

Glucagon like peptide-1 receptor agonists as neuroprotective agents for ischemic stroke: a systematic scoping review

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Abstract:

Stroke mortality and morbidity is expected to rise. Despite considerable recent advances in the treatment of acute ischemic stroke, scope remains for development of widely applicable neuroprotective agents.

Glucagon like peptide-1 receptor agonists (GLP-1RAs), originally licensed for the management of Type 2 Diabetes Mellitus, have demonstrated pre-clinical neuroprotective efficacy in a range of neurodegenerative conditions.

This systematic scoping review aims to report the pre-clinical basis of GLP-1RAs as neuroprotective agents in acute ischemic stroke and their translation into clinical trials.

We included 35 pre-clinical studies, 11 retrospective database studies, 7 cardiovascular outcome trials and 4 prospective clinical studies.

Pre-clinical neuroprotective efficacy was demonstrated in normoglycemic models when administration was delayed by up to 24-hours following stroke induction. Outcomes included infarct volume reduction, reduced apoptosis, oxidative stress and inflammation alongside increased neurogenesis, angiogenesis and cerebral blood flow. Improvement in neurological function and a trend towards increased survival were also reported.

Cardiovascular outcomes trials reported a significant reduction in stroke incidence with long-term semaglutide and dulaglutide. Retrospective database studies show a trend towards neuroprotection. Prospective interventional clinical trials are on-going, but initial indicators of safety and tolerability are favourable.

Ultimately, we propose that repurposing GLP-1RAs is potentially both clinically and economically advantageous.

Five key words: Acute Stroke, Neuroprotection, Reperfusion, Clinical Trials, Animal Models

Introduction:

Stroke accounts for 6.5 million deaths per year globally and by 2030 will result in the annual loss of over 200 million disability adjusted life years.(1,2) With an increasing number of strokes occurring in younger patients, alongside an increased number of stroke survivors, the cost of post-stroke care is increasing. There is, therefore, significant scope to improve upon the current position.

Considerable advances have been made in acute ischemic stroke (AIS) treatment, notably reperfusion therapies, but these are limited to 10-20% of total stroke patients following careful clinical and radiological selection.(3) Even when intravenous thrombolysis and/or endovascular thrombectomy are administered, reduction in disability is highly time dependent.(4,5) Scope remains for further improvement, especially for patients who are unsuitable for reperfusion therapies or those within remote environments.

Using simpler clinical selection processes, neuroprotective therapies could bring benefits to a wider patient group. Neuroprotectants could also enhance the benefits of reperfusion therapies by preservation of the ischemic penumbra and reduction in reperfusion injury. Despite pre-clinical potential, a suitable agent has not yet been identified by translational studies.(6) There remains a multitude of factors affecting the translation from bench-to-bedside. Namely, animal models are not perfect in their representation of the heterogeneity of clinical stroke.(7) Stroke in humans occurs in the context of ageing, co-morbidity (hypertension, diabetes mellitus, atrial fibrillation, pre-existing cerebrovascular disease) and concomitant medication use.(8) Furthermore, factors such as gender, cerebral blood flow, body temperature and glycaemic status may influence stroke mechanism and outcomes associated with therapy.(6,9–11)

Glucagon Like Peptide-1 (GLP-1) are gaining increasing momentum as a possible neuroprotective agent in AIS. GLP-1 is an incretin hormone. Alongside its role in insulin secretion from the pancreas and glucagon suppression, it also crosses the blood-brain barrier (BBB) and promotes synaptic function, enhances neurogenesis, reduces apoptosis and protects neurons from oxidative stress.(12) GLP-1 is produced in the brain and receptors are distributed throughout the central nervous system.(13) GLP-1 Receptor Agonists (GLP-1RAs), licensed for Type 2 Diabetes Mellitus (T2DM) have already demonstrated pre-clinical neuroprotective efficacy in Alzheimer's Disease and clinical trials in neurodegenerative conditions are ongoing.(12,14)

The aim of this systematic scoping review is to report the pre-clinical basis of GLP-1RAs as neuroprotective agents in AIS and their translation into clinical trials. As well as describing the characteristics and quality of studies, objectives are to specifically consider timing of administration, association with glycemic status, neuroprotective outcomes and application to clinical care.

Materials and Methods:

Eligibility criteria:

Pre-clinical: We included pre-clinical *in vivo* studies which administered naturally occurring GLP-1, a mimetic or analogue, before, during or after stroke induction. Normoglycemic, hyperglycemic and induced T2DM models were included.

Studies were excluded if their only focus was hemorrhagic stroke as this does not reflect the proposed mechanism for how GLP-1 is involved in ischemic tissue injury. Those studies which reported incidence of hemorrhagic transformation as a complication of AIS were included as these reflect post-stroke complications.

Clinical: We included all prospective clinical trials which administered GLP-1RAs before, during or after stroke onset with outcome measures defined to identify neuroprotective efficacy by way of stroke volume reduction or improvement in post-stroke function or mortality. We also included any potential feasibility or safety-based studies in this area.

Our scoping searches identified that only a small quantity of prospective clinical trials measuring stroke outcomes were available. Pragmatically, we therefore also included all retrospective database analyses of stroke incidence or composite cardiovascular outcomes in patients treated with GLP-1RAs. Furthermore, we included cardiovascular outcome trials (CVOTs) of GLP-1RAs to evaluate the incidence of stroke in this relatively higher risk cohort.

Studies were excluded if their full-text was not available or not published in English. Efforts were made to contact authors directly to obtain any missing articles or data.

Database search strategy:

After several initial scoping searches(15), we accessed Web of Science on 19/3/2020 to search MEDLINE, Web of Science core collection, BIOSIS and SciELO from 1/1/2000. Keywords were EITHER ‘GLP(-)1, glucagon like peptide(-)1, exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, semaglutide’ AND EITHER ‘stroke, CVA, cerebrovascular, h(a)emorrhage, small vessel disease’. Articles were cross-referenced and references were searched to identify further studies of interest.

All articles/studies were screened independently in an unblinded, standardised manner by MM and HE by way of title and abstract to identify those suitable for full-text review. Queries and disagreements were resolved by discussion. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were applied. Pre-clinical studies were appraised according to Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines,(16) and the updated Stroke Therapy Academic Industry Roundtable Preclinical Recommendations (STAIR) guidelines.(7) Data supporting the findings of this review are available from the corresponding author upon reasonable request.

