 1**). In four of these trials, pancreatic cancer events were adjudicated.

Selected studies were published between 2011 and 2017. The mean trial duration was 1.74 years. The trials included 36,397 patients, 62.77% of whom were men and with a mean age of 58.0 ± 4.3 years. Characteristics of included trials are presented in **table 1.** The results regarding the individual quality of included trials are presented in Supplemental Material (**figure 1s**).

GLP-1 analogues did not increase the risk for pancreatic cancer when compared to the control OR 1.06; 95% CI 0.67 to 1.67; I^2 14%) (**figure 2**). When this analysis was repeated using only adjudicated trials, the results were left unchanged (OR 1.00; 95% CI 0.62 to 1.63; I^2 61%). TSA showed that the ideal sample size was 47,023, which was not reached (36,397). However, as the futility boundary was crossed, there is enough data to exclude the association between GLP-1 agonists treatment and pancreatic cancer (considering a difference of 0.1% between treatment groups) (**figure 3**). Considering results from all patients exposed to GLP-1 agonist use, the medication is safe, and a NNH as high as 1000 can be rejected. Funnel plot analysis did not show any small study bias (p=0.721).

The LEADER trial reported pancreatic cancer incidence in more than one way. The first approach used only the adjudicated cases (GLP-1 n = 13 and placebo n = 5). This analysis is depicted in figure 2. Their second approach identified four additional cases of death, which were attributed to malignancy related to pancreatic cancer (but without histological documentation) by the adjudication committee (GLP-1 n = 13 and placebo n = 9). Repeating the meta-analysis with this additional information did not change the results (OR 0.95 95% CI 0.61 to 1.48; I^2 0%).

To investigate if trial duration influences the outcome, we performed a meta-regression. No association was found (p =0.812; 95% CI -1.12 to 1.37) between trial duration and pancreatic cancer risk, but this analysis lacked power as only 7 studies were included.

DISCUSSION

Our findings reinforce that GLP-1 analogue use is not associated with pancreatic cancer. This conclusion is based on randomised studies with a mean follow-up of 1.74 years (minimum 1 year – maximum 3.5 years) and confirmation through TSA that enough patients have been studied so far to exclude this association for this length of time, long-term associations cannot be analysed with the current studies.

As our findings are based on good quality randomised trials, confounding and attrition bias are controlled, and the risk of unreliable results is diminished. Most importantly, our meta-analysis adds evidence to previous meta-analyses, (12; 18; 21) as it is the only one to incorporate the TSA approach, which allowed us to exclude a clinically relevant magnitude of the association between GLP-1 analogues and pancreatic cancer. In other words, we achieved a number needed to harm (NNH) as high as 1537 patients.

We must acknowledge that our findings are based on studies with different follow-up durations and pancreatic cancer definitions. We explored these limitations with meta-regression, as well as with subgroup analysis, and the results were unchanged. In addition, in a search of clinicaltrials.gov, 87 ongoing trials with GLP-1 analogues were found. To reach a higher NNH, the results of these trials will need to be taken into account. Another point to be considered is the 17 trials with unreported pancreatic cancer events, from which we only received replies of 4 authors.

Compared to previous studies, these results are reassuring. There have been concerns regarding the safety of GLP-1 agonists since Raufman et al. reported in the early 90s that GLP-1 interacted with exendin receptors on dispersed acini from guinea pig pancreas,(22; 23) and these concerns were increased with the results from the LEADER trial showing a numerically greater, although statistically not significant, number of cases of pancreatic cancer in the liraglutide arm compared to the placebo.(20)

This study has some limitations. The follow up duration of the included trials may not be sufficient for the occurrence of carcinogenesis. We performed metarregression to evaluate if there was a trend towards higher incidence of pancreatic cancer in studies with longer duration, however no association between duration and the outcome was observed. Second, the limits of the confidence interval of the main outcome were 0.67 and 1.67, meaning that the real value of the statistics in 95% of the cases is somewhere in between those values, what indicates that besides the lack of association reported in this review, there is a chance that the medication could increase (as well as decrease) the risk of pancreatic cancer. In order to reassure our results, we performed direct meta-analysis and TSA, and in both cases the estimate was close to 1.0. Finally, it would be interesting to analyse the effect of each medication of the class separately, however due to the small number of studies with each one of them this analysis would lack power.

Our findings are relevant to patients with diabetes and obesity, as well as for physicians, as they reinforce the position of the European Medicines Agency, which considers incretins safe for use regarding pancreatic disease. In addition, other studies, most of which were observational, have shown similar results.(24; 25)

Ultimately, our analysis did not find an association of GLP-1 analogue use with pancreatic cancer in a mean follow-up of 1.74 years, and a sufficient number of patients have been randomised to be able to exclude a NNH of more than 1000 patients. To exclude smaller risks (i.e., a larger NNH) and to aside concerns regarding the influence of longer duration of medication exposition and the outcome, further evidence is needed.

Competing Interests: JLG reports grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), during the conduct of the study; the sponsor had no interference on data extracting, analyses and manuscript writing. LCP, DVR, SSB and CBL have declared that no competing interests exist.

Contribution Statement. L.C.P. retrieved the full texts, abstracted the data, performed statistical analysis, wrote the first draft of the manuscript and revised the final version; M. R. F. retrieved the full texts, abstracted the data and revised the final version; D.V.R. abstracted the data, and revised the final version of the manuscript; J. L. G. conceived the study idea, C.B.L. conceived the study idea and revised the final version of the manuscript. L.C.P. is the guarantor for the contents of the article, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Funding:** This work was funded by the Conselho Nacional de Desenvolvimento Científico e tecnológico (CNPq) nº 307015/2010-6 and FIPE – Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre.

REFERENCES

1. Drucker DJ. The biology of incretin hormones. Cell Metab. 2006;3(3):153-65. doi: 10.1016/j.cmet.2006.01.004. PubMed PMID: 16517403.
2. Perfetti R, Zhou J, Doyle ME, Egan JM. Glucagon-like peptide-1 induces cell proliferation and pancreatic-duodenum homeobox-1 expression and increases endocrine cell mass in the pancreas of old, glucose-intolerant rats. Endocrinology. 2000;141(12):4600-5. doi: 10.1210/endo.141.12.7806. PubMed PMID: 11108273.
3. Matveyenko AV, Dry S, Cox HI, Moshtaghian A, Gurlo T, Galasso R, et al. Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin. Diabetes. 2009;58(7):1604-15. Epub 2009/04/29. doi: 10.2337/db09-0058. PubMed PMID: 19403868; PubMed Central PMCID: PMCPMC2699878.
4. Elashoff M, Matveyenko AV, Gier B, Elashoff R, Butler PC. Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. Gastroenterology. 2011;141(1):150-6. doi: 10.1053/j.gastro.2011.02.018. PubMed PMID: 21334333; PubMed Central PMCID: PMCPMC4404515.
5. Administration FaD. FDA Drug Safety Communication: FDA investigating reports of possible increased risk of pancreatitis and pre-cancerous findings of the pancreas from incretin mimetic drugs for type 2 diabetes 2016 [cited 2017 October 26th]. Available from: https://www.fda.gov/Drugs/DrugSafety/ucm343187.htm.
6. Vrang N, Jelsing J, Simonsen L, Jensen AE, Thorup I, Søeborg H, et al. The effects of 13 wk of liraglutide treatment on endocrine and exocrine pancreas in male and female ZDF rats: a quantitative and qualitative analysis revealing no evidence of drug-induced pancreatitis. Am J

