



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

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-003834
Article Type:	Protocol
Date Submitted by the Author:	16-Aug-2013
Complete List of Authors:	Foghsgaard, Signe; Copenhagen University Hospital Gentofte, Diabetes Research Division, Department of Internal Medicine Vedtofte, Louise; Copenhagen University Hospital Gentofte, Diabetes Research Division, Department of Internal Medicine Mathiesen, Elisabeth; Copenhagen University Hospital Rigshospitalet, Center for Pregnant Women with Diabetes, Department of Endocrinology Svare, Jens; Copenhagen University Hospital Herlev, Department of Gynaecology-Obstetrics Gluud, Lise; Copenhagen University Hospital Gentofte, Diabetes Research Division, Department of Internal Medicine Holst, Jens; Faculty of Health Sciences, University of Copenhagen, The NNF Center for Basic Metabolic Research, Department of Biomedical Sciences Damm, Peter; Copenhagen University Hospital Rigshospitalet, Center for Pregnant Women with Diabetes, Department of Obstetrics Knop, Filip; Copenhagen University Hospital Gentofte, Diabetes Research Division, Department of Internal Medicine Vilsbøll, Tina; Copenhagen University Hospital Gentofte, Diabetes Research Division, Department of Internal Medicine
Primary Subject Heading:	Diabetes and endocrinology
Secondary Subject Heading:	Nutrition and metabolism
Keywords:	DIABETES & ENDOCRINOLOGY, Diabetes in pregnancy < DIABETES & ENDOCRINOLOGY, General diabetes < DIABETES & ENDOCRINOLOGY

SCHOLARONE™
Manuscripts

1
2
3
4 **The effect of a glucagon-like peptide-1 receptor agonist on glucose**
5 **tolerance in women with previous gestational diabetes mellitus: Protocol**
6 **for an investigator-initiated, randomised, placebo-controlled, double-**
7 **blinded, parallel intervention trial**
8
9

10
11
12 SIGNE FOGHSGAARD, LOUISE VEDTOFTE, ELISABETH R MATHIESEN, JENS A SVARE, LISE L
13 GLUUD, JENS J HOLST, PETER DAMM, FILIP K KNOP, AND TINA VILSBØLL
14
15

16
17 *Diabetes Research Division, Department of Medicine, Copenhagen University*
18 *Hospital Gentofte, 2900 Hellerup, Denmark: Signe Foghsgaard Ph.D.-student, Louise*
19 *Vedtofte post.doc., Lise L Gluud senior consultant, Filip K Knop consultant, Tina*
20 *Vilbøll professor; Center for Pregnant Women with Diabetes, Department of*
21 *Endocrinology, Rigshospitalet, University of Copenhagen, 2100 Copenhagen Ø,*
22 *Denmark: Elisabeth R Mathiesen professor; Department of Gynaecology-Obstetrics,*
23 *Copenhagen University Hospital Herlev, 2730 Herlev, Denmark: Jens A Svare senior*
24 *consultant; Department of Biomedical Sciences, Faculty of Health Sciences, The*
25 *NNF Center for Basic Metabolic Research, University of Copenhagen, 2200*
26 *Copenhagen N, Denmark: Signe Foghsgaard Ph.D.-student, Jens J Holst professor,*
27 *Filip K Knop consultant; Center for Pregnant Women with Diabetes, Department of*
28 *Obstetrics, Rigshospitalet, University of Copenhagen, 2100 Copenhagen Ø,*
29 *Denmark: Peter Damm professor*
30
31

32
33 **Correspondence to**

34 Professor Tina Vilbøll, MD DMSc
35 Chief Consultant Endocrinologist & Head of Diabetes Research Division
36 Department of Medicine
37 Copenhagen University Hospital Gentofte
38 Niels Andersens Vej 65
39 DK-2900 Hellerup
40 Denmark
41 Tel: +45 4094 0825
42 Fax: +45 3977 7661
43 E-mail: t.vilboll@dadlnet.dk
44
45

46 Word counts

47 Abstract: 300 words

48 Body of manuscript (excl title page, abstract, summary and references) 4,058 words
49
50

51
52 Keywords: gestational diabetes mellitus, incretin hormones, oral glucose tolerance
53 test, GLP-1, type 2 diabetes
54
55
56
57
58
59
60

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-

1
2
3
4 induced insulin secretion from the beginning of a meal (18–20). This potentiation of
5 insulin secretion after oral glucose ingestion is called the incretin effect, and is
6 illustrated when orally administered glucose elicits a greater insulin response than
7 intravenous glucose at identical PG profiles. Patients with T2D exhibit impaired
8 incretin effect. The cause of this pathophysiological trait is unclear (21–23).
9 Understanding the pathophysiology in the early stages of T2D may provide new and
10 effective interventions aiming at preventing the development of the disease. One
11 approach to achieve this is to prospectively follow a high-risk group.
12
13
14
15
16
17
18