Results:

Study selection:

The literature search identified 797 results (see Fig.1) alongside 10 from other sources. After removal of duplicates this left 794 for screening. We excluded 593 articles based upon title and review of abstract leaving 201 full text articles to review. In total, 35 preclinical studies, 11 retrospective database studies, 7 cardiovascular outcome trials and 4 prospective clinical studies met the inclusion criteria.

Pre-clinical studies:

Characteristics of included studies:

As shown in Table 1, 35 pre-clinical studies were included within this review. Studies were completed between 2009 and 2020. Studies were predominantly based upon mouse and rat models of stroke, however, 1 study utilised a gerbil model.(17) Stroke induction was either via transient (range 30 – 120 mins) or permanent common carotid (CCAO) or middle cerebral artery occlusion (MCAO). Most studies induced unilateral occlusion in keeping with spontaneously occurring stroke onset in humans, but 6 studies utilised a bilateral

occlusion model. Cerebral ischemia was induced by either ligation, filament occlusion or ablation of the relevant artery.

Twelve studies administered exendin-4,(17–28) 9 used liraglutide,(29–37) 3 used rhGLP-1 (recombinant human GLP-1),(38–40) 3 used lixisenatide (41–43) and 1 study each reported the utility of semaglutide,(43) PEx-4 (exendin-4 loaded poly-microspheres),(44) proGLP-1 (long acting GLP-1RA),(45) DMB (GLP-1R agonist/modulator),(46) dual GLP-1/Glucose-dependent Insulinotropic Peptide (GIP) agonist (GLP-1/GIP DA),(47) oxyntomodulin (co-activates GLP-1R and glucagon receptor),(48) P7C3 (aminopropyl carbazole compound) (49) and one study directly compared exendin-4 with liraglutide.(50) In 8 studies, GLP-1R antagonists, such as Ex-9-39, were administered to study the role of the GLP-1R in neuroprotective mechanisms.(19,21–23,41,45,46,49)

Two studies investigated multiple doses of GLP-1RAs to compare neuroprotective efficacy and concluded that neuroprotection was dose-dependent.(20,51)

Most studies administered GLP-1RAs via intraperitoneal, subcutaneous or transvenous routes. However, Zhang *et al* reported neuroprotection with both oral DMB,(46) and with intranasal exendin-4.(22)

Some 14 studies administered GLP-1RAs chronically prior to the onset of stroke. Clinically, this would represent those patients who receive GLP-1RAs as part of routine T2DM management and then go on to experience AIS.(18,19,22,23,29,31,38,39,42,44–46,48,51) Chronic pre-treatment occurred for between 14 days prior and 15 minutes prior to stroke onset, from 1 to 3 times daily.

A further 4 studies administered GLP-1RAs prior to and following stroke onset for between 0 and 4 weeks – with dosing schedules of up to twice daily.(17,20,21,30)

Most closely aligned with the proposed clinical application of administering treatment to GLP-1RA naïve patients in the hyper-acute AIS setting, 17 studies only administered the medication following stroke onset.(24–28,32–37,40,41,43,47,49,50) First dose was delayed between 0 minutes and 24 hours post-onset and continued for up to 4 weeks.

Quality of included studies:

Pre-clinical study methodology was appraised according to the STAIR 2009 criteria, with a maximum available score of 7. Median score was 3 (range 2 – 7). Inclusion/exclusion criteria and reporting of potential conflicts of interest/funding was consistently reported in 100% and 94% of studies respectively. Fewer studies commented on randomisation (54%), allocation concealment (17%) and blinded assessment of outcome (43%). Only 4 studies (11%) reported performing a sample size calculation.

Reporting of pre-clinical studies was also assessed using the ARRIVE guidelines, with a maximum score of 36. Median score was 22 (range 14 – 29). Methods, statistical analysis, outcomes and confidence intervals were well reported, as were ethical and funding statements, but the justification for animal models, translation to human biology, limitations and adverse events were frequently not reported.

Impact of hyperglycemia in stroke models:

Twelve studies were based on hyperglycemic rodents, with or without a normoglycemic control group.

Kuroki *et al* demonstrated that hyperglycemia was associated with an increase in infarct volume, cerebral edema and hemorrhagic transformation in a mouse model that underwent transient, unilateral occlusion of the MCAO for 60 minutes.(27) They reported that intraperitoneal administration of Exendin-4 60 minutes after stroke onset was associated with a reduction in these parameters which was not replicated by insulin monotherapy.

Briyal *et al* reported that liraglutide reduced infarct volume by a similar amount in both diabetic and normoglycemic models - but this neuroprotection was again not reproduced in the insulin treatment arm despite resolution of hyperglycemia.(29) Deng *et al* further confirmed neuroprotection was independent of glycemic status prior to stroke onset.(30) Jiang *et al* reported stroke infarct volume in the rhGLP-1 group was significantly reduced when compared to insulin treatment.(38) Metformin was also shown to ameliorate hyperglycemia but did not confer the additional neuroprotective outcomes associated with liraglutide.(31)

One study demonstrated similar neuroprotective outcomes between 2 month old, healthy mice and 14 month old, overweight, diabetic mice treated with Exendin-4.(25)

Whilst GLP-1RAs were associated with a reduction in blood glucose in hyperglycemic models,(40) no study reported hypoglycemia when GLP-1RAs were administered to normoglycemic models. This is to be expected as GLP-1RA associated insulin secretion from the pancreas is glucose dependent.(35) Indeed, Zhang *et al* concluded that neuroprotection was not glucose dependent.(45)

Yang *et al* monitored blood glucose in rats which underwent intraperitoneal administration of semaglutide starting 2 hours following unilateral permanent MCAO occlusion, further demonstrating neuroprotection in the absence of hypoglycemic episodes.(43)

Infarct volume and neuronal survival:

Administration of GLP-1RAs prior to, at the point of, or delayed following stroke onset were associated with reduction in infarct volume. In total, 9 studies demonstrated a reduction in infarct volume with Exendin-4,(18–24,26,27) 8 with liraglutide,(29–33,35–37) 3 with rhGLP-1, 3 with Lixisenatide and 1 with each of PEx-4, proGLP-1, DMB, OXM, GLP-1/GIP DA and semaglutide. Liraglutide was associated with a reduction in infarct volume when the first dose was delayed by up to 1 day.(35) Kim *et al* reported a reduction in infarct volume by up to 75% in a rat model of transient MCAO. Darsalia *et al* reported that Exendin-4 administration was associated with a reduction in stroke volume when administered for 4 weeks prior to, and 2-4 weeks following stroke onset.(20) However in a later study, whereby they only administered Ex-4 following stroke onset, it did not significantly reduce infarct volume but did reduce overall neuronal loss.(25) Reduction in neuronal loss was also reported with semaglutide,(43) rhGLP-1(40) and Lixisenatide.(51)