Physiol Endocrinol Metab. 2012;303(2):E253-64. Epub 2012/05/15. doi: 10.1152/ajpendo.00182.2012. PubMed PMID: 22589391.
7. Tatarkiewicz K, Belanger P, Gu G, Parkes D, Roy D. No evidence of drug-induced pancreatitis in rats treated with exenatide for 13 weeks. Diabetes Obes Metab. 2013;15(5):417-26. Epub 2012/12/07. doi: 10.1111/dom.12040. PubMed PMID: 23163898; PubMed Central PMCID: PMCPMC3654567.
8. Franks AS, Lee PH, George CM. Pancreatitis: a potential complication of liraglutide? Ann Pharmacother. 2012;46(11):1547-53. Epub 2012/11/07. doi: 10.1345/aph.1Q789. PubMed PMID: 23136352.
9. Storgaard H, Cold F, Gluud LL, Vilsbøll T, Knop FK. Glucagon-like peptide-1 receptor agonists and risk of acute pancreatitis in patients with type 2 diabetes. Diabetes Obes Metab. 2017;19(6):906-8. Epub 2017/03/17. doi: 10.1111/dom.12885. PubMed PMID: 28105738.
10. Azoulay L, Filion KB, Platt RW, Dahl M, Dormuth CR, Clemens KK, et al. Incretin based drugs and the risk of pancreatic cancer: international multicentre cohort study. BMJ. 2016;352:i581. Epub 2016/02/17. PubMed PMID: 26888382; PubMed Central PMCID: PMCPMC4772785.
11. Knapen LM, van Dalem J, Keulemans YC, van Erp NP, Bazelier MT, De Bruin ML, et al. Use of incretin agents and risk of pancreatic cancer: a population-based cohort study. Diabetes Obes Metab. 2016;18(3):258-65. Epub 2016/01/08. doi: 10.1111/dom.12605. PubMed PMID: 26537555.
12. Monami M, Nreu B, Scatena A, Cresci B, Andreozzi F, Sesti G, et al. Safety issues with glucagon-like peptide-1 receptor agonists (pancreatitis, pancreatic cancer and cholelithiasis): Data from randomised controlled trials. Diabetes Obes Metab. 2017;19(9):1233-41. Epub 2017/06/20. doi: 10.1111/dom.12926. PubMed PMID: 28244632.
13. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Bmj. 2009;339:b2535. Epub 2009/07/23. doi: 10.1136/bmj.b2535. PubMed PMID: 19622551; PubMed Central PMCID: PMCPmc2714657.
14. Robinson KA, Dickersin K. Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. Int J Epidemiol. 2002;31(1):150-3. PubMed PMID: 11914311.
15. Standards of Medical Care in Diabetes-2016: Summary of Revisions. Diabetes Care. 2016;39 Suppl 1:S4-5. doi: 10.2337/dc16-S003. PubMed PMID: 26696680.
16. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011;343:d5928. PubMed PMID: 22008217; PubMed Central PMCID: PMCPMC3196245.
17. Guyatt GH, Oxman AD, Kunz R, Jaeschke R, Helfand M, Liberati A, et al. Incorporating considerations of resources use into grading recommendations. Bmj. 2008;336(7654):1170-3. Epub 2008/05/24. doi: 10.1136/bmj.39504.506319.80. PubMed PMID: 18497416; PubMed Central PMCID: PMCPmc2394579.
18. Tkáč I, Raz I. Combined Analysis of Three Large Interventional Trials With Gliptins Indicates Increased Incidence of Acute Pancreatitis in Patients With Type 2 Diabetes. Diabetes Care. 2016. doi: 10.2337/dc15-1707. PubMed PMID: 27659407.
19. Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine

tumors. Diabetes. 2013;62(7):2595-604. doi: 10.2337/db12-1686. PubMed PMID: 23524641; PubMed Central PMCID: PMCPMC3712065.
20. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med. 2016;375(4):311-22. Epub 2016/06/14. doi: 10.1056/NEJMoa1603827. PubMed PMID: 27295427; PubMed Central PMCID: PMCPMC4985288.
21. Monami M, Dicembrini I, Nardini C, Fiordelli I, Mannucci E. Glucagon-like peptide-1 receptor agonists and pancreatitis: a meta-analysis of randomised clinical trials. Diabetes Res Clin Pract. 2014;103(2):269-75. Epub 2014/01/14. doi: 10.1016/j.diabres.2014.01.010. PubMed PMID: 24485345.
22. Malhotra R, Singh L, Eng J, Raufman JP. Exendin-4, a new peptide from Heloderma suspectum venom, potentiates cholecystokinin-induced amylase release from rat pancreatic acini. Regul Pept. 1992;41(2):149-56. PubMed PMID: 1279756.
23. Raufman JP, Singh L, Singh G, Eng J. Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. J Biol Chem. 1992;267(30):21432-7. PubMed PMID: 1328231.
24. Romley JA, Goldman DP, Solomon M, McFadden D, Peters AL. Exenatide therapy and the risk of pancreatitis and pancreatic cancer in a privately insured population. Diabetes Technol Ther. 2012;14(10):904-11. Epub 2012/07/30. doi: 10.1089/dia.2012.0075. PubMed PMID: 22845701; PubMed Central PMCID: PMCPMC3513976.
25. Funch D, Gydesen H, Tornøe K, Major-Pedersen A, Chan KA. A prospective, claims-based assessment of the risk of pancreatitis and pancreatic cancer with liraglutide compared to other antidiabetic drugs. Diabetes Obes Metab. 2014;16(3):273-5. Epub 2013/11/26. doi: 10.1111/dom.12230. PubMed PMID: 24199745; PubMed Central PMCID: PMCPMC4237552.

Table 1. Characteristics of Included Trials

Author, Year	GLP-1 Agonist	Events Control	Events Intervention	Patients Control	Patients Intervention
Marso, 2016 (SUSTAIN-6)	Semaglutide	4	1	1649	1648
Pfeffer, 2015 (ELIXA)	Lixizenatide	9	3	3034	3034
Marso, 2016 (LEADER)	Liraglutide	5	13	4672	4668

Holman, 2017 (EXSCEL)	Exenatide 1w*	15	16	7396	7356
Nauck, 2016	Dulaglitide	0	1	101	200
Kramer, 2015	Liraglutide	0	0	25	26
Home, 2015	Albiglutide	1	0	277	271
Diamant, 2014	Exenatide 1w*	0	0	233	223
Sathyanarayana, 2011	Exenatide 2d*	0	0	10	11
Pratley, 2011	Liraglutide	0	1	219	445
Bolli, 2014	Lixizenatide	0	1	160	322
Xu, 2014	Exenatide 2d*	0	0	274	142

*Exenatide 1w = exenatide once weekly; exenatide 2d = exenatide twice a day;

