The effect of a glucagon-like peptide-1 receptor agonist on glucose tolerance in women with previous gestational diabetes mellitus: Protocol for an investigator-initiated, randomised, placebo-controlled, double-blinded, parallel intervention trial

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ABSTRACT

Introduction: Pregnancy is associated with decreased insulin sensitivity, which is usually overcome by a compensatory increase in insulin secretion. Some pregnant women are not able to increase their insulin secretion sufficiently, and consequently develop gestational diabetes mellitus (GDM). The disease normally disappears after delivery. Nevertheless, women with previous GDM have a high risk of developing type 2 diabetes (T2D) later in life. We aim to investigate the early development of T2D in women with previous GDM and to evaluate whether treatment with the glucagon-like peptide-1 receptor (GLP-1R) agonist, liraglutide, may modify their risk of developing T2D.

Methods and analyses: One hundred women with previous GDM will be randomised to either liraglutide or placebo treatment for 1 year (blinded) with an open-label extension for another 4 years. Additionally, 15 women without previous GDM will constitute a baseline control group. Women will be tested with an oral glucose tolerance test (primary endpoint: area under curve for plasma glucose) and an isoglycaemic intravenous glucose infusion at baseline, after one year, and after five years. Additional evaluations include a glucagon test, dual-energy X-ray absorptiometry, imaging of the liver (ultrasound elastography and fibro-scanning), an *ad libitum* meal for food intake evaluation and questionnaires related to appetite, quality of life and alcohol consumption habits.

Ethics and dissemination: The protocol has been approved by the Danish Medicines Agency, the Scientific-Ethical Committee of the Capital Region of Denmark, and the Danish Data Protection Agency and will be carried out under the surveillance and guidance of the GCP unit at Copenhagen University Hospital Bispebjerg in compliance with the ICH-GCP guidelines and in accordance with the Helsinki Declaration. Positive, negative, and inconclusive results will be published at scientific conferences and as one or more scientific manuscripts in peer-reviewed journals.

Registrations: The trial is registered at https://eudract.ema.europa.eu (2012-001371-37) and www.clinicaltrials.gov (NCT01795248).

ARTICLE SUMMARY

Article focus

- This article describes a study protocol for a randomised, placebo-controlled trial evaluating the effect of the glucagon-like peptide-1 receptor agonist, liraglutide, in patients with previous gestational diabetes mellitus
- We hypothesise that the glucose tolerance after an oral glucose challenge, one and five years after study commencement, is superior after liraglutide compared to placebo treatment

Key messages

- Women with previous gestational diabetes mellitus are at high risk of developing type 2 diabetes later in life
- This will be the first study investigating the effect of a glucagon-like peptide-1 receptor agonist on the risk of developing type 2 diabetes in women with previous gestational diabetes mellitus
- Women with previous gestational diabetes mellitus constitute a high-risk group and, thus, a unique opportunity to investigate the early development of type 2 diabetes

Strengths and limitations of this study

- Participants will be monitored extensively during a 1-year blinded phase and throughout a 4-year extension period
- Although all eligible women from a large part of Denmark has been invited to the study, the study population may not be fully representative
- The study may not be powered to conclude on the secondary endpoints

INTRODUCTION

Women with previous gestational diabetes mellitus represent a high-risk group

Pregnancy is associated with insulin resistance (1). This is normally compensated for by increased pancreatic insulin secretion, resulting in retained normal glucose tolerance (NGT) (2). Women with gestational diabetes mellitus (GDM) are not capable of increasing their insulin secretion enough to compensate for pregnancy-induced insulin resistance, resulting in abnormal glucose tolerance. In the majority of women, glucose tolerance is re-established after delivery. Nevertheless, most women with previous GDM are at high risk of developing T2D later in life (3,4). *Bellamy et al*. state that up to 63% of women with GDM develop T2D within 16 years (5), *Bian et al*. describe a risk of 33% for the development of T2D from 5-10 years after GDM (6), and a Danish study reports a risk of 40% for the development of T2D 7 years after diet-treated GDM (3). Insulin dependency, high plasma glucose (PG) levels during oral glucose tolerance test (OGTT), difficult controllable glycaemia (need of insulin-treatment), and high body mass index (BMI) during pregnancy are strong predictors of the risk of T2D after GDM (4).