Cellular function:

Apoptosis represents a chain of enzymatic events resulting in programmed cell death.(52) Whilst controlled apoptosis is essential for the maintenance of homeostasis, dysregulated apoptosis, for example in within the ischemic penumbra, can result in increased cell death.(53) Bcl-2 is an anti-apoptotic protein which functions at least in part by reducing cytochrome C release from the mitochondria. Conversely, Bax is a pro-apoptotic protein and increases cytochrome C levels.(52) Increased Bcl-2 and reduced Bax levels, contributing to an

increased Bcl-2:Bax ratio and representing reduced levels of apoptosis, have been reported in 3 studies of liraglutide(29,33,37) and 1 study for each pro-GLP-1,(45) rhGLP-1,(39) DMB,(22) Ex-4,(50) Lixisenatide,(51) GLP-1/GIP DA(47) and semaglutide.(43) Critically, this reduction of apoptosis was reproduced in normoglycemic models when administered after stroke onset.(33,37,43,47)

Caspase proteins are also pro-apoptotic and increased levels are associated with higher rates of cell death.(54) Liraglutide, Exendin-4, Lixisenatide, rhGLP-1 and semaglutide have been shown to reduce levels of caspase-3 in pre-clinical models of stroke.(33,37,39,41–43,46,50,51) Zhu *et al* have also demonstrated reduced levels of caspase 8 and 9 in models administered liraglutide.(33)

Cleavage of PARP by caspases is a signal of apoptosis and has been implicated in cerebral ischemia.(53) Reduction in PARP has been demonstrated with both liraglutide and exendin-4 treatment.(33,50)

TUNEL assays detect DNA degradation during the later stages of apoptosis and have demonstrated reduced apoptotic activity with liraglutide and exendin-4.(37,50,55)

GLP-1RA administration following stroke induction has been associated with an anti-inflammatory effect.(26) Tumor necrosis factor alpha (TNF- α) is a cytokine synthesised by many cell lines – but particularly by macrophages and microglia.(56) TNF- α is involved with the inflammatory response following stroke onset.(56) Five studies reported a reduction in TNF- α when compared to controls, 3 with lixisenatide(41,42,51) and 1 with each of liraglutide(37) and exendin-4.(27) Indeed, two studies reported reduced microglial activation(17,24) whilst a further study by Darsalia *et al* reported reduced microglial infiltration – but a marginal effect on activation.(20) Reduced levels of other markers of inflammation, such as myeloperoxidase and interleukin, have also been reported.(30,37,51)

One study reported a non-statistically significant reduction in pro-inflammatory markers.(25)

There is also deterioration in markers of oxidative stress following AIS. Twelve studies reported an improvement oxidative stress parameters following GLP-1RA administration in AIS – 5 studies of exendin-4,(19,24,26,27,50) 4 studies of liraglutide,(29,30,32,36) 2 of lixisenatide(41,42) and 1 of rhGLP.(40)

Predominantly, studies reported reduced levels of malondialdehyde and increased concentrations glutathione and superoxide dismutase.

Dong *et al* performed 18-FDG PET imaging in a rat model of unilateral transient MCAO. In the animals treated with subcutaneous liraglutide there was radiological evidence of increased glucose metabolism within the ischemic penumbra.(34)

Neurovascular function:

The neurovascular unit incorporates both cellular and extracellular components involved in the regulation of cerebral blood flow and blood-brain barrier function – it is involved with the maintenance of cerebral homeostasis and control of cerebral blood flow.(57)

GLP-1RAs have been shown to increase cerebral blood flow following AIS when compared with controls.(44)

Li *et al* reported that cerebral microcirculation is reduced following AIS, but improved to a similar degree within 4-12 hours after MCAO in diabetic mice treated with either liraglutide or exendin-4 after stroke induction.(50) Blood-brain barrier integrity has also been shown to improve with GLP-1RA treatment.(26)

Vascular endothelial growth factor (VEGF) is known to promote angiogenesis and protect ischemic neurons from injury – demonstrating a crucial role in the neurovascular remodelling post-AIS.(58) Chen *et al* demonstrated that intraperitoneal liraglutide therapy, administered 24 hours following stroke induction and once daily for 14 days, was associated with increased VEGF expression when compared with normal saline treatment at days 7 and 14 post-AIS in a normoglycemic model.(35) Similarly, Sato *et al* demonstrated upregulation of VEGF in the cerebral cortex of liraglutide treated, normoglycemic rats, however, this was not seen within the striatum.(32)

Markers of neuronal, glial and endothelial function (NeuN, GFAP and vWF respectively) were used by Dong *et al* to investigate for evidence of neurovascular remodelling following AIS. They reported that liraglutide, administered to normoglycemic mice at 1 and 24 hours following bilateral, transient CCAO, was associated with significantly higher levels of each marker – indicating increased remodelling associated with GLP-1RAs following AIS.(34)

Jiang *et al* reported that levels of brain injury markers (S100B, NSE and MBP) were reduced with both rhGLP-1 and nimodipine treatments.(38)

Wang *et al* investigated for evidence of increased neurogenesis associated with GLP-1R activation. They reported that doublecortin (marker of newborn neuroblasts) and β -tubulin III (marker of neurogenesis and neural progenitor activity) levels were significantly upregulated with P7C3 treatment when compared to controls. They also report increased levels of cell proliferation markers (ki67, BrdU) and neurodevelopmental markers (adam11, adamts20) alongside markers of increased neurogenesis (GSK-3 inhibition).(49)

Darsalia *et al* also counted ki67 expressing cells to evaluate stem cell proliferation and reported a 2 fold greater number of proliferating cells in the exendin-4 treatment group compared with controls at 2 weeks following stroke induction – however, there was no detectable difference at 4 weeks. Doublecortin levels were also increased at 2 weeks in the exendin-4 treatment group, with no difference from controls at 4 weeks. When using Neun and BrdU expressing neurons to identify new, mature neurons generated after stroke onset there was no difference between the treatment and control groups.