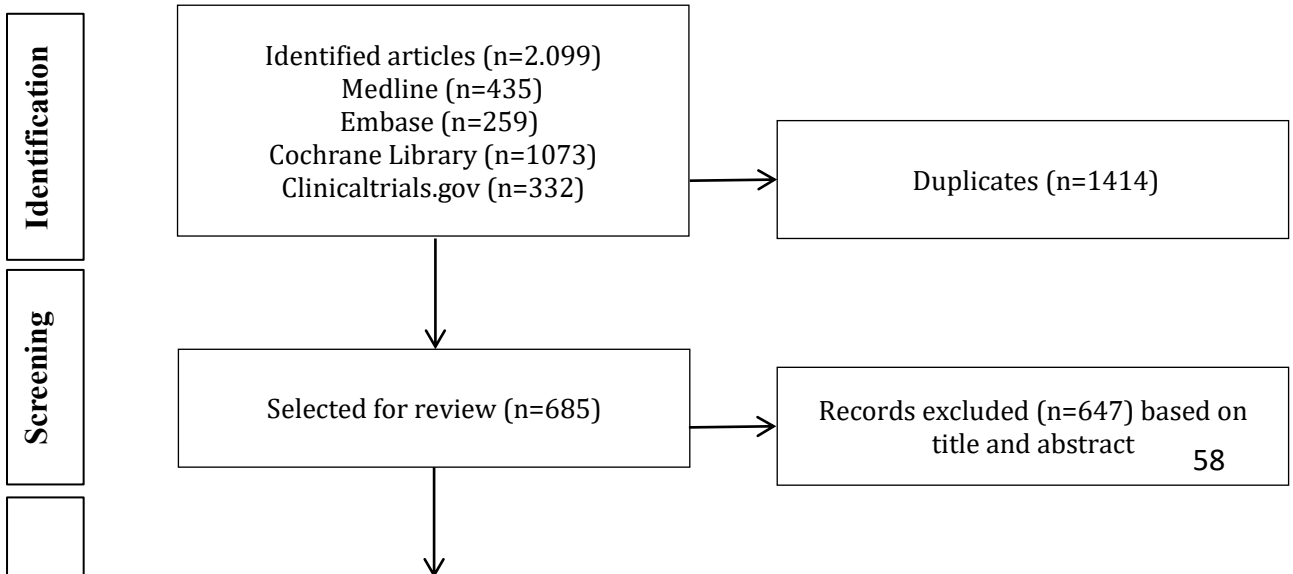

Figure 1. Study Flowchart

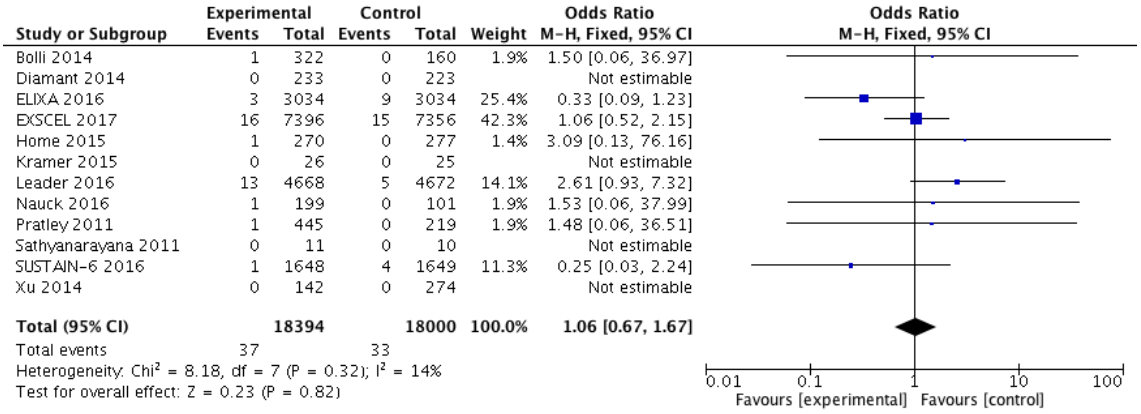

Figure 2. Forest Plot for association between GLP-1 inhibitors and pancreatic cancer

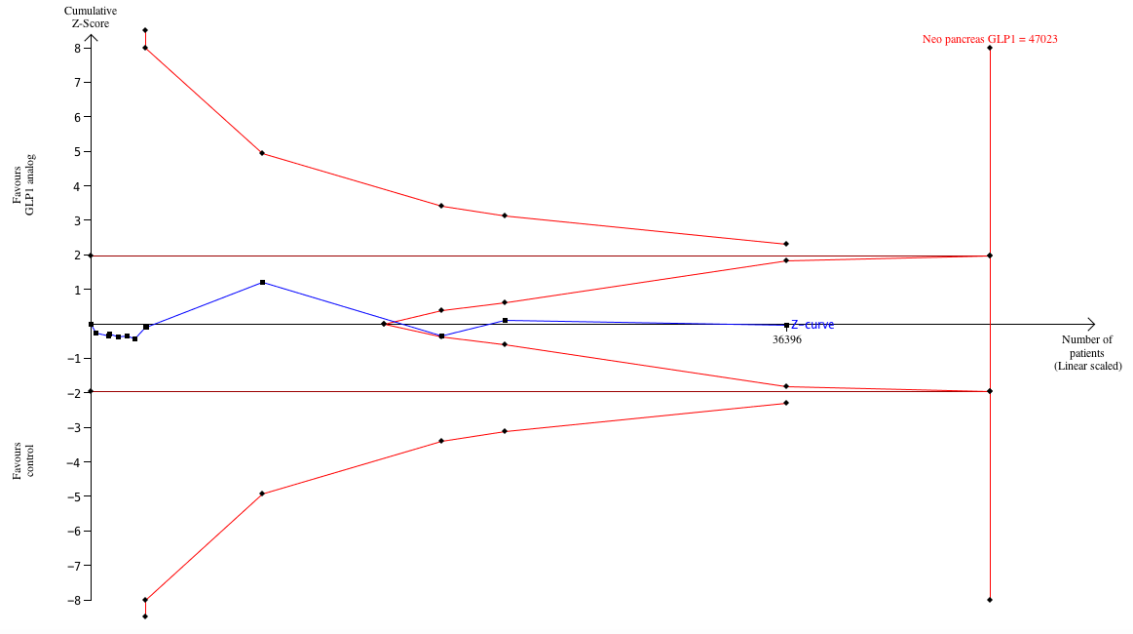

Figure 3. TSA for pancreatic cancer

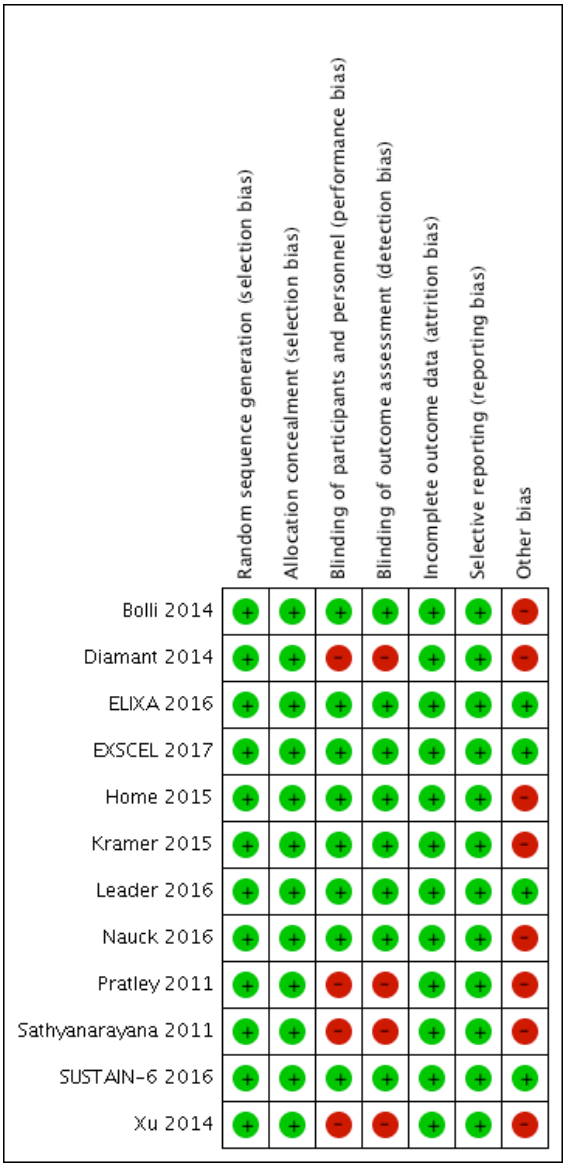

Figure 1S. Risk of bias

Chapter 4

Dose-ranging effects of SGLT2 inhibitors in patients with type 2 diabetes: a systematic review and meta-analysis