The early pathophysiology of type 2 diabetes remains elusive

Studies show that, on average, patients with T2D are diagnosed when their relative beta cell function is approximately 50% of normal (7). The delayed onset of symptoms makes it difficult to characterise the pathogenesis of T2D. There is general agreement that both insulin resistance and abnormal insulin secretion co-exist in T2D. Nevertheless, the order in which the pathophysiological characteristics of T2D occur remains unclear (8). Studies of first-degree relatives of patients with T2D have yielded conflicting results regarding whether abnormal insulin secretion or insulin resistance is the primary defect in the disease (9–17). Other physiological defects have been suggested as primary pathogenic defects. These include decreased first-phase insulin response and impaired incretin effect (decreased beta cell response to the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)). In healthy subjects, both incretin hormones possess strong glucose-dependent insulinotropic properties and enhance glucose-

induced insulin secretion from the beginning of a meal (18–20). This potentiation of insulin secretion after oral glucose ingestion is called the incretin effect, and is illustrated when orally administered glucose elicits a greater insulin response than intravenous glucose at identical PG profiles. Patients with T2D exhibit impaired incretin effect. The cause of this pathophysiological trait is unclear (21–23). Understanding the pathophysiology in the early stages of T2D may provide new and effective interventions aiming at preventing the development of the disease. One approach to achieve this is to prospectively follow a high-risk group.

Liraglutide - a once-daily GLP-1 receptor agonist

GLP-1 receptor (GLP-1R) agonists are used in the treatment of overweight patients with T2D. Preclinical studies show that GLP-1R agonists may have beta cell protective properties (reduced beta cell apoptosis). Clinical studies have described sustained effects (two years) on glucose tolerance, beta cell function, and body weight in obese patients *without* diabetes (24,25).

OBJECTIVE

Because of their high risk of developing T2D, overweight and obese non-diabetic women with previous GDM provide an opportunity for investigating how the early stages of T2D develop and whether GLP-1R agonists prevent or delay the onset of T2D. The primary objective of the present protocol is to assess whether treatment with the GLP-1R agonist liraglutide reduces the risk of T2D in women with previous GDM. Simultaneously, the developmental trajectories of pathophysiological defects known from patients with T2D will be evaluated. Such information will ultimately contribute to a clarification of the pathogenesis of T2D and provide a basis for preventive measures and interventions. We hypothesise that women with previous GDM receiving liraglutide treatment for a year will have a smaller area under the curve (AUC) for PG during an OGTT carried out after one week of wash-out (primary endpoint) than women with previous GDM treated with placebo. The primary endpoint will be re-evaluated after another 4 years of liraglutide treatment or no intervention. Secondary endpoints include metabolic measures, biomarkers and questionnaires as described below.

METHODS AND ANALYSES

Study design, randomisation and blinding

This is an investigator-initiated, randomised, placebo-controlled, double-blinded, two-arm parallel group intervention trial carried out in non-diabetic women with previous GDM. Participants with previous GDM will be randomised to treatment with liraglutide or placebo after the baseline experiments. Adequate randomisation will be ensured by stratification based on a computer-generated random number sequence (1:1) with stratified block (permuted-block size) with participants stratified according to the baseline glucose tolerance: NGT versus non-NGT (impaired fasting glucose (IFG), impaired glucose tolerance (IGT) or combined IFG/IGT). Participants with T2D at baseline will not be included in the trial. The randomisation will be carried out through a central independent unit via an unblinded data manager at Public Health and Quality Improvement, Central Denmark Region, Denmark. The allocation sequence will be concealed from the investigators and healthcare staff enrolling and assessing participants. Trial medication will be allocated via the electronic case report form (eCRF). The unblinded person will be impartial and have no influence or knowledge of the treatment of the participants following randomisation. Participants, investigators and healthcare staff will remain blinded for the allocated treatment and kept masked until the afternoon of visit 10 (OGTT after 1 year). Data analysis will be carried out blinded.