Doublecortin levels were also shown to be increased with semaglutide treatment.(43)

Neurological outcomes:

Assessment of neurological outcomes was heterogeneous amongst studies, with many utilising a range of different techniques or modified adaptations. Overall, selected neurological assessments examined both cognitive and locomotive neurological function.

Eight studies reported a reduction in post-stroke neurological deficit when treated with liraglutide,(29,31–36,50) 6 with exendin-4,(17–19,22,23,26) 2 with rhGLP-1,(38,39) 2 with lixisenatide(41,42) and 1 study for each of PEx-4,(44) proGLP-1,(45) DMB,(46) OXM,(50) GLP-1/GIP DA,(47) P7C3(49) and semaglutide.(43)

Nine studies reported a reduction in neurological deficit when GLP-1RAs were administered to normoglycemic models at least 1 hour after induction of AIS.(32–36,41,43,47,49)

Notably, Filchenko *et al* reported that whilst reduction in neurological deficit was observed in the non-diabetic rat model treated with liraglutide, this trend was not observed in the diabetic groups.(31)

Potential mechanisms:

GLP-1RAs stimulate insulin secretion from the pancreas in a glucose-dependent manner. Neuroprotective efficacy has been demonstrated in both diabetic and normoglycemic models; their neuroprotection is therefore not glucose-dependent.(27,31,45)

Whilst it is well documented that GLP-1R levels increase following AIS, the precise mechanism of neuroprotection is not completely understood. To further understand the role of the GLP-1R, studies have utilised targeted GLP-1R shRNA and the GLP-1R antagonist, Ex-9-39. GLP-1R targeted shRNA has been reported to block the neuroprotective efficacy of GLP-1RAs.(22,45,46) However, whilst there are some reports of Ex-3-9 administration blocking neuroprotection,(21) suggestive of GLP-1R dependence, other studies did not demonstrate this trend – highlighting the possibility of a GLP-1R independent mechanism.(41,46) For example, GLP-1 [28-36] is cleaved from endogenous GLP-1; research has shown this cell-permeable nonapeptide to have antioxidant, anti-apoptotic and mitochondrial regulatory properties in pancreatic cells – further research is required to establish its role in neural tissue (59).

The reduction in apoptosis, oxidative stress and inflammation, alongside increased neurogenesis, angiogenesis and cerebral blood flow are likely to contribute to the neuroprotective outcomes. Multiple signalling cascades have been studied, with P-Akt/p-eNOS,(44,50) cAMP/PKA(22,45,46,49) and P13k/Akt(22,26,33,37,45) pathways implicated in neuroprotection.

Clinical studies:

Stroke risk in cardiovascular outcome (safety) Trials:

All 6 licensed GLP-1RAs (Table 2) have reported Cardiovascular Outcome Trials (CVOTs). SUSTAIN-6 (semaglutide) and REWIND (dulaglutide) demonstrated a significant reduction in stroke incidence, HR 0.61 (95% CI 0.38 - 0.99, p=0.04) and HR 0.76 (95% CI 0.61 - 0.94, p=0.017) respectively. LEADER (liraglutide) reported a non-statistically significant reduction in non-fatal stroke incidence, HR 0.89 (95% CI 0.72 - 1.11, p=0.30). This

was not reported in ELIXA (lixisenatide), EXSCEL (exenatide), HARMONY (albiglutide) and notably not in the oral semaglutide study, PIONEER-6.

Post-hoc analysis of CVOT trial data has identified sub-groups which may potentially derive greater cardiovascular protection. Mann *et al* identified enhanced benefits in patients with chronic kidney disease (60), whilst Kang *et al* reported increased benefit in Asian participants.(61)

Meta-analysis by Barkas *et al* (62) demonstrated a 13% reduction in the risk of total stroke associated with GLP-1RA treatment versus placebo (RR 0.87, 95% CI 0.78–0.98, p=0.021).

GLP-1RAs and stroke in real-world datasets

We identified 11 published retrospective studies analysing cardiovascular outcomes of T2DM patients who had been treated with GLP-1RAs (Table 3). These were conducted using data extracted from insurance/national databases.

Recently, O'Brien *et al* (2018) reported a retrospective cohort study of 132,737 insured adults with T2DM commenced on a second line diabetes medication between 2011 and 2015 (63). Adjusting for confounding variables, GLP-1RAs were associated with a reduction in stroke risk (HR 0.65, 95% CI 0.44-0.97) when compared to treatment with Dipeptidyl Peptidase-4 (DPP-4) inhibitors. Reduction in stroke risk was reported in a further 2 studies(64,65) – however both Svantstrom *et al* and Anyanwagu *et al* did not report this trend.(66,67)

Raparelli *et al* reported an increased GLP-1RA associated reduction in cardiovascular risk in women compared to men.(84)

Prospective interventional studies:

Daly *et al* report the first pilot non-randomised interventional study administering exenatide to 11 patients within 12 hours of stroke onset followed by twice-daily injections until discharge (68). Glucose levels were monitored, followed by a 3-month modified Rankin score. Although mild nausea and vomiting were common, they reported no serious adverse events nor associated hypoglycemic episodes. They concluded that exenatide was safe and

tolerable in AIS patients and did not worsen functional or neurological outcome. This study was not sufficiently powered to assess for improvement in stroke outcomes.

Larsson *et al* report on PROLOGUES, a randomised controlled trial comparing pre-hospital administration of exenatide with standard care for hyperglycemia in patients with suspected stroke (69). Nineteen patients were randomised, of an intended 42, with 8 receiving exenatide. The trial was stopped early due to slow inclusion; due to baseline glucose criteria and obtaining informed consent. No associated adverse events were reported.

Results are awaited for two prospective studies. The Short-Term EXenatide in Acute ischemic Stroke (STEXAS) trial (70) is a randomised, open-label, parallel-group pilot study to investigate the efficacy of exenatide in lowering blood glucose levels in patients with AIS and hyperglycemia. They plan to recruit 30 patients with AIS to either insulin or exenatide for up to 72 hours. They will assess feasibility of administration, incidence of hypoglycemia and functional outcomes at 3 months.

The Treatment with EXenatide in Acute Ischemic Stroke (TEXAIS) trial (71) is a 3 year, phase 2, multi-centre, prospective, randomised, open-label, blinded end-point trial comparing exenatide to standard care and aims to be powered to detect a change in neurological outcome. Primary end-point is defined as major neurological improvement at 7 days (≥ 8 point improvement in NIHSS score, or score 0-1). In contrast to STEXAS, this study will include patients independent of their glycaemic status.