Running title: SGLT2 inhibitors dosage and type 2 diabetes

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ABSTRACT

Introduction: The lowest dosage of empagliflozin (10 mg) produced similar benefits on glycated hemoglobin (HbA1c), body weight, blood pressure, total and cardiovascular mortality in comparison with the highest dose (25 mg) in EMPAREG trial. These findings have not been confirmed in additional analyses and it is uncertain if the other two agents, canagliflozin and dapagliflozin, behave similarly and if one of them is more effective than the others.

Objective: To compare the effect of different doses of SGLT2 inhibitors in HbA1c, body weight, and adverse events of patients with type 2 diabetes.

Methods: MEDLINE, Cochrane and Embase databases were searched for randomised controlled trials of SGLT2 inhibitors in type 2 diabetes patients, lasting at least 12 weeks. HbA1c and body weight variations were described as standard mean difference. We performed direct, indirect meta-analysis, as well as a metarregression with medications' doses as covariates. PROSPERO Registry: CRD42015006975

Results: Eighteen studies were included (16,095 patients). In direct meta-analysis, SGLT2 inhibitors reduced HbA1c by 0.62% (95% CI -0.66 to -0.59) and body weight by 0.60 kg (95% CI -0.64 to -0.55). In indirect meta-analysis canagliflozin 300 mg ranked the best regarding reductions in HbA1c and body weight (-0.79%; 95% CI -0.84 to -0.75; -2.35 kg; 95% CI -2.73 to -1.97). The remaining medications and dosages were clinically similar, despite some statistically significant differences among them (-0.15 to -0.44% in HbA1c and -0.28 to -1.04 kg in body weight in comparison to placebo). All SGLT2 inhibitors in different doses were associated with increased risk for genital infection.

Conclusions: Canagliflozin 300 mg seems to be the most potent SGLT2 inhibitors in reducing HbA1c and body weight in patients with type 2 diabetes, however the differences are not

clinically relevant in comparison to other dosages/agents. The remaining SGLT2 inhibitors in different doses lead to statistically similar effects for both outcomes. Whether these glycemic and weight effects reflect on similar reductions in mortality and cardiovascular events among different agents is still uncertain and may be topic for further studies.

INTRODUCTION

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are a class of antihyperglycemic medications that inhibit renal glucose reabsorption in the proximal convoluted renal tubule and lead to glucosuria.(1; 2) SGLT2 inhibitors also have a beneficial effect on blood pressure (BP) and body weight.(3-5)

Canagliflozin, dapagliflozin and empagliflozin are the three SGLT2 inhibitors currently approved by Food and Drug Administration (FDA) for clinical use and the usual recommended doses are 300 mg, 10 mg and 25 mg, respectively.(6) However, in EMPAREG trial, a smaller dose of empagliflozin (10 mg) produced similar benefits on glycated hemoglobin (HbA1c), body weight and BP in comparison with the highest available dose (25 mg).(7) Most importantly, the reduction in total and cardiovascular mortality was comparable with 10 mg and 25 mg,(8) suggesting no dose-range effect for none of the evaluated outcomes. As there aren't any head-to-head studies among the different SGLT2 inhibitors, it is uncertain if the other two agents, canagliflozin and dapagliflozin, behave similar to empagliflozin.

Thus, the aim of this study was to analyze the efficacy of different SGLT2 inhibitors' doses compared to placebo and among each other in patients with type 2 diabetes regarding HbA1c, body weight and adverse events.

METHODS

Protocol and Registration

This systematic review and meta-analysis follows recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) protocol (9) and it is a part of the

project registered at PROSPERO (the International Prospective Register of Systematic Reviews) (CRD42015006975).

Information sources and search strategy

We performed a systematic literature search for randomised clinical trials (RCTs) that compared SGLT2 inhibitors with placebo. We searched MEDLINE, Embase, Cochrane Central and Clinicaltrials.gov from database inception to January 2018 and abstracts published in the most recent American Diabetes Association and the European Association for the Study of Diabetes meetings. The search strategy combined the Medical Subject Heading (MeSH) terms "2-(3-(4-ethoxybenzyl)-4-chlorophenyl)-6-hydroxymethyltetrahydro-2H-pyran-3,4,5-triol" OR "dapagliflozin" OR "canagliflozin" OR "ipragliflozin" OR "6-((4-ethylphenyl)methyl)-3′,4′,5′,6′-tetrahydro-6′-(hydroxymethyl)spiro(isobenzofuran-1(3H),2′-(2H)pyran)-3′,4′,5′-triol" "ASP1941" OR "SHR3824" OR "empagliflozin" OR "ertugliflozin" OR "henagliflozin" OR "sergliflozin" OR "sergliflozin etabonate" OR "sotagliflozin" OR "remogliflozin etabonate" AND "diabetes mellitus, type 2" AND a validated filter to identify RCTs.(10) All eligible trials were considered for review, regardless of language. Manual search of reference lists of key articles was also performed.

Eligibility criteria

The inclusion criteria were: (I) RCTs, (II) SGLT2 inhibitors as experimental treatment, (III) treatment duration for a minimum of 12 weeks, (IV) description of variation in HbA1c or body weight, and (V) inclusion of adult patients (≥18 y old) with type 2 diabetes.(11)

Study selection and data collection

Two independent investigators (L.C.P. and D.V.R.) selected studies based on titles and abstracts. Studies satisfying inclusion criteria or those with abstracts that lacked crucial information to

decide upon their exclusion were retrieved for full-text evaluation. Both investigators (L.C.P. and D.V.R.) also analyzed the trials selected for detailed analysis and extracted data, and disagreements were resolved by consensus. We extracted the following information: first author's name, year of trial publication, sample size and dropouts, age, gender, mean diabetes duration, trial duration, treatment used prior to randomization, doses of SGLT2 inhibitors, change in HbA1c (mean [SD]), change in body weight in kilograms (mean [SD]) and adverse events: hypoglycemia (any event), bone fractures (any fracture), urinary tract infection and genital mycotic infection. We also aimed to analyze changes in blood pressure, however very few trials reported this outcome (n = 10, 6 trials with canagliflozin and only 1 with dapagliflozin), precluding the analysis.