Study population and study sites

Ninety eight participants will participate in the randomised trial and 15 healthy women without previous GDM will make up a baseline control group. The trial will be conducted at the Diabetes Research Division, Copenhagen University Hospital Gentofte, Denmark. Recruitment will take place by an invitation letter send to all women, who have been diagnosed with GDM at either Center for Pregnant Women with Diabetes, Rigshospitalet, Copenhagen, Denmark or Department of Gynaecology-Obstetrics, Copenhagen University Hospital Herlev, Denmark, within the last 10 years. Moreover, participants will be recruited from advertisements in

newspapers and on the internet. Total number of subjects in the following categories will be recorded: invited, excluded before screening (including reasons for exclusion), declined to participate, not reached, received participants information and declined, screened, excluded at screening, randomised, allocated for intervention, received allocated intervention, completed intervention period, withdrew/dropped out during study period (including reason for withdrawal/dropping out), lost to follow-up, and analysed. Recruitment is planned to proceed until 49 participants in each group have been randomised. Reasons for withdrawal or exclusion will be reported in details. Inclusion and exclusion criteria are presented in Box 1 and Box 2.

BOX 1 – Inclusion criteria

Inclusion criteria - women with previous gestational diabetes mellitus (GDM)

- Informed oral and written consent
- Age ≥18 years
- Body mass index (BMI) between 25 kg/m^2 and 45 kg/m^2
- Previous diagnosis of GDM according to current Danish guidelines (plasma glucose (PG) concentration at 120 min after 75g-oral glucose tolerance test (OGTT) ≥9.0 mmol/l during pregnancy) within the last ten years
- Normal glucose tolerance (NGT), impaired fasting glucose (IFG), and/or impaired glucose tolerance (IGT)
- Use of safe contraception or sterilisation
- Negative pregnancy test

Inclusion criteria - women without previous GDM

- Informed oral and written consent
- Age ≥18 years
- BMI between 25 kg/m^2 and 45 kg/m^2
- NGT (fasting PG (FPG) ≤6.0 mmol/l *and* PG concentration at 120 min after 75g-OGTT <7.8 mmol/l)
- Pregnancy within the last ten years without GDM
- No family history of type 2 diabetes
- Negative pregnancy test

BOX 2 – Exclusion criteria

Exclusion criteria - women with previous gestational diabetes mellitus (GDM)

- Pregnancy or breast feeding
- Anaemia (haemoglobin <7 mmol/l)
- Diabetes (fasting plasma glucose (FPG) >7.0 mmol/l *or* plasma glucose (PG) concentration at 120 min after 75g-oral glucose tolerance test ≥11.1 mmol/l *or* glycated haemoglobin A_{1c} (HbA_{1c}) ≥48 mmol/mol (6.5%))
- Previous pancreatitis
- Cancer within the last five years
- Desire to become pregnant within the next five years
- Treatment with statins, corticosteroids, or other hormone therapy (except estrogens and gestagens)
- Ongoing abuse of alcohol or narcotics
- Impaired hepatic function (liver transaminases >3 times upper normal limit)
- Impaired renal function (se-creatinine >120 µM and/or albuminuria)
- Uncontrolled hypertension (systolic blood pressure >180 mmHg, diastolic blood pressure >100 mmHg)
- Receiving any investigational drug within the last 3 months
- Any condition that the investigator feels would interfere with trial participation

Exclusion criteria - women without previous gestational diabetes mellitus

- Pregnancy or breastfeeding
- Anaemia (haemoglobin <7 mmol/l)

Experimental design

Eligible participants will receive detailed oral and written information about the study. Sufficient time for reflection will be allowed before written informed consent and authorisation are obtained. Both groups will attend the same visits unless stated otherwise. An initial screening visit will be followed by a baseline OGTT and an isoglycaemic intravenous glucose infusion (IIGI) (Fig. 1).

Following baseline OGTT, IIGI and other baseline procedures (including blood sampling, full body dual energy X-ray absorptiometry (DXA) scanning, glucagon test, imaging of the liver (ultrasound elastography and fibro-scanning) (Fig. 1)), the participants are randomised to once-daily injections of either liraglutide 1.8 mg or placebo. In the 1-year intervention period, the participants will be monitored at regular clinical control visits and phone calls. At the end of the intervention period, the OGTT will be repeated, as well as all other procedures made at baseline, with an additional OGTT after 1-week washout in the liraglutide-treated group. Finally, an IIGI, matching the blood glucose profile of the latter OGTT, will be conducted in both groups. Both groups will attend biannual control visits in the 4-year follow-up period and terminate the study with the same experimental days as at the end of the intervention period. The women without previous GDM will only participate in the study for the baseline OGTT and IIGI experimental days and their related baseline procedures. An outline of the trial visits and experimental procedures is shown in Box 3.