Discussion:

As survival following stroke increases, so does the impact of post-stroke disability. Alongside the ongoing development in reperfusion therapies as part of the gold standard of stroke care, a safe, widely applicable, well tolerated and cost-effective neuroprotective agent – aimed at improving post stroke function – becomes increasingly attractive.

We report a systematic review of the pre-clinical research to support the neuroprotective properties of GLP-1RAs targeting the ischemic-reperfusion injury in animal models of AIS. These have been shown to be safe and effective in normoglycemic models and demonstrate neuroprotective outcomes by way of reduced infarct volume, apoptosis, oxidative stress, inflammation and increased neurogenesis, angiogenesis and cerebral blood

flow. Crucially, they have demonstrated an improvement in post-stroke functional outcomes – including within both locomotive and cognitive domains.

In keeping with the real-world potential of GLP-1RAs as a neuroprotectant in the management of AIS, pre-clinical trials have now demonstrated reproducible benefits in normoglycemic models with delayed administration up to 24 hours after stroke induction.

Their neuroprotective mechanism is not fully understood, with further research required. However, emerging trends towards both GLP-1R dependent, and independent, pathways have been reported. Following direct comparison studies with metformin and insulin, their effects are due to more than restoration of glucose homeostasis. Additional research is required to fully understand the role of cleaved GLP-1 subunits, including GLP-1 [28-36] and could go some way to explain the difference in stroke reduction between GLP-1RAs in CVOTs.

The economic benefits of repurposing existing treatments should not be overlooked. Especially as the CVOTs have demonstrated their cardiovascular safety, alongside a reduced incidence of non-fatal stroke. Retrospective database studies show a trend towards a reduction in cardiovascular events – providing an important perspective from ‘real-world’ usage.

Prospective clinical trials are ongoing, with initial outcomes suggestive of safety, tolerability and that GLP-1RAs are feasibly administered in the AIS cohort.

We acknowledge the limitations of this study. Pre-clinical studies were generally conducted on a homogenous population of young animals without representing the co-morbidities often afflicting the human stroke population. However, one study did demonstrate comparable neuroprotective outcomes in both young mice and older, obese, diabetic models.(25)

Further research into the impact of factors such as age, hypertension and gender is required. In addition, studies relied on a uniform etiology of stroke onset, vascular territory and duration of occlusion – constants which are not representative of the diverse, heterogenous clinical manifestation of AIS. However, the inter-study

differences in stroke induction protocols (e.g. unilateral vs. bilateral, duration and vessel occlusion) do provide some mitigation to this. Furthermore, some studies utilised simultaneous bilateral occlusion of the carotid arteries to cause global ischemia which would not normally occur in human stroke; however, this does still provide a valuable model of ischemic injury.

We also acknowledge the effect of publication bias favouring positive outcome and the limitations presented by the results of our STAIR and ARRIVE assessments. The potential for under-powered research is appreciated.

Overall, pre-clinical studies are restricted by small numbers, limited standardisation, disparity between study dose and the licensed clinical dose and having a limited ability to replicate the numerous confounding variables encountered in real-world stroke practice.

Of course, CVOTs report stroke incidence rather than stroke outcome. Other limitations include short trial duration, complex multi-morbid patient characteristics, and the potential for pharmacological interactions with concomitant medications affecting outcomes. GLP-1RAs are known to reduce blood pressure, body weight and cholesterol, all potentially mitigating stroke risk.

Retrospective studies generally included large numbers and are based upon real-world use and adherence. They reported encouraging results; often demonstrating a reduction in cardiovascular risk and/or a modest reduction in stroke risk. Eloquent attempts were often made to adjust for confounding variables; but these do remain a significant limitation, as does incomplete extraction of events from databases (65). These studies are also limited by heterogeneity in treatment duration, dose, and by the grouping together of GLP-1RAs.

There remains an unfulfilled requirement for neuroprotective agents in the management of AIS. Ultimately, many agents have previously failed to translate from bench to bedside.

However, we propose that further research into the repurposing GLP-1RAs – already licensed, with established side effect profiles, cardiovascular safety record and clear indicators from pre-clinical research of their functional benefit - is both potentially clinically and economically advantageous. Further research is required to establish optimal dosing, interaction with reperfusion therapies, timing of administration, and to fully

understand the underlying mechanism of action – with consideration of age, gender and co-morbidity - in order to guide larger clinical trial development.

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Ethical approval: This systematic review does not contain any new studies with human participants or animal performed by any of the authors.

No supplementary material present on JCBFM website.

Figure Legends:

Fig.1: PRISMA flow chart demonstrating the selection of studies

Fig.2: Proposed neuroprotective mechanism of action for GLP-1RAs in AIS. Direct GLP-1R activation by GLP-1RA. 2. Potential indirect receptor activation by GLP-1RA. 3. GLP-1 sub-units are cell permeable and may affect mitochondrial function, or act on extra-cellular receptors. 4. GLP-1RAs reduce excitotoxicity