Risk of bias in individual studies and the quality of meta-analysis

The quality of the studies was assessed according to the Cochrane Collaboration tool for risk of bias. (12; 13) The quality of each meta-analysis was evaluated by the GRADE approach (Grading of Recommendations Assessment, Development and Evaluation),(13) considering factors that may decrease or increase the quality of evidence. As recommended, each meta-analysis was rated as high, moderate, low or very low.(14)

Synthesis of results

First, we analyzed different SGLT2 inhibitors′ doses versus placebo. The outcomes of interest were absolute changes in HbA1c, body weight and adverse events. Continuous variables were expressed as standard mean differences and 95% confidence interval (CI). Discrete events (urinary tract infections, genital infections, hypoglycemia) were expressed as relative risk (RR) and 95% CI. All analyses were performed using the random effects model. Direct meta-analyses were used to compare individual SGLT2 inhibitor doses with placebo. The Cochran Q test was

used to evaluate heterogeneity among studies, and a threshold *p*-value of 0.1 was considered statistically significant; the I^2 test was also conducted to evaluate the magnitude of the heterogeneity among studies. If heterogeneity in the meta-analysis was high (I^2 >75%), we planned to use metarregression to assess the variables involved in this heterogeneity. A separate indirect meta-analysis was conducted for both change in HbA1c and body weight to compare the different doses with placebo, as well as among each other, by a frequentist approach and random model.

We assessed the possibility of small-study bias by a funnel plot of each trial's effect size against the standard error. Funnel plot asymmetry was also evaluated by Begg's and Egger's tests, and a bias was considered present if the *p*-value was <0.1 The trim-and-fill computation was used to estimate whether the if unpublished would influence the interpretation of results.(14; 15)

The analyses were made using Stata version 12.0 (Stata Inc., College Station, Texas, USA). Indirect meta-analyses were performed with R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria). The packages used to achieve this aim were "meta", "metafor" and "netmeta". Risk of bias was analyzed with RevMan software version 5.3 (Cochrane Collaboration, Copenhagen, Denmark).

RESULTS

Literature search

Our search retrieved 1,539 articles. After removal of duplicated papers and scanning titles and abstracts, 99 articles remained for whole-text evaluation. Subsequently, 18 RCTs were included for analysis (**Figure 1**).

Study characteristics and risk of bias

The included trials were published from 2009 to 2018. These analyses included 16,095 patients, of whom 10,043 were men (62.39%). Detailed studies' characteristics are shown in table 1.

Details regarding the assessment of quality for individual studies and across studies are presented in the additional material (**Figure 1S - Supplemental Material**). Random sequence generation, allocation concealment and blinding of outcome assessment were clear in most studies; blinding of participants and personnel, incomplete outcome data and selective reporting were considered as having a low chance of bias in most studies.

Main outcomes

The overall reduction in HbA1c was 0.62% (95% CI -0.66% to -0.59%; I^2 92%) when all medications and dosages were analyzed together. In direct meta-analysis, canagliflozin 300 mg produced the greatest numerical reduction in HbA1c (-0.79%; 95% CI: -0.84% to -0.75%; I^2 97%) whilst dapagliflozin 2.5 mg resulted in the smallest one (-0.35%; 95% CI -0.45% to -0.26%; I^2 0%). Regarding body weight, canagliflozin 300 mg also had the greatest reduction in body weight (-2.36 kg; 95% CI -2.74 kg to -1.98 kg; I^2 76%) and dapagliflozin 2.5 mg had the smallest benefit (-1.31 kg; 95% CI -1.78 kg to -0.84 kg; I^2 71.6%).

The results of indirect and network meta-analysis are similar to the ones found in direct meta-analysis: in terms of HbA1c reduction, canagliflozin 300 mg was superior to all other SGLT2 in different doses, dapagliflozin 10 mg was similar to empagliflozin 10 mg, but inferior to empagliflozin 25 mg, and both doses of empagliflozin (10 mg and 25 mg) were similar to canagliflozin 100 mg. The rank analysis showed that canagliflozin 300 mg had 95% chance of being the best in reducing HbA1c, followed by empagliflozin 25 mg (73% chance) and

canagliflozin 100 mg (61% chance). None of this analysis showed inconsistency among results of direct, indirect and network meta-analysis.

Regarding body weight reduction, canagliflozin 300 mg also had the greatest benefit, however it was not different from canagliflozin 100 mg, empagliflozin 25 mg, dapagliflozin 10 mg and dapagliflozin 5 mg. The rank analysis showed that canagliflozin 300 mg had a 96% chance of being the best option for body weight reduction, empagliflozin 25 mg had 76% chance and canagliflozin 100 mg had 60% chance. The only analysis that showed significant inconsistency was the comparison between canagliflozin 100 mg and 300 mg. The results regarding both direct (against placebo) and indirect meta-analyses are shown in **table 2**.

Some of the included trials did not discriminate incidence in adverse events concerning different doses of SGLT2 inhibitors, therefore this analysis was made using the trials in which it was discerned by dosage. None of the SGLT2 inhibitors, in all of the studied doses, increased risk for urinary tract infection (number of trials = 8; n=4770) and for bone fractures (number of trials = 8; n=4416). Only dapagliflozin 2.5 mg increased the risk for hypoglycemia (number of trials = 5;n=3690), this result is probably due to one study that used this dose of dapagliflozin and patients were on high doses of insulin(16) . All SGLT2 inhibitors in different doses were associated with increased risk for genital mycotic infection (number of trials = 10; n=5865) (**Figure 2S – Supplemental material**).

Most of trials did not analyze ketoacidosis nor Fournier's gangrene, so the meta-analysis for both the outcomes could not be performed.

As heterogeneity among trials was high, we performed metarregression using medication's doses as a covariate and it did not explain the heterogeneity found.

Meta-analysis quality evaluation

The GRADE quality of evidence was considered high but was downgraded one point due to indirectness. No publication bias was identified in the meta-analysis (p=0.441).

DISCUSSION

The present study shows that SGLT2 inhibitors have similar effects on HbA1c and body weight regardless the agent used and the employed dosages. Some minor differences were found in indirect analysis for canagliflozin 300 mg, however the clinical significance of this difference (0.2% in HbA1c and less than 500 g in body weight) is questionable. Regarding adverse events, all SGLT2 inhibitors in different doses were associated with genital mycotic infections, but not with bone fractures nor urinary tract infection.

Other meta-analyses had similar findings to ours regarding effects of SGLT2i in HbA1c (17; 18). Both included trials that lasted more than 24 weeks and one analyzed also the efficacy of SGLT2 inhibitors against other agents. (17) However, these previous studies also did not explore the effects of different doses of SGLT2 inhibitors nor compared their effectiveness among each other. Our results are in accordance with a large trial with a SGLT2 inhibitor, the EMPAREG trial, (8) where the two tested doses of empagliflozin had the same effect on cardiac outcomes, body weight and HbA1c. The other published cardiovascular trial with SGLT-2 inhibitors, the CANVAS trial, did not show the results for canagliflozin 100 mg and 300 mg separately, so their results were not included in this analysis. (19)

The finding of greater reduction in HbA1c and body weight with canagliflozin 300 mg should be interpreted carefully. This reduction is expected since canagliflozin is the least selective among these three SGLT2 inhibitors, leading also to SGLT1 inhibition in the distal part of the

convoluted proximal tubule (S3 segment) and intestine,(20; 21) and this particular characteristic may increase the amount of glucosuria and/or decrease intestinal absorption of glucose. However, it is important to highlight that the greater benefits found with canagliflozin 300 mg, even though statistically significant in relation to other medications/ doses, may not be clinically relevant, as it represent a reduction of approximately 0.2% in HbA1c and less than 500 g in body weight. So, the differences reported herein should not be a factor taken in consideration when choosing a particular SGLT2 inhibitor. The findings of CANVAS trial should also be taken into account, while the greater incidence of amputations in patients randomized to canagliflozin remains not explained. (19) There was a small increase in risk of hypoglycemias with dapagliflozin 2.5 mg that might be related to a study included, which randomized patients on high doses of insulin to SGLT2 inhibitors.(16)

We must stress some strengths of our results: we performed thorough search of databases, the findings were consistent across the outcomes and the quality of primary studies was high. As heterogeneity across included trials was high, we also performed metarregression using studied doses of canagliflozin, dapagliflozin and empagliflozin as covariates and they did not explain the heterogeneity found, reassuring the results. Our results have practical and economic implications. It is not worthwhile to increase SGLT2 inhibitors to higher dosages in the intent to further decrease HbA1c or body weight. Even more, in the light of our and others results (8) we believe that these medications should be produced in only one dosage formulation.

