

BMJ Open Cardiovascular, renal and gastrointestinal effects of incretin-based therapies: an acute and 12-week randomised, double-blind, placebo-controlled, mechanistic intervention trial in type 2 diabetes

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ABSTRACT

Introduction: Incretin-based therapies, that is, glucagon-like peptide (GLP)-1 receptor agonists and dipeptidyl peptidase (DPP)-4 inhibitors, are relatively novel antihyperglycaemic drugs that are frequently used in type 2 diabetes management. Apart from glucose-lowering, these agents exhibit pleiotropic actions that may have favourable and unfavourable clinical consequences. Incretin-based therapies have been associated with heart rate acceleration, heart failure, acute renal failure and acute pancreatitis. Conversely, these agents may reduce blood pressure, glomerular hyperfiltration, albuminuria and hepatic steatosis. While large-sized cardiovascular safety trials can potentially identify the clinical significance of some of these pleiotropic actions, small-sized mechanistic studies are important to understand the (patho) physiological rationale of these findings. The current protocol describes a mechanistic study to assess cardiovascular, renal and gastrointestinal effects, and mechanisms of incretin-based therapies in type 2 diabetes.

Methods and analyses: 60 patients with type 2 diabetes will undergo acute and prolonged randomised, double-blind, intervention studies. The acute intervention will consist of intravenous administration of the GLP-1 receptor agonist exenatide or placebo. For the prolonged intervention, patients will be randomised to 12-week treatment with the GLP-1 receptor agonist liraglutide, the DPP-4 inhibitor sitagliptin or matching placebos. For each examined organ system, a primary end point is defined. Primary cardiovascular end point is change in resting heart rate variability assessed by beat-to-beat heart rate monitor and spectral analyses software. Primary renal end point is change in glomerular filtration rate assessed by the classic inulin clearance methodology. Primary gastrointestinal end points are change in pancreatic exocrine function assessed by MRI-techniques (acute intervention) and faecal elastase-1 levels (12-week

Strengths and limitations of this study

- This study includes three randomised, placebo-controlled, double-blind clinical trials to assess both acute and prolonged (12 weeks) effects of incretin-based therapies on the cardiovascular, renal and gastrointestinal system.
- Multiple secondary end points are studied in order to provide an integrative view on the examined organ systems.
- The study may not be sufficiently powered to draw conclusions on secondary end points.
- The duration of the prolonged intervention study is 12 weeks and can, therefore, not be considered as a long-term exposure to the study drugs.

intervention). Secondary end points include systemic haemodynamics, microvascular function, effective renal plasma flow, renal tubular function, pancreatic volume and gallbladder emptying-rate.

Medical ethics and dissemination: The study is approved by the local Ethics Review Board (VU University Medical Center, Amsterdam) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Trial registration number: NCT01744236.

INTRODUCTION

The global prevalence of type 2 diabetes has reached alarming proportions, which is strongly related to ageing and the obesity pandemic. Long-term intensive glycaemic control has reduced the incidence of its microvascular and macrovascular complications.^{1 2} However, approximately 25% of patients with type 2

diabetes do not reach glycaemic targets with currently available antihyperglycaemic treatment options.³ Intensified treatment schedules may be associated with adverse effects, including weight gain and hypoglycaemia. Thus, besides improving and implementing public health initiatives and preventive treatment strategies, the development of novel antihyperglycaemic agents is needed. Over the past decade, several antihyperglycaemic drug classes have been introduced into the market. However, as with every novel drug, there are potential safety risks that need exploration in clinical trials. In spite of stringent regulatory rules that enforce the conduction of large-scaled registration programmes, rare adverse effects may only become apparent after marketing authorisation, when a large group of patients has been treated for a longer period of time. In addition to ongoing long-term cardiovascular safety trials for all novel antihyperglycaemic drugs,⁴ there is a growing need to improve the postmarketing monitoring of potential risks and benefits of these drugs.

The 7th-Framework Programme funded European project 'Safety Evaluation of Adverse Reactions in Diabetes' (SAFEGUARD) is a pharmacovigilance project designed to assess, quantify and understand safety aspects of antihyperglycaemic drugs in type 2 diabetes, with a focus on incretin-based therapies.⁵ The SAFEGUARD-project consists of eight work packages, including pharmacovigilance database studies, observational database studies, meta-analyses and mechanistic studies. Here, we describe one of the protocols of the mechanistic work package that explores some of the pleiotropic actions that have been associated with the use of incretin-based diabetes therapies.

GLP-1 and DPP-4

Incretin-based therapies are based on the gut-derived incretin hormone glucagon-like peptide (GLP)-1. GLP-1 is released by intestinal L-cells on food ingestion and regulates glucose homeostasis by influencing pancreatic islet-cell function, including glucose-dependent stimulation of insulin and suppression of glucagon secretion.⁶ However, native GLP-1 is rapidly metabolised by the serine protease dipeptidyl peptidase (DPP)-4, leading to a plasma half-life of ~2 min. In type 2 diabetes, the effect of this incretin on endocrine pancreatic function is impaired. However, when native GLP-1 is administered at pharmacological doses, it lowers fasting and postprandial glucose, improves islet-cell function, delays gastric emptying and induces bodyweight loss.⁷ Therefore, GLP-1 was regarded as an attractive therapeutic option for type 2 diabetes.⁸ To date, two incretin-based drug classes have been developed and marketed: injectable DPP-4-resistant GLP-1 receptor agonists which mimic the effects of native GLP-1, and oral DPP-4 inhibitors which prolong the actions of endogenously secreted GLP-1.⁹ Both incretin-based drug classes improve glycaemic control in patients with type 2 diabetes, with minor risk of hypoglycaemia in clinical practice.^{2 9}

Pleiotropic effects of incretin-based therapies

Interestingly, the receptor for GLP-1 has been identified in many non-pancreatic organ systems, such as the heart, blood vessels, kidneys, gastrointestinal system and central nervous system.^{10 11} It was, therefore, not unexpected to find pleiotropic effects of GLP-1 and related therapies. Although some of the pleiotropic actions of incretin-based therapies may be beneficial, these could also cause adverse effects. For example, clinical use of incretin-based therapies has been associated with heart rate acceleration,¹² heart failure,¹³ sporadic cases of acute renal failure¹⁴ and acute pancreatitis.¹⁵⁻¹⁷ However, to date, a causal relationship between the use of incretin-based therapies and the occurrence of adverse effects is unclear (as type 2 diabetes per se is associated with these conditions), and the underlying mechanisms remain hitherto largely unexplored.