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TABLE 1: Overview of the pre-clinical studies										
Author	Year	Animal model	Co-morb.	Drug	Admin route	Sub-studies	Stroke induction (time/mins)	Administration time (mins)		Main outcomes (NP – Neuroprotective)
								Pre-ischemia	Post-ischemia	
GLP-1RA administered prior to stroke onset (including chronic pre-treatment)										
Li(18)	2009	Rat		Ex-4	i.c.v.		u.1 tMCAO (60)	15		NP – ↓ infarct volume and improves functional outcome
Briyal(19)	2012	Rat		Ex-4	i.p.	BQ123	u.1. pMCAO	BD - 7 days		NP – ↓ infarct volume, ↓ motor deficit, ↓ oxidative stress parameters (↓MDA, ↑ GSH, ↑ SOD) NP via ↓ oxidative stress and independent of endothelin receptor.
Briyal(29)	2014	Rat	DM	Liraglutide	s.c.	Non-diabetic, insulin	u.1. pMCAO	OD - 14 days		NP - ↓ infarct volume, ↓ motor deficit, ↓ oxidative stress parameters (↓ MDA, ↑ GSH, ↑ SOD), ↓ apoptosis (↑ Bcl-2, ↓ Bax). NP in both non-diabetic & DM models with similar infarct volume reduction, insulin treatment did not reduce infarct volume. NP via ↓ oxidative stress and ↓ apoptosis
Chien(44)	2015	Rat	DM	PEX-4	s.c.	Non-diabetic, Ex-4	b.1 tCCAO (10) & hypotension	OD - 14 days		NP - ↓ brain oedema, ↓ cognitive deficit, ↓ oxidative stress, ↑ cerebral blood flow PEX-4 more NP than Ex-4 in DM.
Zhang(45)	2015	Mouse		Pro-GLP-1	i.p.	shRNA	u.1. tMCAO (90)	OD - 7 days		NP – ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↑ Bcl-2, ↓ Bax). NP not glucose dependent. NP blocked by shRNA (suggesting GLP-1R mediated NP)
Jiang(38)	2016	Rat	DM	rhGLP-1	i.p.	nimodipine, insulin	u.1. tMCAO (90)	TDS - 14 days		NP in DM - ↓ infarct volume (more than insulin group), ↓ neurological deficit, ↓ brain injury markers (↓ S100B, ↓NSE, ↓ MBP)
Zhang(46)	2016	Mouse		DMB	p.o.	Ex-4, Ex-9-39, shRNA	u.1. tMCAO (60)	30		NP given orally - ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↑ Bcl-2, ↓ Bax). No impact on plasma insulin and glucose levels in non-diabetic mice DMB activation of GLP-1R different to Ex-4. NP blocked by shRNA but not by Ex-9-39.
Zhang(22)	2016	Mouse		Ex-4	i.n.	Intraperitoneal Ex-4, shRNA	u.1. tMCAO (90)	OD – 7 days		NP given intranasally - ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↓ caspase-3) Intranasal route produced ↑ CNS concentration than i.p. No impact on plasma insulin and glucose levels and NP was blocked by shRNA suggesting NP is GLP-1R mediated.
Kim(23)	2017	Rat		Ex-4	i.c.v	Ex-9-39	u.1. MCAO (60)	30		NP - ↓ infarct volume (by up to 75%), ↓ neurological deficit, ↓ oxidative damage/inflammation (↓ COX-2, ↓ PE2)
Li(48)	2017	Rat		OXM	i.c.v.		u.1. tMCAO (60)	15		NP – ↓ infarct volume, ↑ locomotive activity

Abdel-latif(42)	2018	Rat	DM	Lixisenatide	i.p.	glimepiride	b.l. tCCAO (30)	OD – 14 days		NP - ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress (↓ MDA, ↑ GSH, ↑ catalase), ↓ inflammation/apoptosis (↓ caspase-3, ↓ TNF-α) Lixisenatide more NP than glimepiride.
Fang(39)	2018	Rat	DM	rhGLP-1	i.p.	nimodipine	u.l. tMCAO (90)	TDS – 14 days		NP - ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress parameters (↓ MDA, ↑ GSH, ↑ SOD), ↓ apoptosis (↑ Bcl-2, ↓ Bax, ↓ caspase-3), ↑ EAAT2,
Filchenko(31)	2018	Rat	DM	Liraglutide	s.c.	Non-diabetic, metformin	u.l. tMCAO (30)	OD – 7 days		NP - ↓ infarct volume, ↓ neurological deficit only in non-DM model, NP not associated with glycemic control amelioration (Metformin not NP)
Gad(51)	2020	Rat		Lixisenatide	i.p.	0.7 and 7 nmol/kg lixisenatide	b.l. tCCAO (60)	OD - 14 days		NP - ↓ oxidative stress parameters (↓ MDA, ↑ GSH, ↑ SOD), ↓ apoptosis (↑ Bcl-2, ↓ Bax, ↓ caspase-3), ↓ inflammatory markers (↓ MPO, ↓ IL-1β, ↓ TNF-α), ↑ viable hippocampal neurons on histological staining. Higher dose Lixisenatide ↑ NP
GLP-1RA administered prior to and following stroke onset										
Hyun Lee(17)	2011	Gerbil		Ex-4	i.p.		b.l. tCCAO (5)	120	60	NP - ↓ neurological deficit, ↑ GLP-1R immunoreactivity, ↓ microglial activation
Darsalia(20)	2012	Rat	DM	Ex-4	i.p.	0.1, 2 or 5µg/kg of Ex-4	u.l. tMCAO (90)	BD - 4 weeks	BD – 2 - 4 weeks	NP - ↓ infarct volume (no difference between 2 & 4 weeks post MCAO), ↓ microglial infiltration but marginal effect on activation, ↑ stem cell proliferation & neuroblast formation. NP is dose dependent.
Jia(21)	2015	Rat		Ex-4/Catapol	i.c.v.	Ex-9-39	u.l. tMCAO (60)	15	0	NP - ↓ infarct volume, ↓ neurological deficit, ↑ hippocampal β-endorphin expression, NP blocked by Ex-9-39, β-endorphin anti-serum and naloxone
Deng(30)	2018	Rat	DM	Liraglutide	i.p.	Non-DM	u.l. pMCAO	BD – 7 days	BD – 7 days	NP - ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress (↑ SOD), ↓ inflammation (↓ MPO), ↑ Nrf2, ↑ HO-1 (antioxidative stress signalling pathway) No significant difference in NP between DM & non-DM
GLP-1RA administered following stroke onset (including delayed administration)										
Teramoto(24)	2011	Mouse		Ex-4	t.v.		u.l. tMCAO (60)		0/60/180	NP - ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress, ↓ inflammation (↓ microglial activation), ↑ intracellular cAMP levels (due to GLP-1R activation). NP probably mediated via raised intracellular cAMP levels
Sato(32)	2013	Rat		Liraglutide	i.p.		u.l. tMCAO (90)		60	NP - ↓ infarct volume, ↓ neurological deficit, ↓ oxidative stress, ↑ VEGF in cortex but not in the striatum.
Darsalia(25)	2014	Mouse	DM	Ex-4	i.p.	2 month old & 14 month obese/DM mice	u.l. tMCAO (30)		90, 180 or 270 then OD 1 week	NP - ↑ neuronal survival, ↑ M2 microglial markers Non statistically significant reduction in pro-inflammatory markers NP in non-diabetic/DM at 50µg/kg at 90/180 mins & at 90 mins for 5µg/kg.