BOX 3 – Trial visits and examinations

	Informed consent	Physical examination	Full body DXA scan	Ultrasound and fibroscan of liver	Blood samples	Glucagon test	*Ad libitum* meal and questionnaire	Appetite questionnaire	Quality of life questionnaire	Adverse event assessment

Screening, week -1	X	X			X					
OGTT, week 0					X			X		
IIGI, week 0			X	X	X	X	X	X	X	
Telephone, week 1										X
Control, week 4		X			X					X
Telephone, week 8										X
Control, week 12		X			X					X
Control, week 26		X			X					X
Control, week 38		X			X					X
OGTT, week 52					X	X		X		X
OGTT, week 53 (only liraglutide group)					X			X		X
IIGI, week 53			X	X	X	X	X	X	X	X
Control, week 78		X			X					X
Control, week 104		X			X					X
Control, week 130		X			X					X
Control, week 156		X			X					X
Control, week 182		X			X					X
Control, week 208		X			X					X
Control, week 234		X			X					X
OGTT, week 260					X	X		X		X
OGTT, week 261 (only liraglutide group)					X			X		
IIGI, week 261			X	X	X	X	X	X	X	

On the initial screening day, fasting blood samples (Table 1) will be collected to verify that the participant fulfils the inclusion and exclusion criteria, medical history will be recorded and a full physical examination will be performed. All participants are required to use adequate contraceptive methods throughout the intervention and follow-up period. Intrauterine device will be offered.

Analysis	Sampling period
• Albumin • ALT, AST, amylase • Cholesterol, triglycerides • Creatinine, potassium, sodium • Erythrocytes • Glycated haemoglobin (HbA_{1c}) • Haemoglobin • Leucocytes • TSH	Screening and control visits
• Glucose • Insulin, proinsulin, C-peptide	Screening, control visits, OGTT, IIGI and glucagon test
• Glucose-dependent insulinotropic polypeptide (GIP) • Glucagon • Glucagon-like peptide-1 (GLP-1) • Glucagon-like peptide-2 (GLP-2)	OGTT and IIGI
• Beta hCG • Coagulation factor 2, 7 and 10 • Urine albumin:creatinine ratio	Screening

Table 1. *Blood samples. ALT: alanine aminotransferase, AST: aspartate aminotransferase, hCG: human chorionic gonadotrophin, IIGI: isoglycaemic intravenous glucose infusion, OGTT: oral glucose tolerance test, TSH: thyroid-stimulating hormone*

Within 10 days of the screening visit, a 4h 75g-OGTT will be performed. The participant will ingest 75 g glucose dissolved in 300 ml water over 5 minutes and, subsequently; repeated blood samples for measurements of PG, insulin, C-peptide, glucagon, GLP-1, and GIP will be drawn throughout the test. At baseline and once an hour afterwards, the participants will answer a questionnaire on appetite using a visual analogue scale (VAS) on a tablet-computer. If the 2h PG value is below 11.1 mmol/l, the participant will be randomised. At visit 10 (after 1 year) and 20 (after 5 years) a glucagon test will be carried out at the end of the OGTT. Within 10 days of