Incretin-based therapies and the cardiovascular system

GLP-1 receptor agonists have been associated with resting heart rate acceleration (mean increase of 2-4 bpm),¹² an established risk factor for cardiovascular and all-cause mortality.¹⁸ The mechanisms underlying this finding remain unclear, but alterations in cardiac autonomic nervous system balance may be involved.^{19 20} Other potential mechanisms include changes in baroreceptor sensitivity, natriuresis and reduced systemic vascular resistance. Furthermore, although mechanistic trials with incretin-based therapies showed improvement in parameters associated with heart failure,^{21 22} recent large-sized clinical trials revealed signs of increased hospitalisation for heart failure with the DPP-4 inhibitors saxagliptin and alogliptin, but not sitagliptin.^{13 23 24}

Conversely, long-term GLP-1 receptor agonist administration decreases systolic and diastolic blood pressure in clinical trials.¹² Moreover, it improves endothelial dysfunction, measured as flow-mediated vasodilation,^{25 26} decreases macrophage foam cell formation and atherosclerosis in animals,²⁷ and reduces carotid intimal-media thickness in humans.²⁸ Also, in animal models of myocardial infarction, native GLP-1 administration reduced infarction size²⁹ and improved postinfarction myocardial function.³⁰ Similar cardiovascular improvements have been noted in small-sized clinical studies.³¹⁻³³

Incretin-based therapies and the renal system

Several case reports have described the occurrence of acute renal failure in patients with type 2 diabetes treated with incretin-based therapies.^{34 35} However, associations between the use of incretin-based therapies and renal failure were not supported by a large-sized database analysis.³⁶ Moreover, to date, clinical studies have not given rise to concerns regarding renal adverse events.

More recently, incretin-based therapies have been associated with renoprotective properties. In animal models, administration of GLP-1 and associated therapies reduced systemic hypertension and albuminuria, and

ameliorated renal damage, as verified by histology.^{37–40} In clinical studies, GLP-1 receptor agonists have been associated with decreased development and progression of albuminuria.^{41–42} Moreover, the clinical use of the DPP-4 inhibitors saxagliptin and linagliptin were associated with a glucose-independent reduction in albuminuria by approximately 25%.^{43–45} Collectively, these data suggest a renoprotective effect with the use of incretin-based therapies. However, the mechanisms by which these antihyperglycaemic drugs may improve renal outcome beyond glycaemic control are unclear, but may involve improvements in body weight, blood pressure, renal haemodynamics and albuminuria or lipid profiles.^{40–46}

Incretin-based therapies and the gastrointestinal system

GLP-1 receptor agonists and DPP-4 inhibitors have been associated with acute pancreatitis,^{15–17} and histological inflammatory changes of the pancreas have been shown in rodents.⁴⁷ Also, it has been hypothesised that incretin-based therapies increase the risk of chronic pancreatitis or pancreatic cancer.⁴⁸ However, available data are conflicting since many other studies fail to find such adverse effects with incretin-based therapies.^{49–51} As type 2 diabetes, and frequently associated comorbidities, is by itself associated with pancreatitis, it is difficult to distinguish the sole contribution of incretin-based therapies.

In contrast, beneficial gastrointestinal effects of GLP-1 have been described, contributing to improved glycaemic control through decreased (postprandial) glycaemic excursions.⁵² Moreover, mounting evidence suggests therapeutic effects of incretin-based therapies on gastrointestinal diseases, including non-alcoholic fatty liver disease and irritable bowel syndrome.^{53–54} Mechanistically, GLP-1 peptide infusion decreases stomach and bowel motility,⁵⁵ and secretion of gastric acid and exocrine pancreatic enzymes.^{56–58} Many of these effects are likely to be mediated through changes in vagal nerve function.⁵⁶ In addition, the GLP-1 receptor agonist exenatide reduces gallbladder emptying rate.⁵⁹ Hepatic effects of incretin-based therapies are still unclear, but may include reduced endogenous glucose and lipid production.⁶⁰

Rationale and aim

Since a decade, GLP-1 receptor agonists and DPP-4 inhibitors have been implemented for glucose-lowering treatment of type 2 diabetes, and are now widely used in clinical practice. However, there remain many gaps in our understanding about various pleiotropic effects of these antihyperglycaemic drugs. Importantly, incretin-based therapy use has been associated with potential adverse events, among others, affecting the cardiovascular, renal and gastrointestinal systems. While large-sized randomised trials and database studies patients with type 2 diabetes will provide evidence on their clinical risks, mechanistic studies are needed to identify the underlying mechanisms of the adverse effects. Therefore, the

current study aims to investigate the acute and prolonged effects of incretin-based therapies on the cardiovascular, renal and gastrointestinal system of patients with type 2 diabetes.

METHODS AND ANALYSES

Study design

In order to assess acute and prolonged effects of incretin-based therapies on the cardiovascular, renal and gastrointestinal system, the current study integrates three double-blind, randomised, placebo-controlled trials in patients with type 2 diabetes. In the main parallel-group study, 60 patients will be randomised and treated for 12 weeks with the GLP-1 receptor agonist liraglutide, the DPP-4 inhibitor sitagliptin or matching placebos (figure 1). After the baseline measurements of the 12-week intervention study, two acute intervention-studies are performed to assess the effect of intravenous administration of the GLP-1 receptor agonist exenatide versus placebo: 1 parallel-group trial in all 60 patients to assess acute cardiovascular and renal effects, and 1 cross-over trial in a subset of 12 male patients to assess acute pancreatic effects. Placebo is chosen as comparator for all three trials, since this allows for the study of drug-induced changes per se, instead of comparing changes with an active comparator.

All examinations will be performed at the Clinical Research Unit (CRU) of the Department of Internal Medicine/Diabetes Centre of the VU University Medical Center in Amsterdam, the Netherlands.

Outcome measures

Outcome measures will be studied after acute intravenous drug-administration, and after 12 weeks of prolonged drug-intervention. In addition, predefined outcome measures will be evaluated after 2 and 6 weeks of prolonged intervention (safety visits; table 1). Outcome measures will be compared between the intervention and placebo groups.

Cardiovascular outcomes

Primary outcome measure is resting heart rate variability (HRV) assessed with a beat-to-beat heart rate monitor and spectral analyses software. Secondary outcome measures include systemic haemodynamics (heart rate, blood pressure, cardiac output, vascular resistance), arterial stiffness and microvascular function (see online supplementary table A).

Renal outcomes

Primary outcome measure is glomerular filtration rate (GFR), assessed by using the inulin clearance technique, based on timed urine sampling. Secondary outcome measures include para-amino hippuric acid (PAH)-measured effective renal plasma flow, tubular function, and glomerular and tubular damage markers (see online supplementary table B).