										Stroke volume not affected by Ex-4 administration Not NP when Ex-4 administered 4.5hrs after stroke onset NP in both young and old animal models
Zhao(40)	2015	Rat	DM	rhGLP-1	i.p.	nimodipine	u.l. tMCAO (120)		0	NP - ↓ infarct volume, ↑ neuronal survival, ↓ oxidative stress (↓ MDA, ↑ GSH, ↑ SOD), ↓ nitric oxide damage (iNOS, eNOS) Statistically significant reduction in blood glucose in high dose group (no hypoglycemia)
Han(47)	2016	Rat		GLP-1/GIP (DA)	i.p.	GLP-1RA (SA)	u.l. tMCAO (60)		60	NP - ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↑ Bcl-2, ↓ Bax), ↓ nitric oxide damage (iNOS) DA more NP than SA.
Chen(26)	2016	Mouse		Warfarin & Ex-4	t.v.	P13K inhibitor	u.l. tMCAO (45)		0	NP - ↓ infarct volume, ↓ neurological deficit, ↑ BBB integrity, ↓ oxidative stress (↓ 8-OHdG, ↓ HHE), ↓ inflammation, NP blocked by P13K inhibitor. Ameliorated warfarin associated hemorrhagic transformation.
Kuroki(27)	2016	Mouse	DM	Ex-4	i.p.	insulin	u.l. tMCAO (60)		60	Hyperglycemia associated with ↑ infarct volume, ↑ cerebral oedema, ↑ hemorrhagic transformation. Ex-4 NP - ↓ infarct volume, ↓ cerebral oedema, ↓ oxidative stress, ↓ inflammation (↓ TNF- α), ↓ MMP-9 activation, ↓ Iba-1 positive microglia, NP in hyperglycemic model. Insulin not NP.
Li(50)	2016	Mouse	DM	Ex-4 or Liraglutide	i.p.		u.l. tMCAO (60)		0, 3, 6 or 12hrs	NP - ↓ neurological deficit including ↓ bladder dysfunction, ↑ cerebral microcirculation. ↓ cerebral oedema, ↓ oxidative stress (↓ DHE, ↓ ROS, ↑ SOD), ↓ apoptosis (↓ caspase-3, ↓ TUNEL, ↓ Bax, ↓ PARP, ↑ Bcl-2), ↓ inflammation (↓ ICAM-1, NF-2 κ B, p50, p65).
Yang(28)	2016	Rat		Ex-4	t.v.		u.l. tMCAO & b.l. tCCAO (60)		0 & 24hrs	NP - ↓ DNA damage (↑ APE1, ↓ TUNEL)
Zhu(33)	2016	Rat		Liraglutide	s.c.		u.l. pMCAO		1hr, then OD for 1, 3 and 7 days	NP - ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (↓ ROS, ↓ caspase-3, -8, -9, ↓ PARP, ↑ Bcl-2, ↓ Bax), ↓ DNA damage (↓ TUNEL), ↓ stress related hyperglycemia without causing hypoglycemia.
Dong(34)	2017	Rat		Liraglutide	s.c.		u.l. MCAO (90)		24hrs, then OD for 4 weeks	NP - ↓ neurological deficit, ↑ GLP-1R expression ↑ glucose metabolism in ischemic penumbra (18-FDG-PET) ↑ neurovascular remodelling (↑ NeuN, ↑ GFAP, ↑ vWF)

										NP when first dose delayed by 1 day
Abdel-latif(41)	2018	Rat		Lixisenatide	i.p.	Ex-9-39, pentoxifylline	b.l. tCCAO (30)		1 & 24hrs	NP - ↓ infarct volume, ↓ neurological deficit, ↓ apoptosis (caspase-3), ↓ oxidative stress (↓ GSH, ↓ MDA, ↓ catalase, ↓NO), ↓ inflammation (TNF-α) – these effects not inhibited by GLP-1R antagonist Ex-39 suggesting GLP-1R independent pathway.
Chen(35)	2018	Mouse		Liraglutide	i.p.		u.l. pMCAO		24hrs, then OD for 14 days	NP - ↓ infarct volume, ↓ neurological deficit, ↑ angiogenesis (↑VEGF) No significant difference in blood glucose levels between Liraglutide and Controls (as expected, stimulated insulin release is in a blood-glucose dependent manner) NP when first dose delayed by 1 day
Wang(49)	2018	Mouse		P7C3	t.v.	Ex-9-39	u.l. tMCAO (40)		120	NP - ↓ neurological deficit, ↑ neurogenesis (↑ doublecortin, ↑ β-tub3, ↑ adam11, ↑ adams20, ↓ GSK-3) NP is GLP-1R dependent.
Yang(43)	2019	Rat		Semaglutide	i.p.	Blood glucose monitoring	u.l. pMCAO		2hrs, then alternate days 1,7,14 or 21 days	NP - ↓ infarct volume, ↓ neurological deficit, ↓ neuronal loss, ↓ apoptosis (↑Bcl-2, ↓Bax, ↓ caspase-3), ↑ neurogenesis, improved growth factor signalling (↑ ERK1, ↑ ERS-1), No hypoglycemia in normoglycemic model.
He(36)	2019	Mice		Liraglutide	i.p.		u.l. pCCAO		OD for 7 days	NP - ↓ neurological deficit, ↓ oxidative stress
Zeng(37)	2020	Rat		Liraglutide	i.p.	P13k inhibitor	u.l. pMCAO		0 then OD	NP - ↓ infarct volume, ↓ cerebral oedema, ↓ apoptosis (↑ Bcl-2, ↓ caspase-3, ↓ TUNEL), ↓ inflammation (↓ TNF-α, IL-18, IL-1β, IL-6, COX-2) P13k inhibitor partially reversed NP effects.