the OGTT, the participants will attend an IIGI. In the morning of the IIGI, they are first DXA scanned, and their liver will be assessed by ultrasound scanning and fibro-scanning (26). Over the next 4 hours, the PG curve from the OGTT day will be mimicked using a 20% glucose intravenous infusion. Again, repeated blood samples will be drawn and questionnaires answered as on the OGTT day, although PG may be sampled more frequently to adjust the glucose infusion rate. Two additional questionnaires, one regarding quality of life (QoL) and one regarding alcohol consumption habits, will be filled out once during the IIGI. At the end of the IIGI, a glucagon test will be performed; at time point 0 min 1 mg glucagon will be injected and at time points 2, 6, and 10 minutes, blood will be sampled for measurements of PG, insulin and C-peptide. Next, the participants are offered an *ad libitum* meal consisting of pasta, minced meat, vegetables and cream, which they are instructed to eat as much of "until comfortably satisfied" in addition to drinking 500 ml water. The distribution of energy in the *ad libitum* meal is 50 energy percent (E%) carbohydrates, 37 E% fat, and 13 E% protein. Additional palatability ratings (palatability, taste, aftertaste, smell, and visual appeal) of the meal are filled in immediately after the first few bites and after consumption of the *ad libitum* meal using VAS scores on a tablet. The participants are then instructed in how to inject the trial medication, and how to measure blood glucose (*Contour;* Bayer HealthCare, Copenhagen, Denmark) twice a month and record it in a diary. One and eight weeks after the IIGI day, the participants will be contacted by phone for clarification of any questions they may have and any adverse events are recorded in the eCRF. Four, 12, 26 and 38 weeks after the IIGI, and biannually in the follow-up period, participants will meet in the fasting state for clinical control visits. Measurements of weight, waist-hip-ratio, blood pressure and pulse, compliance, and adverse events will be recorded, and trial medication will be dispensed. At 52 weeks, the OGTT will be repeated for both groups and placebo will be initiated for one week in both groups. An additional OGTT day will be carried out in the liraglutide-group to evaluate glucose tolerance off trial medication. Finally, an IIGI, as described above, will be conducted in both groups, matching the blood glucose profile of the latest OGTT. The exact same setup will be repeated at the end of the 4 year follow-up period.

Intervention

Trial medication will be initiated at the end of the IIGI day in a dosage of 0.6 mg sc. once daily. All participants will be instructed to inject the medicine in the abdomen once daily. Trial medication dose will be escalated over three weeks with once-weekly dose adjustments in increments of 0.6 mg to a maximal dose of 1.8 mg. If intolerable adverse events occur, the dose may be reduced to 1.2 mg, or the participant may remain on 1.2 mg for more than a week before increasing the dose. Trial medication will be delivered in boxes of 5 pre-filled, disposable pen-injectors each containing 3 ml of a premixed colourless solution. The active pens will contain liraglutide (6 mg/ml) mixed with sterile water, disodium phosphate dihydrate, propylene glycol, and phenol. Pens containing placebo will be visually identical to those with the active component and be composed of sterile water, disodium phosphate dihydrate, propylene glycol, and phenol.

Outcome measures and analysis methods

Primary endpoint

The primary endpoint is the change in glucose tolerance from baseline to week 52 as measured by the AUC for the PG excursion following a 4h 75g-OGTT. Additional endpoints regarding glucose tolerance include changes from baseline to week 53 (after trial medication wash-out), to week 260 (end of extension period), and to week 261 (after trial medication washout). The primary endpoint will be analysed by the intention-to-treat approach. In case of missing data points, values will be imputed. Safety measures will be analysed in the per protocol population. In case the full intervention period has not been completed, we will use the 'last observation carried forward' method.

Secondary endpoints

All secondary endpoints will be assessed as changes from baseline until 52 weeks, and until the end of the extension period (260 weeks). In addition, most endpoints will

also be assessed as changes from baseline until week 53 and week 261 (after trial medication wash-out).The secondary endpoints are listed in box 4 and 5.

- Worsening of glycaemia assessed by PG, expressed as percentage of participants in each treatment arm with NGT at inclusion who develop
  - Prediabetes
  - T2D
- Progression, assessed by PG, expressed as percentage of participants in each treatment arm with isolated IFG or isolated IGT who progress to
  - Combined IFG/IGT
  - T2D
- Progression, assessed by PG, expressed as percentage of participants with combined IFG/IGT who progress to
  - T2D
- Improvement in glycaemic status, assessed by PG, expressed as percentage of participants in each treatment arm going from
  - IFG to NGT
  - IGT to NGT
  - Combined IFG/IGT to NGT or isolated IFG or IGT
- Worsening of glycaemia, assessed by changes in HbA_{1c}, expressed as percentage of participants who progress from
  - Normoglycaemia to prediabetes
  - Normoglycaemia to T2D
  - Prediabetes to T2D
- Improvement of glycaemia, assessed by changes in HbA_{1c}, expressed as percentage of participants who progress from
  - Prediabetes to normoglycaemia