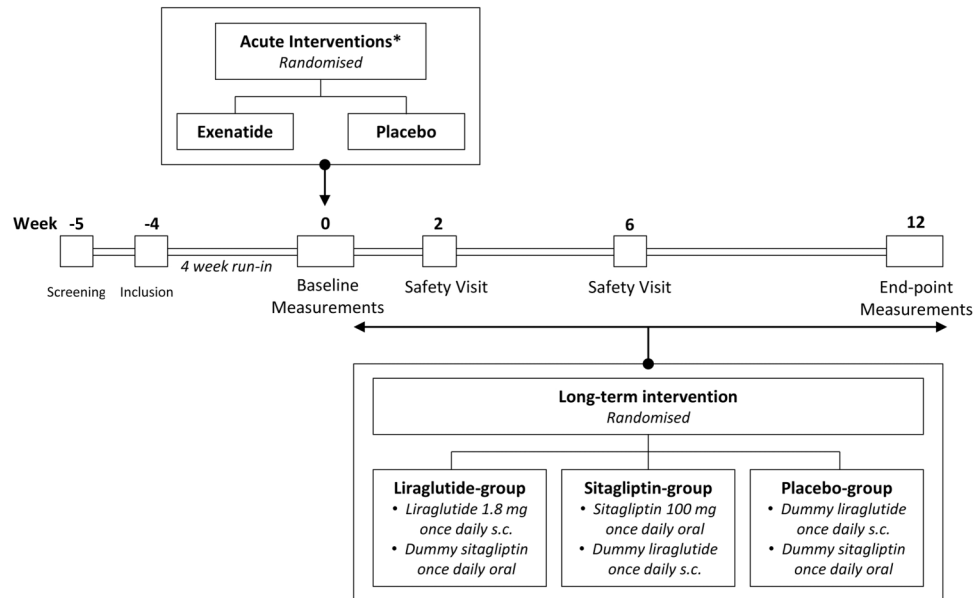


Figure 1 Study design: Subjects will be screened for eligibility. Included subjects will undergo a run-in period of at least 4 weeks after which the baseline end point measurements for the 12-week study and the acute intervention studies are performed*. Subsequently, subjects are randomised for the prolonged intervention. After 2 and 6 weeks, a safety visit is performed. At week 1 and 9, telephone follow-up is performed (not depicted). After 12 weeks of treatment, the end point measurements are performed. *For the acute cardiovascular and renal study, subjects are randomised to exenatide or placebo. For the acute pancreatic study, 12 patients will receive intravenous exenatide and placebo in a randomised cross-over design.

Gastrointestinal outcomes

Primary outcome measure is pancreatic exocrine function. For the acute intervention study, this is measured by exocrine secreted volume assessed by secretin-enhanced MR cholangiopancreatography. For the 12-week study, this is measured by faecal elastase-1 levels. Secondary outcome measures for the 12-week study consist of exocrine pancreatic function, pancreatic structure/volume, gallbladder emptying, gastric emptying, hepatic fat content and gastrointestinal damage markers (see online supplementary table C).

Other and exploratory outcome measures

Additional outcome measures include body anthropometrics, postprandial glucose excursions, glycated haemoglobin, plasma lipids and body fat content (see online supplementary table D). Moreover, urine, faeces and blood (plasma and serum) are collected and stored at -80°C for at least 15 years, to allow for determination of additional biomarkers for potential future research questions.

Participants

Volunteers with type 2 diabetes will be recruited using established recruitment methods: (1) participants in the previous studies of the VU University Diabetes Centre will be contacted (if informed consent was obtained); (2) advertisements in local newspapers, folders and posters; (3) affiliated healthcare workers (internal medicine, general practitioners) will inform patients of the existence of this study; and (4) websites. After providing extensive printed and oral information, a hand-signed

informed consent form will be attained by a clinical research physician. Eligibility will be assessed during a screening visit, comprising of a medical interview and physical, blood, urine and ECG examination. Postvoiding bladder residue will be assessed using ultrasonic bladder scan. Inclusion and exclusion criteria are listed in box 1. After inclusion, participants will receive an unique study number.

Intervention

Acute intervention studies

The GLP-1 receptor agonist exenatide (AstraZeneca, London, UK) or placebo (isotonic 0.9% saline) will be infused for 5 h. One dose of exenatide 10 μg will be diluted in 46 mL isotonic 0.9% saline and 4 mL of the participant's blood to prevent binding of the drug to the infusion material. For placebo, no study drug will be added. The solution will be administered with a calibrated syringe pump at an infusion-rate of 50 ng/min for 30 min; this is then followed by 25 ng/min infusion for the remainder of the test procedures to target the steady-state plasma concentrations of exenatide within the therapeutic range (100–150 pg/mL).⁶¹

Twelve-week intervention study

The GLP-1 receptor agonist liraglutide (Novo Nordisk A/S, Bagsværd, Denmark) and matching liraglutide-placebo prefilled pens for subcutaneous use will be provided by Novo Nordisk. A dose increment scheme is employed, in which patients will inject liraglutide/-placebo at a dose of 0.6 mg once daily during the first week, 1.2 mg once daily during the second week, and

Table 1 Trial visits and end points

End point	Baseline				Safety				Prolonged treatment 12-week				
	CV/R		CV/R acute	PP	GI	MRI	MRI acute*	2-week	6-week	CV/R	PP	GI	MRI
	CV/R	acute											
Cardiovascular													
Resting heart rate variability	X	X	X							X	X		
Systemic haemodynamics	X	X	X							X	X		
Arterial stiffness	X	X	X							X	X		
Microvascular function	X	X	X							X	X		
Renal													
Glomerular filtration rate	X	X								X			
Estimated glomerular filtration rate	X	X						X	X	X			
Effective renal plasma flow	X	X								X			
Fractional electrolyte excretion	X	X								X			
Urine osmolality	X	X								X			
Urine pH	X	X								X			
Glomerular damage parameter (albumin)	X	X								X			
Tubular damage parameters (KIM-1, NGAL)	X	X								X			
Body water percentage	X	X								X			
Gastrointestinal													
Faecal elastase-1					X								X
Faecal chymotrypsin					X								X
Lipase/amylase-levels	X	X	X					X	X	X			
¹³ C-MTG breath test					X								X
Pancreatic bicarbonate secretion						X	X						X
Pancreatic structure/volume					X								X
Trypsinogen blood/urine	X							X		X			
Gallbladder emptying					X								X
Gastric emptying					X								X
Hepatic fat content					X								X
Plasma albumin/AST/ALT/γGT/ALP	X								X	X			
GI damage parameters (I-FABP, L-FABP, calprotectin)	X							X		X			
Microbiome					X								X
General													
HbA1c	X										X		
Fasting glucose	X									X			
Postprandial glucose			X								X		
Fasting lipid spectrum	X									X			
Anthropometrics (length/height/waist/hip)					X			X	X				X
Body fat percentage					X								X
Subcutaneous and visceral fat volume						X							X

*The acute pancreatic MRI study is performed in a subset of 12 patients.

¹³C-MTG, ¹³C-labeled mixed triglyceride breath test; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase CV/R, cardiovascular and renal testing day; GI, gastrointestinal testing day; HbA1c, glycated haemoglobin; I-FABP, intestinal fatty acid binding protein; L-FABP, liver-type fatty acid binding protein; PP, postprandial.