It is important to highlight the inconsistency found in the indirect comparison between canagliflozin 100 mg and 300 mg regarding body weight. The source of this inconsistency is not clear and it is a potential limitation of this particular analysis. Also, some additional information was lacking in the majority of the studies and we were no able to explore some interesting

additional topics, such as BP reduction, mortality, and cardiovascular events. In addition, a potential limitation in the adverse events analysis is that out of the 18 included trials, only few of them evaluated adverse events by dose of medication, therefore this analysis may lack power.

In conclusion, the current review shows that the smallest commercially available doses of SGLT2 inhibitors have similar clinically effects on HbA1c and body weight as the higher ones. More evidence is needed to find out if the effects of different doses on BP, major cardiovascular events and death. Whether these glycemic and weight effects reflect on mortality and cardiovascular events is still uncertain and may be topic for further studies.

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References

1. Komoroski B, Vachharajani N, Feng Y, Li L, Kornhauser D, Pfister M: Dapagliflozin, a novel, selective SGLT2 inhibitor, improved glycemic control over 2 weeks in patients with type 2 diabetes mellitus. Clin Pharmacol Ther 2009;85:513-519
2. List JF, Woo V, Morales E, Tang W, Fiedorek FT: Sodium-glucose cotransport inhibition with dapagliflozin in type 2 diabetes. Diabetes Care 2009;32:650-657
3. Lavalle-González FJ, Januszewicz A, Davidson J, Tong C, Qiu R, Canovatchel W, Meininger G: Efficacy and safety of canagliflozin compared with placebo and sitagliptin in patients with type 2 diabetes on background metformin monotherapy: a randomised trial. Diabetologia 2013;56:2582-2592
4. Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF: Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomised, double-blind, placebo-controlled, phase 3 trial. Diabetes Care 2010;33:2217-2224
5. Rosenstock J, Seman LJ, Jelaska A, Hantel S, Pinnetti S, Hach T, Woerle HJ: Efficacy and safety of empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, as add-on to metformin in type 2 diabetes with mild hyperglycaemia. Diabetes Obes Metab 2013;15:1154-1160
6. Qaseem A, Barry MJ, Humphrey LL, Forciea MA, Physicians CGCotACo: Oral Pharmacologic Treatment of Type 2 Diabetes Mellitus: A Clinical Practice Guideline Update From the American College of Physicians. Ann Intern Med 2017;166:279-290

7. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE, Investigators E-RO: Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-2128
8. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE: Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117-2128
9. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Bmj. 2009;339:b2535. Epub 2009/07/23. doi: 10.1136/bmj.b2535. PubMed PMID: 19622551; PubMed Central PMCID: PMCPmc2714657.
10. Robinson KA, Dickersin K. Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. Int J Epidemiol. 2002;31(1):150-3. PubMed PMID: 11914311.
11. American Diabetes Association: Standards of Medical Care in Diabetes-2017 Abridged for Primary Care Providers. Clin Diabetes 2017;35:5-26
12. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA: The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. Bmj 2011;343:d5928
13. Guyatt GH, Oxman AD, Kunz R, Jaeschke R, Helfand M, Liberati A, Vist GE, Schünemann HJ, Group GW: Incorporating considerations of resources use into grading recommendations. BMJ 2008;336:1170-1173
14. Higgins JP, Thompson SG, Deeks JJ, Altman DG: Measuring inconsistency in meta-analyses. BMJ 2003;327:557-560
15. Duval S, Tweedie R: Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000;56:455-463
16. Wilding JP, Woo V, Soler NG, Pahor A, Sugg J, Rohwedder K, Parikh S, Group DS: Long-term efficacy of dapagliflozin in patients with type 2 diabetes mellitus receiving high doses of insulin: a randomised trial. Ann Intern Med 2012;156:405-415
17. Zaccardi F, Webb DR, Htike ZZ, Youssef D, Khunti K, Davies MJ: Efficacy and safety of sodium-glucose co-transporter-2 inhibitors in type 2 diabetes mellitus: systematic review and network meta-analysis. Diabetes Obes Metab 2016;18:783-794
18. Shyangdan DS, Uthman OA, Waugh N. SGLT-2 receptor inhibitors for treating patients with type 2 diabetes mellitus: a systematic review and network meta-analysis. BMJ Open. 2016;6(2):e009417.
19. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med. 2017;377(7):644-57.
20. Ferrannini G, Hach T, Crowe S, Sanghvi A, Hall KD, Ferrannini E: Energy Balance After Sodium-Glucose Cotransporter 2 Inhibition. Diabetes Care 2015;38:1730-1735
21. Grempler R, Thomas L, Eckhardt M, Himmelsbach F, Sauer A, Sharp DE, Bakker RA, Mark M, Klein T, Eickelmann P: Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab 2012;14:83-90

Table 1. Characteristics of included trials

Author Year	SGLT2i	*n*	Follow-up (wk)	Men (%)	Mean age (y)	Mean Diabetes duration (y)	Mean HbA1c (%)	Mean weight (kg)	Background treatment
Bailey 2013	Dapa	546	24	53.48	59.9	NR	8.05	85.91	Metformin
Wilding 2012	Dapa	808	48	47.28	59.3	13.6	8.53	93.82	Insulin and/or OAD
Wilding 2009	Dapa	71	12	59.15	56.7	12.3	8.43	102.10	Insulin
Rosenstock 2012	Dapa	420	48	49.52	53.4	NR	8.37	86.30	Pioglitazone
Bode 2013	Cana	714	102	55.46	52.9	11.7	7.70	89.50	Naïve or OAD
Wilding 2013	Cana	343	12	51.02	57.4	5.9	7.76	89.76	Metformin
Zinman 2015	Empa	7020	192	71.45	63.1	NR	8.07	86.3	Any
Yang 2018	Dapa	275	24	47.8	57.5	12.5	8.55	71.8	Insulin ± OAD
Januzzi 2017	Cana	714	26	55.4	63.6	NR	NR	NR	OAD
Haering 2016	Empa	666	76	50.9	57.1	NR	8.1	76.9	Metformin + Sulphonylurea

Jabbour 2014	Dapa	447	24	54.80	54.8	5.67	7.95	90.1	Sitagliptin ± Metformin
Matthaei 2015	Dapa	216	24	49.07	61	9.4	8.16	89.35	Metformin + Sulphnylurea
Leiter 2014	Dapa	964	24	67.01	63.7	NR	8.0	93.8	OAD
Stenlof 2013	Cana	584	26	44.18	55.4	4.3	8.00	86.8	Diet + Exercise
Roden 2013	Empa	899	24	61	55	NR	7.88	78.4	No treatment for at least 12 weeks
Tikkanen 2015	Empa	823	12	60.1	60.2	NR	7.90	NR	Diet + Exercise
Yale 2013	Cana	269	52	60.59	68.5	16.3	8.00	NR	Insulin
Fulcher 2016	Cana	316	18	65.50	63.0	12.6	8.1	NR	DPP-4 inhibitor

Abbreviation: wk = week; y = years; OAD = oral antidiabetic drugs.