Box 4. *Secondary endpoints related to glycaemic status.* ***NGT****: normal glucose tolerance: FPG ≤6.0 mmol/l* and *2h PG <7.8 mmol/l;* ***normoglycaemia****: HbA_{1c} ≤42 mmol/mol (6.0%);* ***prediabetes****: IFG and/or IGT* or *43≤ HbA_{1c} ≤47 mmol/mol (6.1-6.4%);* ***IFG****: impaired fasting glucose: 6.1 mmol/l ≤ FPG <7.0 mmol/l* and *2h PG after 75 g-OGTT <7.8 mmol/l;* ***IGT****: impaired glucose tolerance: FPG ≤6.0 mmol/l* and *7.8 mmol/l ≤ 2h PG after 75 g-OGTT ≤11.0 mmol/l;* ***T2D****: FPG ≥7mM* or *2h PG ≥11.1 mmol/l; HbA_{1c} ≥48 mmol/mol;* ***OGTT****: oral glucose tolerance test;* ***IIGI****: isoglycaemic intravenous glucose infusion;* ***PG****: plasma glucose;* ***FPG:*** *fasting plasma glucose*

Changes in

- Anthropometric measurements (BMI, absolute body weight, and waist: hip ratio)
- Beta cell secretory responses during OGTT, IIGI, and glucagon test (AUC for plasma insulin, C-peptide and pro-insulin)
- Insulin sensitivity (assessment of insulin resistance ($HOMA_{IR}$) and Matsuda insulin sensitivity index)
- Hormone secretion (fasting plasma concentrations and AUC for plasma GLP-1, GLP-2, GIP, and glucagon) during OGTT
- Incretin effect and insulin secretory rate (AUC for plasma insulin and C-peptide responses after OGTT and IIGI)
- Cardio-metabolic risk measures (changes in ELF test, intrahepatic fat, whole body and visceral fat mass/fat-free mass, circulating lipids, and cardiovascular biomarkers (hs-CRP, NT-proBNP, TNF-α, adiponectin, and PAI-1)
- Subjective appetite (measured by VAS)
- QoL (measured by validated questionnaire)
- Alcohol consumption (measured by validated questionnaire)

Box 5. Additional secondary endpoints
***BMI**: body mas index; **AUC**: area under the curve; **OGTT**: oral glucose tolerance test; **IIGI**: isoglycaemic intravenous glucose infusion; **HOMA**: homeostasis model assessment; **GLP-1**: glucagon-like peptide-1; **GLP-1**: glucagon-like peptide-2; **GIP**: glucose-dependent insulinotropic peptide; **ELF**: enhanced liver fibrosis; **hs-CRP**: highly sensitive C-reactive protein; **NT-proBNP**: N-terminal prohormone of brain natriuretic peptide; **TNF-α**: tumour necrosis factor-alpha; **PAI-1**: plasminogen activator inhibitor-1; **VAS**: visual analogue scale; **QoL**: quality of life*

Sample size

The primary outcome measure was used in the sample size calculation. With expected end-of-treatment values of 1,713 mmol/l*min (standard deviation (SD): 212) and 1,853 mmol/l*min (SD: 212) in the intervention- and placebo group (27), respectively, and with alpha set to 5% and power to 90%, the estimated sample size was 98 participants (49 participants in each arm).

Data analysis

Continuous data will be presented by descriptive statistics with the number of observations (n), mean, standard deviation, standard error of the mean, minimum,

median, and/or maximum. Categorical data will be summarised in frequency tables using count and percentages. All participants will be presented in separate data listings. Data from participants screened, but not randomised, will not be presented in any tables or listings. Comparisons of data from the two treatment groups will be performed using two-tailed *t* test (paired within groups, unpaired between groups) if the data are normally distributed. For data which are not normally distributed, the significance of differences between the groups will be tested using Mann-Whitney U-test. For within-group comparisons, Wilcoxon test for paired differences will be used. One-way ANOVA will be used to compare means of several groups. Categorical data will be analysed by chi-square test or Fisher´s exact test. The relationship between an effect of liraglutide and hip, waist, and weight measures, visceral fat mass, FPG, HbA_{1c}, degree of steatosis, QoL, alcohol consumption, appetite, respectively, will be examined by correlation analysis. Multivariable linear regression analysis will be performed to evaluate the potential influence of predictors of primary outcomes. All tests will be carried out at a significance level of 5%. Adverse events (AEs) will be summarised qualitatively and quantitatively.