1.8 mg once daily during the remainder of the study. Based on the participants' tolerance to the trial product, the time interval between the dose increments can be extended, and drug dose can be reduced when regarded to be clinically necessary. Subjects are instructed to inject liraglutide/-placebo in the abdominal region, and always at the same time of the day (preferably evening).

The DPP-4 inhibitor sitagliptin (Merck, Whitehouse Station, New Jersey, USA) and matching sitagliptin-

placebo will be encapsulated by an independent GMP-certified clinical research organisation (ACE Pharmaceuticals BV, Zeewolde, the Netherlands). Sitagliptin/-placebo 100 mg will be taken orally once daily for 12 weeks, at the same time as the liraglutide/-placebo injection. No dose escalation is needed for sitagliptin/-placebo.

Participants randomised to the *liraglutide-arm* will receive sitagliptin-placebo capsules, while participants

Box 1 Inclusion and exclusion criteria*Inclusion criteria*

- ▶ Type 2 diabetes
 - Stable dose of oral antihyperglycaemic drugs (metformin and/or sulfonyleurea) for at least 3 months prior to inclusion
 - HbA1c 6.5–9.0% DCCT or 48–75 mmol/mol IFCC
- ▶ Age between 35 and 75 years
- ▶ Females must be postmenopausal (defined as: no menses >1 year)
- ▶ Caucasian
- ▶ Body mass index 25–40 kg/m²

Exclusion criteria

- ▶ Use of the following medication: thiazolidinediones, GLP-1 receptor agonists, DPP-4 inhibitors, insulin, glucocorticoids, non-steroidal anti-inflammatory drugs, antimicrobial agents, chemotherapeutics or immune suppressants. Patients on diuretics will only be excluded when these drugs (eg, hydrochlorothiazide) cannot be stopped for the duration of the study
- ▶ History of pancreatic disease or impaired pancreatic exocrine function (defined as: use of pancreatic enzymes)
- ▶ Active liver disease or a threefold elevation of liver enzymes (AST/ALT) at screening
- ▶ (History of) malignancy (with the exception of basal cell carcinoma)
- ▶ Estimated-GFR <60 mL/min/1.73 m²; Current urinary tract infection and active nephritis
- ▶ Recent (<6 months) history of cardiovascular disease, including acute coronary syndrome, stroke, transient ischaemic neurological disorder; Chronic heart failure (New York Heart Association grade II–IV) or atrial fibrillation
- ▶ Alcohol abuse, defined as >4 units day
- ▶ Allergy to any of the test agents
- ▶ Complaints compatible with or established gastroparesis and/or neurogenic bladder
- ▶ History of or present (severe) mental illness
- ▶ Inability to understand the study protocol and/or to give written informed consent
- ▶ Contraindications for MRI; claustrophobia or presence of metal objects/implants

randomised to the *sitagliptin-arm* will receive liraglutide-placebo prefilled pens. Participants randomised to the *placebo-arm* will receive liraglutide-placebo prefilled pens and sitagliptin-placebo capsules.

Patients using sulfonyleurea will be instructed to frequently check their blood glucose levels, and in case of clinically significant hypoglycaemia, sulfonyleurea dose will be decreased based on the discretion of a research physician. In general, patients and caregivers will be instructed not to change comedication without clinical need.

Randomisation

Randomisation will be performed by an institutional trial pharmacist. For the acute studies, block randomisation will be performed with an allocation ratio of 1:1, and a block-size of 6 (acute cardiovascular and renal study) and 4 (acute pancreatic study). For the 12-week study, block randomisation will be performed with an

allocation ratio of 1:1:1, and a block-size of 6. Stratification will be applied, thereby evenly dividing the number of patients receiving acute intravenous administration of exenatide or placebo among the three prolonged intervention groups (figure 2).

Blinding

Study medication will be provided by the trial pharmacist, and all study personnel and participating patients will remain blinded with regards to the study medication. The blind shall not be broken, unless information concerning the study medication is considered medically necessary. If the investigator is unblinded, study medication will be stopped and the participant will be withdrawn from the study. The blind will be broken by the institutional trial pharmacist after the last patient has completed the last study visit and all data are entered into the online clinical trial database.

Compliance

For the 12-week study, participants will receive extensive printed and oral study, and drug-related instructions by a clinical research physician. The research physician will be in contact with the participants after 1, 2 and 6 weeks of intervention, during which safety and drug accountability will be monitored.

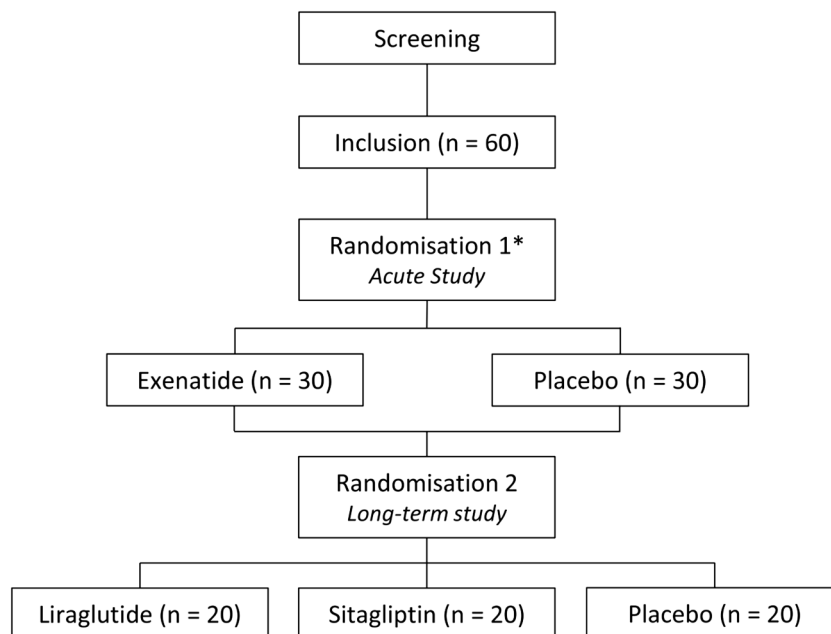
Data collection and study procedures

Data will be collected during designated end point visits at baseline and after 12 weeks of treatment: 1 for the cardiovascular and renal end points, 1 to collect gastrointestinal data, and 1 visit to perform the MRI (table 1). These visits will be planned in no particular order. During the baseline cardiovascular and renal end point visit, the acute study will be performed. At least 24 h will be observed between the acute intervention and the next visit, to allow for washout of the study drug. Patients participating in the acute pancreatic study will undergo two additional MRI prior to the start of the 12-week intervention.