Dapa 2.5	0.10 [-0.04; 0.26]	0.25 [0.10; 0.40]	-0.35 [-0.49; -0.21]	0.23 [0.05; 0.42]	0.29 [0.11; 0.47]	0.30 [0.13; 0.48]	0.48 [0.30; 0.66]
0.56 [-0.04; 1.17]	**Dapa 5**	0.14 [0.01; 0.27]	-0.46 [-0.59; -0.34]	0.12 [-0.04; 0.30]	0.18 [0.01; 0.35]	0.19 [0.03; 0.36]	0.37 [0.21; 0.54]
0.58 [0.02; 1.14]	0.02 [-0.47; 0.51]	**Dapa 10**	-0.61 [-0.70; -0.52]	-0.01 [-0.16; 0.13]	0.04 [-0.10; 0.18]	0.05 [-0.08; 0.19]	0.22 [0.08; 0.36]
-1.30 [-1.83; -0.77]	-1.86 [-2.34; -1.39]	-1.89 [-2.21; -1.57]	**Placebo**	0.59 [0.47; 0.71]	0.65 [0.53; 0.77]	0.66 [0.56; 0.77]	0.84 [0.73; 0.95]
0.47 [-0.19; 1.13]	-0.08 [-0.70; 0.53]	-0.11 [-0.62; 0.39]	1.77 [1.38; 2.17]	**Empa 10**	-0.05 [-0.06; 0.17]	0.06 [-0.08; 0.22]	0.24 [0.08; 0.40]
0.74 [0.08; 1.41]	0.18 [-0.43; 0.80]	0.16 [-0.34; 0.67]	2.05 [1.65; 2.45]	0.27 [-0.06; 0.61]	**Empa 25**	-0.01 [-0.14; 0.17]	0.18 [0.03; 0.34]
0.63 [-0.02; 1.29]	0.07 [-0.54; 0.68]	0.04 [-0.45; 0.55]	1.94 [1.55; 2.32]	0.16 [-0.39; 0.71]	-0.11 [-0.66; 0.44]	**Cana 100**	0.17 [0.06; 0.28]
1.06 [0.38; 1.74]	0.50 [-0.12; 1.14]	0.48 [-0.04; 1.01]	2.37 [1.95; 2.79]	0.59 [0.01; 1.17]	0.32 [-0.25; 0.90]	0.43 [-0.00; 0.87]	**Cana 300**

Table 2. Network meta-analysis for each SGLT2 inhibitor dose regarding effects on HbA1c (grey rectangles) and body weight (white rectangles). Values which are underlined mean $p<0.05$.

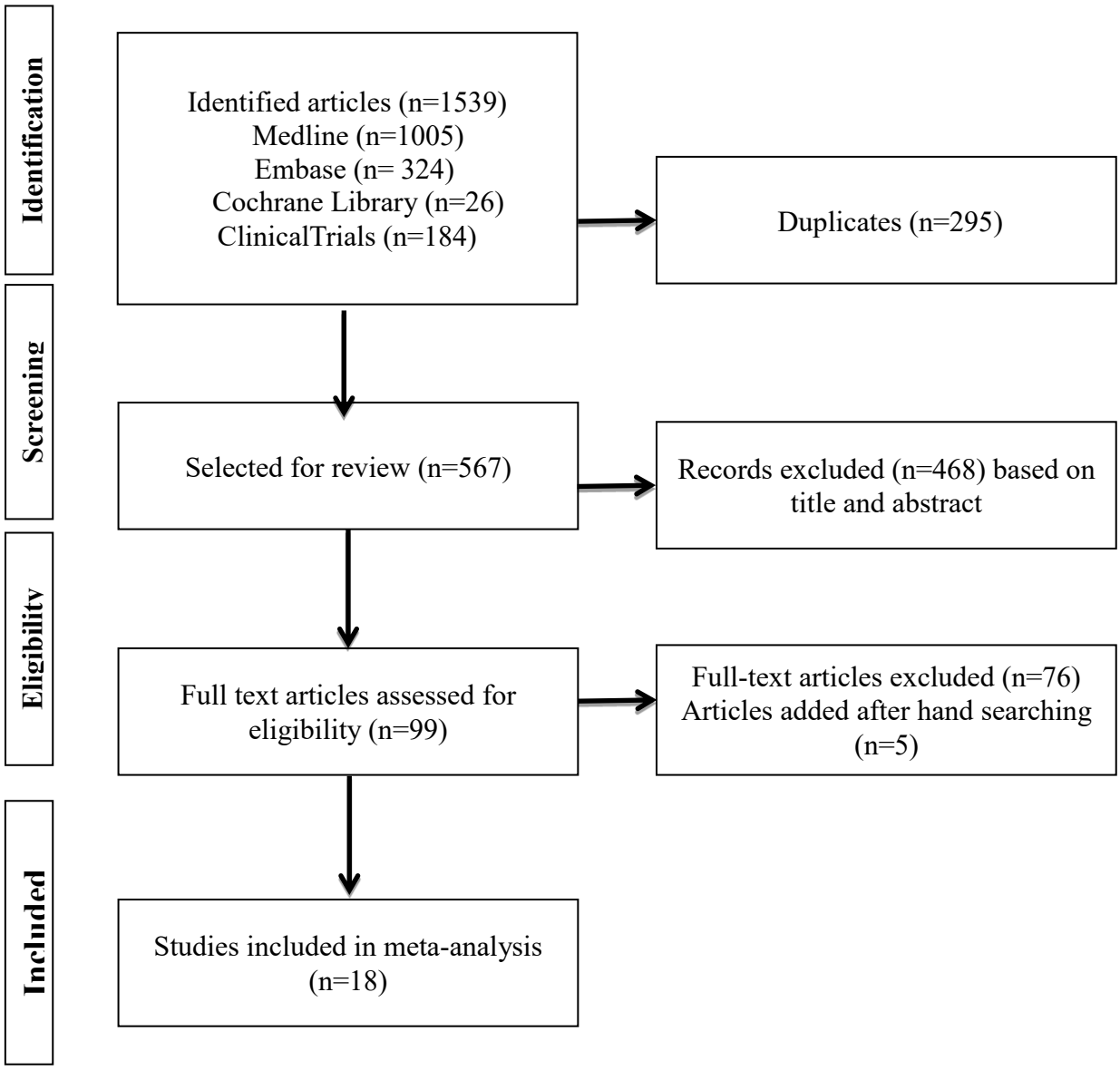


Figure 1. Study flowchart

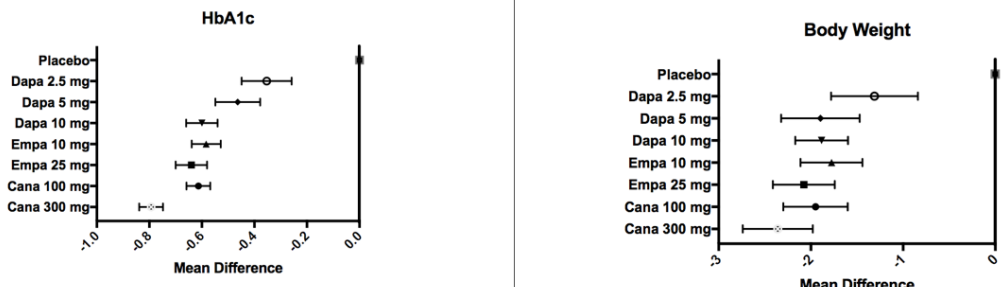


Figure 2. Mean difference in HbA1c and Body Weight according to SLGT2 inhibitor and dose.