19 **Liraglutide - a once-daily GLP-1 receptor agonist**

20 GLP-1 receptor (GLP-1R) agonists are used in the treatment of overweight patients
21 with T2D. Preclinical studies show that GLP-1R agonists may have beta cell
22 protective properties (reduced beta cell apoptosis). Clinical studies have described
23 sustained effects (two years) on glucose tolerance, beta cell function, and body
24 weight in obese patients *without* diabetes (24,25).
25
26
27
28
29

30 **OBJECTIVE**

31 Because of their high risk of developing T2D, overweight and obese non-diabetic
32 women with previous GDM provide an opportunity for investigating how the early
33 stages of T2D develop and whether GLP-1R agonists prevent or delay the onset of
34 T2D. The primary objective of the present protocol is to assess whether treatment
35 with the GLP-1R agonist liraglutide reduces the risk of T2D in women with previous
36 GDM. Simultaneously, the developmental trajectories of pathophysiological defects
37 known from patients with T2D will be evaluated. Such information will ultimately
38 contribute to a clarification of the pathogenesis of T2D and provide a basis for
39 preventive measures and interventions. We hypothesise that women with previous
40 GDM receiving liraglutide treatment for a year will have a smaller area under the
41 curve (AUC) for PG during an OGTT carried out after one week of wash-out (primary
42 endpoint) than women with previous GDM treated with placebo. The primary
43 endpoint will be re-evaluated after another 4 years of liraglutide treatment or no
44 intervention. Secondary endpoints include metabolic measures, biomarkers and
45 questionnaires as described below.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4 newspapers and on the internet. Total number of subjects in the following categories
5 will be recorded: invited, excluded before screening (including reasons for exclusion),
6 declined to participate, not reached, received participants information and declined,
7 screened, excluded at screening, randomised, allocated for intervention, received
8 allocated intervention, completed intervention period, withdrew/dropped out during
9 study period (including reason for withdrawal/dropping out), lost to follow-up, and
10 analysed. Recruitment is planned to proceed until 49 participants in each group have
11 been randomised. Reasons for withdrawal or exclusion will be reported in details.
12 Inclusion and exclusion criteria are presented in Box 1 and Box 2.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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/m² and 45 kg/m²
- 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/m² and 45 kg/m²
- 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
<i>Ad libitum</i> 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
<ul style="list-style-type: none"> • Albumin • ALT, AST, amylase • Cholesterol, triglycerides • Creatinine, potassium, sodium • Erythrocytes • Glycated haemoglobin (HbA_{1c}) • Haemoglobin • Leucocytes • TSH 	Screening and control visits
<ul style="list-style-type: none"> • Glucose • Insulin, proinsulin, C-peptide 	Screening, control visits, OGTT, IIGI and glucagon test
<ul style="list-style-type: none"> • Glucose-dependent insulinotropic polypeptide (GIP) • Glucagon • Glucagon-like peptide-1 (GLP-1) • Glucagon-like peptide-2 (GLP-2) 	OGTT and IIGI
<ul style="list-style-type: none"> • 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

1
2
3
4 the OGTT, the participants will attend an IIGI. In the morning of the IIGI, they are first
5 DXA scanned, and their liver will be assessed by ultrasound scanning and fibro-
6 scanning (26). Over the next 4 hours, the PG curve from the OGTT day will be
7 mimicked using a 20% glucose intravenous infusion. Again, repeated blood samples
8 will be drawn and questionnaires answered as on the OGTT day, although PG may
9 be sampled more frequently to adjust the glucose infusion rate. Two additional
10 questionnaires, one regarding quality of life (QoL) and one regarding alcohol
11 consumption habits, will be filled out once during the IIGI. At the end of the IIGI, a
12 glucagon test will be performed; at time point 0 min 1 mg glucagon will be injected
13 and at time points 2, 6, and 10 minutes, blood will be sampled for measurements of
14 PG, insulin and C-peptide. Next, the participants are offered an *ad libitum* meal
15 consisting of pasta, minced meat, vegetables and cream, which they are instructed to
16 eat as much of “until comfortably satisfied” in addition to drinking 500 ml water. The
17 distribution of energy in the *ad libitum* meal is 50 energy percent (E%) carbohydrates,
18 37 E% fat, and 13 E% protein. Additional palatability ratings (palatability, taste,
19 aftertaste, smell, and visual appeal) of the meal are filled in immediately after the first
20 few bites and after consumption of the *ad libitum* meal using VAS scores on a tablet.
21 The participants are then instructed in how to inject the trial medication, and how to
22 measure blood glucose (*Contour*; Bayer HealthCare, Copenhagen, Denmark) twice a
23 month and record it in a diary. One and eight weeks after the IIGI day, the
24 participants will be contacted by phone for clarification of any questions they may
25 have and any adverse events are recorded in the eCRF. Four, 12, 26 and 38 weeks
26 after the IIGI, and biannually in the follow-up period, participants will meet in the
27 fasting state for clinical control visits. Measurements of weight, waist-hip-ratio, blood
28 pressure and pulse, compliance, and adverse events will be recorded, and trial
29 medication will be dispensed. At 52 weeks, the OGTT will be repeated for both
30 groups and placebo will be initiated for one week in both groups. An additional OGTT
31 day will be carried out in the liraglutide-group to evaluate glucose tolerance off trial
32 medication. Finally, an IIGI, as described above, will be conducted in both groups,
33 matching the blood glucose profile of the latest OGTT. The exact same setup will be
34 repeated at the end of the 4 year follow-up period.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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.