Cardiovascular and renal study procedures

Two days prior to this study visit, participants will be asked to adhere to an standardised sodium chloride (9–12 g/day) and protein (1.5–2.0 mg/kg/day) intake in order to minimise diet-induced variation in renal physiology.^{76 77} In addition, they will be asked to refrain from vigorous physical activity and alcohol ingestion for at least 24 h, and from consuming caffeine for at least 12 h. After an overnight fast, participants will be instructed to drink 500 mL of water. Intake of all morning medications, except for metformin, will be delayed until conclusion of the examination day. After arrival at the CRU at 7:30, intravenous catheters will be placed in both arms. Blood and urine will be collected, and the participant will assume a semirecumbent position. Then, resting HRV will be measured using a

Figure 2 Flow chart of the acute and prolonged intervention study. *For the acute cardiovascular and renal intervention study, patients are randomised to receive exenatide or placebo (parallel-group design). For the acute pancreatic intervention study, patients receive exenatide and placebo, in a randomised order (cross-over design).



beat-to-beat heart rate monitor. Systemic haemodynamic parameters will be assessed using an oscillometric blood pressure measurement device and beat-to-beat blood pressure monitor. Arterial stiffness will be measured using applanation tonometry, and microvascular function with capillary videomicroscopy and laser Doppler fluxmetry. Bio-impedance analysis (BIA) will be performed to measure body water and fat content.

Subsequently, the renal tests will start. A 10 min priming with inulin and para-aminohippurate sodium (PAH) will be followed by continuous infusion of these renal tracer substances. After 90 min of equilibration, urine will be collected by spontaneous voiding every 45 min for two time periods, while blood will be collected before and after each collection period. Samples will be used to measure GFR (inulin clearance), renal plasma flow (PAH clearance) and tubular function. Diuresis will be stimulated by oral intake of 10 mL/kg (maximal 1000 mL) tap water during the equilibration phase, followed by 200 mL of tap water per hour (figure 3).

Subsequently, during the baseline end point visit, but not during the end point visit after 12 weeks of treatment, the acute cardiovascular and renal intervention study with intravenous administration of exenatide or placebo will start. After an equilibration period of 60 min to ensure steady-state plasma exenatide concentrations,⁶¹ urine will be collected by spontaneous voiding every 45 min for two time periods while blood will be collected before and after each collection period for determination of inulin and PAH. In addition, the cardiovascular tests will be repeated intermittently (see online supplementary table A and B). Resting HRV will be assessed after 30, 90 and 165 min. Blood pressure and systemic haemodynamics will be assessed after 30, 60, 90, 120 and 165 min of intervention. Arterial stiffness

will be assessed after 30, 60, 90 and 160 min, and microvascular function after 110 min.

Finally, at the end of the renal protocol, a meal-test protocol will start, during which intravenous administration of exenatide or placebo is continued. A standardised high-fat mixed meal of 905.7 kcal (50 g fat, 75 g carbohydrates and 36.8 g protein) will be consumed within 15 min, and cardiovascular and metabolic changes in the postprandial state will subsequently be examined. The same meal-test protocol will be performed during the end point visit after 12 weeks of treatment without intravenous exenatide or placebo administration. Resting HRV will be measured after 30, 60 and 120 min, and blood pressure, systemic haemodynamics and arterial stiffness will be measured every 30 min post meal. Microvascular function will be measured 90 min after the start of the meal.

Gastrointestinal study procedures

During this visit, three experimental protocols will be performed simultaneously: (1) a ¹³C-labelled mixed triglyceride (¹³C-MTG) breath test (pancreatic digestive function), (2) a meal-stimulated gallbladder ultrasound examination (gallbladder emptying rate) and (3) an acetaminophen absorption kinetic test (gastric emptying rate). Two days prior to the study visit, participants are instructed not to take any product that is naturally enriched with ¹³C such as corn products, cane sugar, pineapple and tequila. After an overnight fast, participants will be instructed to delay all morning medications, apart from metformin, until conclusion of the examination day, and arrive at the CRU at 7:30. Body anthropometrics, including weight, height, waist-circumference and hip-circumference are measured. Participants will then assume a semirecumbent position, after which BIA is used to assess body composition. An intravenous

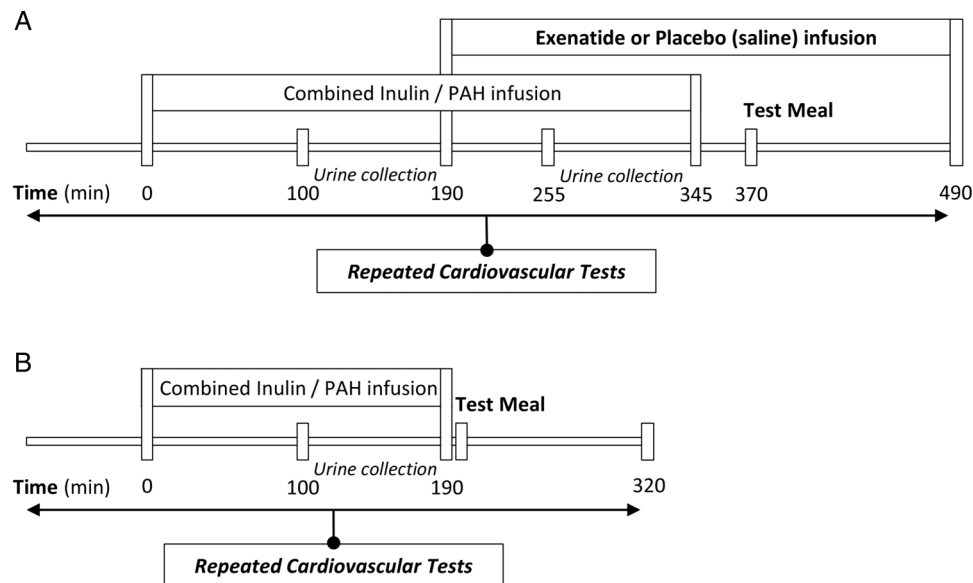


Figure 3 End point visit cardiovascular and renal tests. Schematic overview of the cardiovascular and renal end point visits. (A) At the baseline end point visit, the acute cardiovascular and renal intervention study is performed; (B) At the 12-week end point visit, no acute intervention study is performed. PAH, para-amino hippuric acid.

catheter is inserted into an antecubital vein for blood sampling. The gallbladder will be visualised by ultrasonographic imaging, recording the length, height and width of three measurements to calculate baseline gallbladder volume. Blood will be sampled to determine baseline serum acetaminophen, and reference breath samples will be collected using straw and gas collection tubes. Then, participants will be served a high-fat mixed meal (420 kcal, 22.4 g fat, 38.6 g carbohydrates and 14.6 g protein), containing the ^{13}C -MTG stable isotopes (Euriso-Top, Saint-Aubin Cedex, France) and a standardised acetaminophen solution (Daro, Remark Groep, Rogat, the Netherlands). This high-fat mixed meal triggers gallbladder contraction. Subsequently, gallbladder ultrasonographic imaging will be performed every 15 min for 3 h. Postprandially, blood will be drawn every 30 min for 3 h to examine serum acetaminophen levels, and at set times to assess glucose levels. Every 30 min, a breath sample will be collected for the ^{13}C -MTG breath test for 6 h.