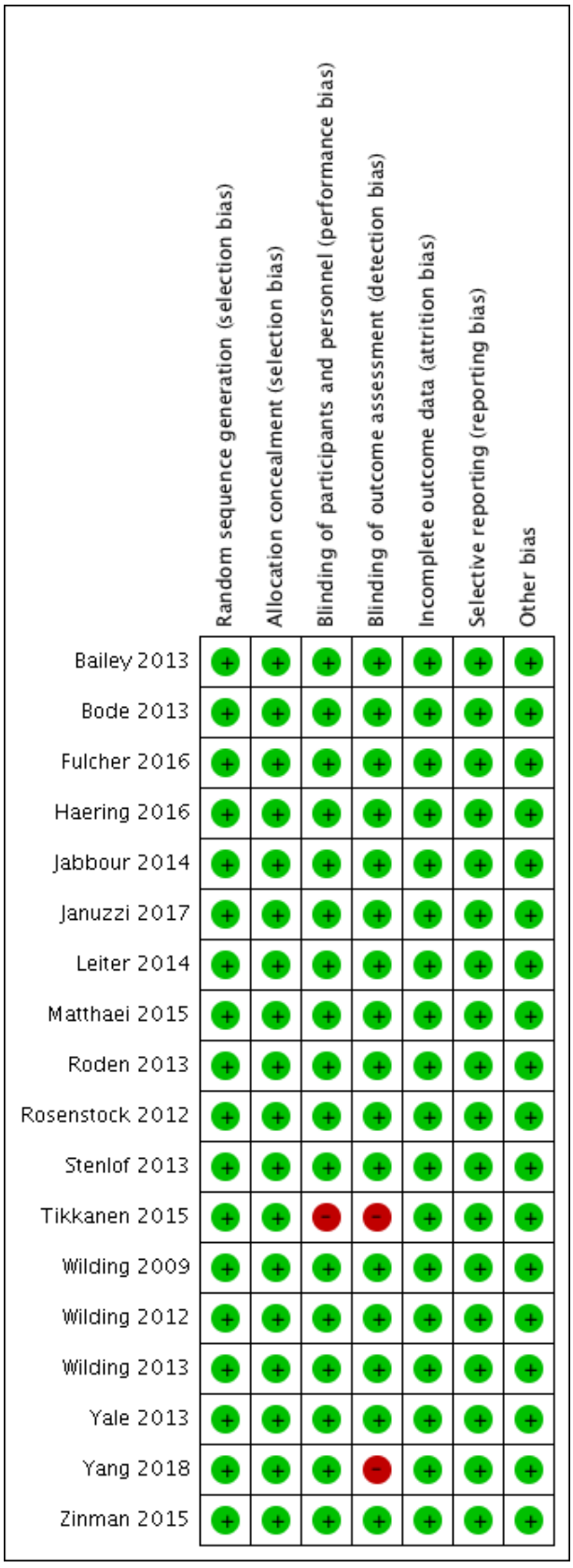
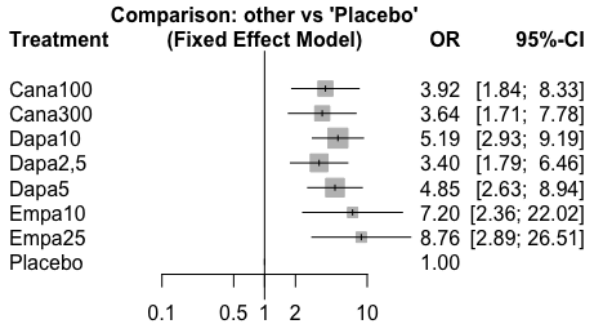


Figure 1S. Risk of bias

A



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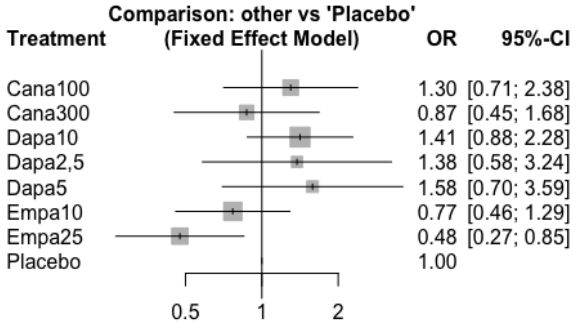

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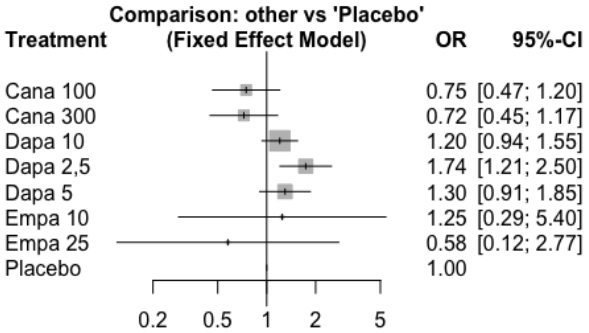

D

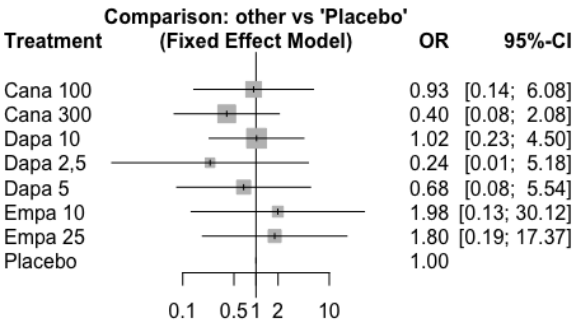

Figure 2S – Adverse events related to SGLT2 inhibitors use. A. Genital mycotic infection; B. Urinary tract infection; C. Hypoglycemia; D. Bone fracture.

FINAL CONSIDERATIONS

This thesis reinforce the safety of the newest antihyperglycemic agents. The first study suggested an increased risk of pancreatitis with DPP-4 inhibitors use, however of small magnitude based on a large NNH. Regarding pancreatic cancer, the first two studies were able to exclude an association of DPP-4 inhibitors and GLP-1 analogues with the outcome, for at least a NNH of 1000 patients. For larges NNHs and guarantee of long-term safety, further studies are required.

The third study assured the safety of SGLT-2 inhibitors, as the only adverse event observed was genital mycotic infection. Notably, we showed that SGLT-2 inhibitors do not have a clinically significant dose range effect on HbA1c or body weight and these two variables should not be used as a guidance for increments in medications dosages of these particular agents.

Finally, besides the safety outcomes demonstrated here, benefits on cardiovascular events and mortality, such as those demonstrated on cardiovascular outcomes trials should be considered when selecting anti-hyperglycemic medications.