<p>Abbreviations: Drugs: SA – single agonist DA – dual agonist Ex-4- exendin-4 PEx-4- Exendin-4 loaded poly-microspheres Ex-9-39- GLP-1R antagonist pro-GLP-1- long acting GLP-1RA rhGLP-1- recombinant human GLP-1 DMB- 6,7-dichloro-2-methyl-sulfonyl-3-N-tert-butylaminoquinoxaline OXM- oxyntomodulin shRNA- small hairpin RNA (targets GLP-1R) BQ123- endothelin receptor antagonist P7C3- aminopropyl carbazole compound</p>	<p>Administration: i.p. - intraperitoneal s.c. – subcutaneous p.o. – oral i.n. – intranasal t.v. – transvenous i.c.v. intracerebroventricular</p> <p>Frequency: OD/BD/TDS – once/twice/three times daily</p> <p>Models: DM – diabetes mellitus Non-DM – non diabetes mellitus (healthy)</p>	<p>Induction methods: u.l. – unilateral b.l. – bilateral MCAO – middle cerebral artery occlusion CCAO – common carotid artery occlusion (t/p)MCAO – transient/permanent</p> <p>Oxidative stress parameters: SOD – superoxide dismutase MDA - malondialdehyde GSH – glutathione 8-OHdG – 8-hydroxy-2-deoxyguanosine HHE – 4-hydroxyhexenal DHE – dihydroethidium ROS – reactive oxygen species</p> <p>Apoptosis markers: Bcl-2 – B cell lymphoma 2 Bax – Bcl-2-like protein 4 Caspase-3 PARP – poly-ADP ribose polymerase TUNEL – terminal deoxynucleotidyl transferase dUTP nick end labelling</p>	<p>Others: S100b – s100- calcium binding protein B NSE – Neuron specific enolase MBP – Myelin basic protein COX-2 – cyclo-oxygenase-2 PE2 – Prostaglandin E2 EAAT2 – Excitatory amino acid transporter-2 MPO – myeloperoxidase HO-1 – heme oxygenase-1 Nrf2 – nuclear factor erythroid-2 NOS – nitric oxide synthase BBB – blood brain barrier MMP-9 – Matrix metalloproteinase-9 ICAM-1 – intracellular adhesion molecule-1 NeuN – Neuronal Nuclear Protein (neuronal marker) GFAP – Glial fibrillary acidic protein (glial marker) vWF – von Willebrand factor (endothelial marker) β-tub3 – beta tubulin III GSK-3 – glycogen synthase kinase 3 IL – interleukin</p>
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TABLE 2: GLP-1RA characteristics and stroke incidence in CVOTs							
	ELIXA(72)	LEADER(73)	SUSTAIN-6(74)	EXSCEL(75)	HARMONY(76)	REWIND(77)	PIONEER-6(78)
GLP-1RA	Lixisenatide	Liraglutide	Semaglutide	Exenatide (extended release)	Albiglutide	Dulaglutide	Semaglutide
Participants	6 068	9 340	3 297	14 752	9 463	9 901	3 183
Median follow-up (years)	2.1	3.8	2.1	3.2	1.6	5.4	1.3
Characteristics							
Administration	subcutaneous	subcutaneous	subcutaneous	subcutaneous	subcutaneous	subcutaneous	Oral
Dose	10 - 20µg	1.8mg	0.5 - 1.0mg	2mg	30 - 50mg	1.5mg	14mg
Frequency	once-daily	once-daily	once-weekly	once-weekly*	once-weekly	once-weekly	Once-daily
Non-fatal stroke incidence							
Treatment group – no. (%)	67 (2.2)	159 (3.4)	27 (1.6)	169 (2.3)	76 (1.6)	135 (2.7)	12 (0.8)
Placebo – no. (%)	60 (2.0)	177 (3.8)	44 (2.7)	193 (2.6)	91 (1.9)	175 (3.5)	16 (1.0)
Hazard ratio (95% CI)	1.12 (0.79-1.58)	0.89 (0.72 - 1.11) P=0.30	0.61 (0.38 - 0.99) P=0.04	0.88 (0.72 - 1.08)	0.84 (0.62 - 1.13)	0.76 (0.61 - 0.94) P=0.017	0.74 (0.35 - 1.57)

*Exenatide standard release requires twice-daily administration

TABLE 3: Retrospective database studies

<i>Author</i>	<i>Years studied</i>	<i>Participants included</i>	<i>Groups</i>	<i>Participants on GLP-1RA</i>	<i>GLP-1RA CVD risk (composite) - HR(95% CI) unless otherwise specified</i>	<i>GLP-1RA Stroke Risk - HR(95% CI)</i>	<i>HR Compared to</i>
Best(79)	2005 - 2009	383 525	Exenatide, non-exenatide	21 754	0.80 (0.74 - 0.86)	NR	Non-exenatide
Paul(64)	2005 - 2009	39 225	Exenatide, exenatide & insulin, insulin	10 674	Exenatide 0.50 (0.32 - 0.79) Exenatide & insulin 0.44 (0.34 - 0.57)	Exenatide 0.50(0.28-0.84) Exenatide & insulin 0.38 (0.27 - 0.54)	Insulin
Gejl(80)	1977 - 2011	10 073	Liraglutide, exenatide, insulin, biguanides, glitazones, DPP-4i, SFU	1 749	Liraglutide OR 0.48 (0.38 - 0.62) Exenatide OR 1.72 (1.20 - 2.48)	NR	N/A
Patomo(81)	2005 - 2013	219 810	GLP-1RA, DPP-4i, SFU, insulin	29 542	i) 1.20 (0.76 - 1.89) ii) 1.05 (0.63 - 1.74) iii) 1.01 (0.73 - 1.41)	NR	i) DPP-4i ii) SFU iii) insulin

Ekström(82)	2005 - 2012	20 422	GLP-1RA, acarbose, insulin, DPP-4i, meglitinide, SU, TZD	219	0.26 (0.10 - 0.67)	NR	Sulphonylurea
Zimmerman(65)	2005 - 2014	105 862	GLP-1RA, non-GLP-1RA	8 362	0.82 (0.74 - 0.91)	0.82 (0.74 - 0.91)	Non-GLP-1RA
Anyanwagu(83)	2006 - 2014	3 586	Insulin & GLP-1RA, insulin	1 793	0.76 (0.41 -1.42)	0.93 (0.54 - 1.61)	Insulin
O'Brien(63)	2011 - 2015	132 737	GLP-1RA, DPP-4i, SGLT- 2i, TZD, SFU, insulin	11 351	0.78 (0.63 - 0.96)	0.65 (0.44 - 0.97)	DPP-4i
Svanstrom(66)	2010 - 2016	46 804	Liraglutide, DPP-4i	23 402	0.90 (0.83 -0.98)	0.88 (0.77 - 1.01)	DPP-4i
Raparelli(84)	2011 - 2017	167 254	GLP-1RA, DPP-4i, SGLT- 2i, SFU	14 697	0.70 (0.62 - 0.78)	NR	SFU
					Women only: 0.57 (0.48 - 0.68)		

Notes: Frison(85) excluded as too small to calculate HR. NR- Not recorded, DPP-4i-dipeptidyl peptidase-4 inhibitor, SFU-sulphonylurea, SGLT-2i-sodium-glucose cotransporter-2, TZD-thiazolidinedio