ETHICS AND DISSEMINATION

Ethics

The treatment is associated with minimal discomfort for the participants comprising blood sample collection and daily injection of liraglutide or placebo in the subcutis of the abdomen, in the thigh, or the upper arm. Common adverse events associated with the active comparator (liraglutide) are mild to moderate transient gastrointestinal symptoms (nausea, vomiting and diarrhoea), affecting around 10-15% of treated patients, and headache. The injection is practically pain-free but may leave a small haemorrhage which will resolve spontaneously. Less commonly, participants may experience stomach pain, constipation, fever, reflux, gastritis, dizziness, tiredness, and upper airway infection. When collecting blood, some participants may experience minor discomfort when the needle penetrates the skin and rarely a small bleeding occurs. The volume of blood collected during the entire study period will amount to a

maximum of 1,800 ml (over 5 years) and only participants with a normal haemoglobin level will be included. Severe systemic AEs are not expected.

A DXA scan will be performed 3 times during the study with the objective of determining the distribution of bone and adipose tissue. DXA scanning takes 15 minutes is a painless procedure with no expected side effects, and results in a modest radiation dose (approximately equivalent to 2-3 times the dose received from a dental X-ray). Scanning of the liver will be carried out using ultrasound. Ultrasound creates images using sound waves and no side effects are expected from this procedure.

The participants will receive thorough verbal and written information about the risk of developing the mentioned AEs. Verbal and written informed consent will be obtained from participants prior to participation in accordance with ICH-GCP guidelines. The declaration of consent will emphasize that participation in the project is voluntary and that participants may withdraw their consent to participate at any time without providing a reason and without any consequences for the patient's current or future treatment by the health service. The participants will receive a randomisation number after the baseline OGTT. All data forms and blood samples will only be labelled with the patient's initials and study number. The sponsor-investigator is responsible for keeping a list separately for all randomised participants containing patient numbers, full names and date of birth. Extra plasma, white blood cells, and urine will be stored for up to 15 years after the end of the study for repeated measurements in case of error analysis or the need for more analyses. The protocol is registered at the Danish Data Protection Agency (01714 GEH-2012-024).

The advantage for the participant, of participating in this trial, is the initial screening for diabetes and the subsequent monitoring of her blood glucose, which also allows for detection of diabetes, and hence early treatment. Serious adverse events are rare. Research will gain more understanding of the pathophysiology in the early stages of T2D. This may provide new and effective interventions aiming at preventing the development of the disease.

Dissemination

At the end of the trial, one or more manuscripts will be prepared for publication in scientific journals in accordance with the CONSORT 2010 Statement (28). Positive, negative, as well as inconclusive results will be published. Novo Nordisk will be given 4 weeks to review and comment on any manuscript/abstract or other means intended for publication or presentation of the data. The investigators will have the right to decide to publish the trial and the final responsibility for the result. All authors should qualify for authorship according to International Committee of Medical Journal Editors, 1997. Each author should have participated sufficiently in the work to take public responsibility for the content. The final decision on the order of authorship will be decided when the study has been finalised. The results from the study may, moreover, be presented as posters or oral presentations at national and/or international conferences.

STUDY APPROVAL

The study has been approved by the Danish Medicines Agency (EudraCT number: 2012-001371-37), the Scientific-Ethical Committee of the Capital Region of Denmark (H-2-2012-073), and the Danish Data Protection Agency (01714 GEH-2012-024). The study is registered at ClinicalTrials.gov (NCT01795248) and will be carried out under the surveillance and guidance of the GCP unit at Copenhagen University Hospital in compliance with the ICH-GCP guidelines conducted in accordance with the Helsinki Declaration.