MRI procedures

After an overnight fast, participants will be instructed to delay all morning medications. Participants will arrive at the CRU between 7:00 and 9:00, and an intravenous catheter will be inserted into an antecubital vein for secretin administration. A negative oral contrast agent (Lumirem, Guerbet, Gorinchem, the Netherlands) is given to enhance visualisation of the pancreatic duct system. The MRI protocol will consist of (1) structural sequences to assess organ anatomy, (2) spectroscopy sequences to assess hepatic lipid content and (3) MR cholangiopancreatography (MRCP) sequences for pancreatic duct morphology. Moreover, secretin (Secrelux,

Sanochemia Pharmazeutika AG, Vienna, Austria) will be administered intravenously to induce pancreatic excretion, which is measured and quantified using MRCP sequences.

For the acute pancreatic study, the same MRI protocol is used during concomitant infusion of exenatide or placebo. After arrival at the CRU between 7:00 and 9:00, infusion of the study drug or placebo is started. After an equilibration period of 60 min, the MRI protocol will start. At least 1 week gap is observed between the MRI visits.

Safety visits (weeks 2 and 6) and telephone follow-up (weeks 1 and 9)

At week 2 and 6, after an overnight fast, participants will be instructed to delay all morning medications, apart from metformin. Medical history is taken, and drug accountability is monitored and stimulated. Body anthropometrics and blood pressure are recorded. Fasting blood samples will be collected to assess renal and pancreatic safety parameters at week 2 and 6, whereas blood, urine and faeces will be stored at week 2 only. At week 1 and 9, medical history is taken and drug accountability is monitored and stimulated.

Early-term assessments

During all study visits and telephone follow-up, compliance and participant retention will be promoted. However, in case patients withdraw their participation, they will be asked to participate in early-term assessments aiming to study as many predefined outcome measures as possible, with a particular focus on primary outcomes.

Quality assurance: data management and monitoring

Outcome data will be collected on hard-copy case report forms (CRF). The CRF files will be anonymous, only indicating the participants study number. There will be double entry of all data into a secure online clinical trial database programme (OpenClinica LLC, V.3.3, Waltham, Massachusetts, USA), which complies with all regulations proposed by the International Conference on Harmonization of Good Clinical Practice (GCP). The final data set will be exported from OpenClinica, containing anonymous data, and will principally be available to study physicians and the principal investigator only.

An independent monitor, provided by the institutional Clinical Research Bureau of the VU University Medical Center, will oversee the progress of the clinical trial and ensure that it is conducted, recorded and reported in accordance with the protocol, standard operating procedures, GCP and the applicable regulatory requirements.

Sample size

Based on previous animal and clinical data on the effects of GLP-1 administration on HRV, the estimated GFR and duodenal aspiration measured exocrine pancreatic function,^{56 62 63} assuming 2-sided significance level of 0.05 and a power (1- β) of 80%, we considered 15 participants per treatment arm to be sufficient to detect statistically significant changes in each of our outcome measures. To allow for a dropout rate of 15%, we decided to include a total of 60 patients with type 2 diabetes, with 20 per treatment arm for the 12-week study and 30 participants per treatment arm for the acute cardiovascular and renal intervention study. Using the same assumptions, we calculated that 12 patients would be sufficient for the acute cross-over pancreatic study. Sample size calculations were performed using an ANOVA-model with SAS-software (V.9.2, Cary, North Carolina, USA) for comparing liraglutide and sitagliptin to placebo. A detailed power analysis is available in the online supplementary material.

Statistical analyses

A per-protocol analysis is considered the most appropriate approach to examine biological effects of incretin-based therapies on different organs systems.

To test treatment effects versus placebo, we will perform multivariable linear regression models (for single measured end points) and linear mixed models (for repeatedly measured end points). All analyses will be corrected for potential between-group baseline differences. This strategy will be used for both acute and 12-week studies. Log-transformation will be applied before analysis of non-Gaussian distributed data, as assessed by visual inspection of histograms and Q-Q plots. For each end point, we will report the between-group difference with its 95% CI or SE of the mean and p value. All analyses will be performed using SPSS V.22.0 (IBM SPSS Inc, Chicago, Illinois, USA).

MEDICAL ETHICS AND DISSEMINATIONS

The original protocol and all amendments to this protocol were approved by the local Ethics Review Board (2012/391) and the National Central Committee on Research Involving Human Subjects (NL41701.029.12). The study will be conducted in accordance with the Declaration of Helsinki and GCP. All participants will provide written informed consent before participation. The study is registered at ClinicalTrials.gov (ID: NCT01744236).

All patient data will be handled confidentially and anonymously. Data acquired during the eligibility visit will be coupled to a screening-visit number. All data acquired during the study will be coupled to a participant study number. A code list, with identifier data, screening numbers and study numbers, will be stored securely on the institutional server and protected by passwords only known to the responsible study physicians and principal investigator.

During the study, participants will be monitored by safety visits and telephone follow-up. All antihyperglycaemic study drugs (exenatide, liraglutide and sitagliptin) have been approved by the Food and Drug Administration and the European Medicines Agency for the treatment of type 2 diabetes, and are considered to be safe. Known adverse effects are nausea, vomiting, diarrhoea and constipation (especially for exenatide and liraglutide) and nasopharyngitis (especially for sitagliptin). These adverse events are usually mild and transient. Both GLP-1 receptor agonists and DPP-4 inhibitors may induce hypoglycaemia, especially when combined with sulfonylurea treatment. As described above, care will be taken to frequently measure blood glucose levels and sulfonylurea dose will be decreased if regarded necessary. Given the short treatment duration in this cohort of patients with type 2 diabetes without serious complications, we expect heart failure and pancreatic risk to be low. Any reported adverse event will be recorded, while serious adverse events will additionally be reported to the Ethics Review Board. Standard clinical care will be provided to manage the adverse event. In case of possible damage as a result of participating in this trial, the loss is covered by institutional insurance.

The protocol has been developed by experts in the field of endocrinology, vascular medicine, nephrology, gastroenterology and radiology. Members of the SAFEGUARD consortium have been involved in the development of this study protocol, and will be involved in the drafting of the manuscripts. Novo Nordisk A/S has not been involved in the development of the study protocol, but will be allowed to comment on medical accuracy and confidentiality of manuscripts before publication. The Dutch Kidney Foundation was not involved in the development of the study protocol, nor will it be involved in the drafting of the manuscripts. The findings from this study will be disseminated through international peer-reviewed publications, at scientific conferences, and when considered publically interesting,

through mass media. Authorship eligibility will be determined using the guidelines of the International Committee of Medical Journal Editors.

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†Deceased. This paper is in memory of Professor Michaela Diamant, whose experience and expertise were crucial for the design of this study.

MMS and LT have contributed equally.

Contributors MMS, LT and MHAM drafted the manuscript. TH, MHHK, ICP, DLC, DHvR read the draft critically to make contributions and approved the final text. All authors were involved in the development of the study protocol. We commemorate MD, the original primary principal investigator of this project, whose experience, expertise and capacity for inventive thought and understanding were crucial for the design of this protocol.

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Competing interests Before her passing away, on 9 April 2014, MD was a consultant for Abbott, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Gl Dynamics, Merck Sharp & Dohme, Novo Nordisk, Poxel Pharma and Sanofi. She was a speaker for AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Novo Nordisk and Sanofi and through MD, the VU University Medical Center received research grants from Abbott, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, Medtronic, Merck Sharp & Dohme, Novo Nordisk and Sanofi. MD received no personal payments in connection to the aforementioned activities: all funds were directly transferred to the Diabetes Centre's non-profit Research Foundation. Through MHHK, the VU University Medical Center received research grants from Boehringer Ingelheim, Novo Nordisk and Sanofi.

Patient consent Obtained.

Ethics approval Institutional Ethics Review Board ('METC').

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REFERENCES

- National Kidney Foundation. KDOQI clinical practice guideline for diabetes and CKD: 2012 update. *Am J Kidney Dis* 2012;60:850–86.
- Inzucchi SE, Bergenstal RM, Buse JB, *et al.* Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia* 2015;58:429–42.
- Morsink LM, Smits MM, Diamant M. Advances in pharmacologic therapies for type 2 diabetes. *Curr Atheroscler Rep* 2013;15:302.
- Holman RR, Sourij H, Califf RM. Cardiovascular outcome trials of glucose-lowering drugs or strategies in type 2 diabetes. *Lancet* 2014;383:2008–17.
- <http://www.safeguard-diabetes.org>.
- Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev* 2007;87:1409–39.
- Zander M, Madsbad S, Madsen JL, *et al.* Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 2002;359:824–30.
- Nauck M, Stöckmann F, Ebert R, *et al.* Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986;29:46–52.
- Lovshin JA, Drucker DJ. Incretin-based therapies for type 2 diabetes mellitus. *Nat Rev Endocrinol* 2009;5:262–9.
- Pyke C, Heller RS, Kirk RK, *et al.* GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* 2014;155:1280–90.
- Körner M, Stöckli M, Waser B, *et al.* GLP-1 receptor expression in human tumors and human normal tissues: potential for in vivo targeting. *J Nucl Med* 2007;48:736–43.
- Robinson L, Holt T, Rees K. Effects of exenatide and liraglutide on heart rate, blood pressure and body weight: systematic review and meta-analysis. *BMJ Open* 2013;3:pii: e001986.
- Standl E, Erbach M, Schnell O. Dipeptidyl-peptidase-4 inhibitors and heart failure: class effect, substance-specific effect, or chance effect? *Curr Treat Options Cardiovasc Med* 2014;16:353.
- Weise WJ, Sivanandy MS, Block CA, *et al.* Exenatide-associated ischemic renal failure. *Diabetes Care* 2009;32:e22–3.
- Ahmad SR, Swann J. Exenatide and rare adverse events. *N Engl J Med* 2008;358:1970–1; discussion 1971–2.
- Singh S, Chang H-Y, Richards TM, *et al.* Glucagonlike peptide 1-based therapies and risk of hospitalization for acute pancreatitis in type 2 diabetes mellitus: a population-based matched case-control study. *JAMA Intern Med* 2013;173:534–9.
- Elashoff M, Matveyenko AV, Gier B, *et al.* Pancreatitis, pancreatic, and thyroid cancer with glucagon-like peptide-1-based therapies. *Gastroenterology* 2011;141:150–6.
- Jensen MT, Marrott JL, Allin KH, *et al.* Resting heart rate is associated with cardiovascular and all-cause mortality after adjusting for inflammatory markers: the Copenhagen City Heart Study. *Eur J Prev Cardiol* 2012;19:102–8.
- Barragán JM, Eng J, Rodríguez R, *et al.* Neural contribution to the effect of glucagon-like peptide-1-(7-36) amide on arterial blood pressure in rats. *Am J Physiol* 1999;277:E784–91.
- Gardiner SM, March JE, Kemp PA, *et al.* Mesenteric vasoconstriction and hindquarters vasodilatation accompany the pressor actions of exendin-4 in conscious rats. *J Pharmacol Exp Ther* 2006;316:852–9.
- Shigeta T, Aoyama M, Bando YK, *et al.* Dipeptidyl peptidase-4 modulates left ventricular dysfunction in chronic heart failure via angiogenesis-dependent and -independent actions. *Circulation* 2012;126:1838–51.
- Nathanson D, Ullman B, Löfström U, *et al.* Effects of intravenous exenatide in type 2 diabetic patients with congestive heart failure: a double-blind, randomised controlled clinical trial of efficacy and safety. *Diabetologia* 2012;55:926–35.
- Scirica BM, Bhatt DL, Braunwald E, *et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013;369:1317–26.
- Suh S, Seo GH, Jung CH, *et al.* Increased risk of hospitalization for heart failure with newly prescribed dipeptidyl peptidase-4 inhibitors and pioglitazone using the Korean Health Insurance Claims Database. *Diabetes Metab J* 2015;39:247–52.
- Nyström T, Gutniak MK, Zhang Q, *et al.* Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 2004;287:E1209–15.
- Ceriello A, Esposito K, Testa R, *et al.* The possible protective role of glucagon-like peptide 1 on endothelium during the meal and evidence for an 'endothelial resistance' to glucagon-like peptide 1 in diabetes. *Diabetes Care* 2011;34:697–702.
- Tashiro Y, Sato K, Watanabe T, *et al.* A glucagon-like peptide-1 analog liraglutide suppresses macrophage foam cell formation and atherosclerosis. *Peptides* 2014;54:19–26.
- Rizzo M, Chandalia M, Patti AM, *et al.* Liraglutide decreases carotid intima-media thickness in patients with type 2 diabetes: 8-month prospective pilot study. *Cardiovasc Diabetol* 2014;13:49.
- Dokken BB, La Bonte LR, Davis-Gorman G, *et al.* Glucagon-like peptide-1 (GLP-1), immediately prior to reperfusion, decreases