AUTHOR'S CONTRIBUTIONS

SF and LV contributed to the design of the study and wrote the manuscript. ERM, JAS, LLG, JJH and PD contributed to the design of the study and reviewed the manuscript. FKK and TV conceived and designed the study and reviewed the manuscript. TV sponsors the trial. SF, LV, and FKK are co-investigators at Diabetes Research Division, Copenhagen University Hospital Gentofte; ERM and PD are co-investigators at Center for Pregnant Women with Diabetes, Copenhagen University Hospital Rigshospitalet; JAS is co-investigator at Department of Obstetrics and

Gynaecology, Copenhagen University Hospital Herlev; and JJH is co-investigator at Department of Biomedical Sciences, Faculty of Health Sciences, The NNF Center for Basic Metabolic Research, University of Copenhagen, Denmark. All authors have read and approved the final version of the manuscript.

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DECLARATION OF INTERESTS

Louise Vedtofte and Jens A. Svare have no conflict of interests in relation to the present paper. Signe Foghsgaard has received research support from Novo Nordisk. Lise L. Gluud has participated in a trial sponsored by Merck. Filip K. Knop has received lecture fees from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Gilead Sciences, Merck Sharp & Dohme, Novo Nordisk, Ono Pharmaceuticals, Sanofi, and Zealand Pharma, is a member of the Advisory Boards of Eli Lilly, Bristol-Myers Squibb/AstraZeneca and Zealand Pharma, and has consulted for AstraZeneca, Gilead Sciences, Ono Pharmaceuticals and Zealand Pharma. Elisabeth R. Mathiesen and Peter Damm have received lecture fees from Novo Nordisk and have been members of an Advisory Board of Novo Nordisk, Jens J. Holst has consulted for Merck Sharp and Dome, Novo Nordisk and Roche, Tina Vilsbøll has received lecture fees from AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Novartis, Sanofi, and Zealand Pharma, and is a member of the Advisory Boards of Novo Nordisk, Merck Sharp & Dohme and Bristol-Myers Squibb/AstraZeneca.

Figure legend

Figure 1. *Schematic illustration of study design. The first year is blinded and the remaining four years are open-label. Arrow: screening visit; star: oral glucose tolerance tests (OGTT); triangle: isoglycaemic intravenous glucose infusion, full body dual energy X-ray absorptiometry scanning, ultrasound scanning of the liver, fibro-scanning, glucagon test, and* ad libitum *meal test; dot: OGTT and subsequent glucagon test, light blue arrows: clinical control visits.*

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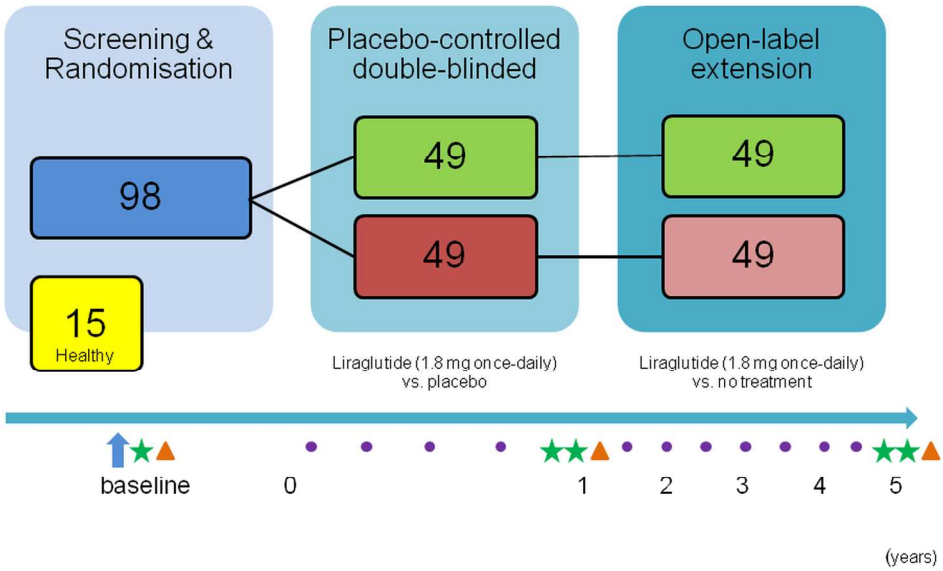

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254x190mm (300 x 300 DPI)