- neurophil activation and reduces myocardial infarct size in rodents. *Horm Metab Res* 2011;43:300–5.
30. Timmers L, Henriques JPS, de Kleijn DPV, *et al.* Exenatide reduces infarct size and improves cardiac function in a porcine model of ischemia and reperfusion injury. *J Am Coll Cardiol* 2009;53:501–10.
 31. McCormick LM, Hoole SP, White PA, *et al.* Pre-treatment with glucagon-like Peptide-1 protects against ischemic left ventricular dysfunction and stunning without a detected difference in myocardial substrate utilization. *JACC Cardiovasc Interv* 2015;8:292–301.
 32. Read PA, Hoole SP, White PA, *et al.* A pilot study to assess whether glucagon-like peptide-1 protects the heart from ischemic dysfunction and attenuates stunning after coronary balloon occlusion in humans. *Circ Cardiovasc Interv* 2011;4:266–72.
 33. Lønborg J, Vejlstrop N, Kelbæk H, *et al.* Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction. *Eur Heart J* 2012;33:1491–9.
 34. Filippatos TD, Elisaf MS. Effects of glucagon-like peptide-1 receptor agonists on renal function. *World J Diabetes* 2013;4:190–201.
 35. Kao DP, Kohrt HE, Kugler J. Renal failure and rhabdomyolysis associated with sitagliptin and simvastatin use. *Diabet Med* 2008;25:1229–30.
 36. Pendergrass M, Fenton C, Haffner SM, *et al.* Exenatide and sitagliptin are not associated with increased risk of acute renal failure: a retrospective claims analysis. *Diabetes Obes Metab* 2012;14:596–600.
 37. Yu M, Moreno C, Hoagland KM, *et al.* Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003;21:1125–35.
 38. Liu Q, Adams L, Broyde A, *et al.* The exenatide analogue AC3174 attenuates hypertension, insulin resistance, and renal dysfunction in Dahl salt-sensitive rats. *Cardiovasc Diabetol* 2010;9:32.
 39. Park CW, Kim HW, Ko SH, *et al.* Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J Am Soc Nephrol* 2007;18:1227–38.
 40. Muskiet MHA, Smits MM, Morsink LM, *et al.* The gut-renal axis: do incretin-based agents confer renoprotection in diabetes? *Nat Rev Nephrol* 2014;10:88–103.
 41. Bergenstal RM, Wysham C, Macconell L, *et al.* Efficacy and safety of exenatide once weekly versus sitagliptin or pioglitazone as an adjunct to metformin for treatment of type 2 diabetes (DURATION-2): a randomised trial. *Lancet* 2010;376:431–9.
 42. The Evaluation of Lixisenatide in Acute Coronary Syndrome—The Results of ELIXA. Session 3-CT-SY28. In: *American Diabetes Association 2015 Scientific Sessions*. 2015.
 43. Mosenzon O, Bhatt DL, Litwak LE, *et al.* Effects of saxagliptin on renal outcome. *EASD Annual Meeting*; Vienna, Austria, 15–19 September 2014. Abstract #183.
 44. Groop P-H, Cooper ME, Perkovic V, *et al.* Linagliptin lowers albuminuria on top of recommended standard treatment in patients with type 2 diabetes and renal dysfunction. *Diabetes Care* 2013;36:3460–8.
 45. Udell JA, Bhatt DL, Braunwald E, *et al.* Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus and moderate or severe renal impairment: observations from the SAVOR-TIMI 53 trial. *Diabetes Care* 2015;38:696–705.
 46. Muskiet MHA, Tonneijck L, Smits MM, *et al.* Pleiotropic effects of type 2 diabetes management strategies on renal risk factors. *Lancet Diabetes Endocrinol* 2015;3:367–81.
 47. Nachnani JS, Bulchandani DG, Nookala A, *et al.* Biochemical and histological effects of exendin-4 (exenatide) on the rat pancreas. *Diabetologia* 2010;53:153–9.
 48. Butler PC, Matveyenko AV, Dry S, *et al.* Glucagon-like peptide-1 therapy and the exocrine pancreas: innocent bystander or friendly fire? *Diabetologia* 2010;53:1–6.
 49. Thomsen RW, Pedersen L, Møller N, *et al.* Incretin-based therapy and risk of acute pancreatitis: a Nationwide Population-Based Case-Control Study. *Diabetes Care* 2015;38:1089–98.
 50. Egan AG, Blind E, Dunder K, *et al.* Pancreatic safety of incretin-based drugs—FDA and EMA assessment. *N Engl J Med* 2014;370:794–7.
 51. Fiorentino TV, Owston M, Abrahamian G, *et al.* Chronic continuous exenatide infusion does not cause pancreatic inflammation and ductal hyperplasia in non-human primates. *Am J Pathol* 2015;185:139–50.
 52. Meier JJ, Gallwitz B, Salmen S, *et al.* Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. *J Clin Endocrinol Metab* 2003;88:2719–25.
 53. Carbone LJ, Angus PW, Yeomans ND. Incretin-based therapies for the treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2015. Published Online First: 25 June 2015. doi:10.1111/jgh.13026
 54. Hellström PM, Hein J, Bytzer P, *et al.* Clinical trial: the glucagon-like peptide-1 analogue ROSE-010 for management of acute pain in patients with irritable bowel syndrome: a randomized, placebo-controlled, double-blind study. *Aliment Pharmacol Ther* 2009;29:198–206.
 55. Marathe CS, Rayner CK, Jones KL, *et al.* Effects of GLP-1 and incretin-based therapies on gastrointestinal motor function. *Exp Diabetes Res* 2011;2011:279530.
 56. Wettergren A, Schjoldager B, Mortensen PE, *et al.* Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig Dis Sci* 1993;38:665–73.
 57. Wettergren A, Wojdemann M, Meisner S, *et al.* The inhibitory effect of glucagon-like peptide-1 (GLP-1) 7-36 amide on gastric acid secretion in humans depends on an intact vagal innervation. *Gut* 1997;40:597–601.
 58. Schjoldager BT, Mortensen PE, Christiansen J, *et al.* GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in humans. *Dig Dis Sci* 1989;34:703–8.
 59. Keller J, Trautmann ME, Haber H, *et al.* Effect of exenatide on cholecystokinin-induced gallbladder emptying in fasting healthy subjects. *Regul Pept* 2012;179:77–83.
 60. Seghieri M, Rebelos E, Gastaldelli A, *et al.* Direct effect of GLP-1 infusion on endogenous glucose production in humans. *Diabetologia* 2013;56:156–61.
 61. Fehse F, Trautmann M, Holst JJ, *et al.* Exenatide augments first- and second-phase insulin secretion in response to intravenous glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2005;90:5991–7.
 62. Griffioen KJ, Wan R, Okun E, *et al.* GLP-1 receptor stimulation depresses heart rate variability and inhibits neurotransmission to cardiac vagal neurons. *Cardiovasc Res* 2011;89:72–8.
 63. Gutzwiller J-P, Tschopp S, Bock A, *et al.* Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J Clin Endocrinol Metab* 2004;89:3055–61.