For peer review only

- 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 $43 \leq \text{HbA}_{1c} \leq 47$ mmol/mol (6.1-6.4%); **IFG:** impaired fasting glucose: $6.1 \text{ mmol/l} \leq \text{FPG} < 7.0$ mmol/l and 2h PG after 75 g-OGTT < 7.8 mmol/l; **IGT:** impaired glucose tolerance: $\text{FPG} \leq 6.0$ mmol/l and $7.8 \text{ mmol/l} \leq 2\text{h PG after } 75 \text{ g-OGTT} \leq 11.0$ mmol/l; **T2D:** $\text{FPG} \geq 7\text{mM}$ or $2\text{h PG} \geq 11.1$ mmol/l; $\text{HbA}_{1c} \geq 48$ 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 mass 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-2:** 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,

1
2
3
4 median, and/or maximum. Categorical data will be summarised in frequency tables
5 using count and percentages. All participants will be presented in separate data
6 listings. Data from participants screened, but not randomised, will not be presented in
7 any tables or listings. Comparisons of data from the two treatment groups will be
8 performed using two-tailed *t* test (paired within groups, unpaired between groups) if
9 the data are normally distributed. For data which are not normally distributed, the
10 significance of differences between the groups will be tested using Mann-Whitney U-
11 test. For within-group comparisons, Wilcoxon test for paired differences will be used.
12 One-way ANOVA will be used to compare means of several groups. Categorical data
13 will be analysed by chi-square test or Fisher's exact test. The relationship between
14 an effect of liraglutide and hip, waist, and weight measures, visceral fat mass, FPG,
15 HbA_{1c}, degree of steatosis, QoL, alcohol consumption, appetite, respectively, will be
16 examined by correlation analysis. Multivariable linear regression analysis will be
17 performed to evaluate the potential influence of predictors of primary outcomes. All
18 tests will be carried out at a significance level of 5%. Adverse events (AEs) will be
19 summarised qualitatively and quantitatively.
20
21
22
23
24
25
26
27
28
29
30

31 32 **ETHICS AND DISSEMINATION**

33 34 35 **Ethics**

36
37 The treatment is associated with minimal discomfort for the participants comprising
38 blood sample collection and daily injection of liraglutide or placebo in the subcutis of
39 the abdomen, in the thigh, or the upper arm. Common adverse events associated
40 with the active comparator (liraglutide) are mild to moderate transient gastrointestinal
41 symptoms (nausea, vomiting and diarrhoea), affecting around 10-15% of treated
42 patients, and headache. The injection is practically pain-free but may leave a small
43 haemorrhage which will resolve spontaneously. Less commonly, participants may
44 experience stomach pain, constipation, fever, reflux, gastritis, dizziness, tiredness,
45 and upper airway infection. When collecting blood, some participants may experience
46 minor discomfort when the needle penetrates the skin and rarely a small bleeding
47 occurs. The volume of blood collected during the entire study period will amount to a
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4 maximum of 1,800 ml (over 5 years) and only participants with a normal haemoglobin
5 level will be included. Severe systemic AEs are not expected.
6
7

8
9 A DXA scan will be performed 3 times during the study with the objective of
10 determining the distribution of bone and adipose tissue. DXA scanning takes 15
11 minutes is a painless procedure with no expected side effects, and results in a
12 modest radiation dose (approximately equivalent to 2-3 times the dose received from
13 a dental X-ray). Scanning of the liver will be carried out using ultrasound. Ultrasound
14 creates images using sound waves and no side effects are expected from this
15 procedure.
16
17
18
19
20
21

22 The participants will receive thorough verbal and written information about the risk of
23 developing the mentioned AEs. Verbal and written informed consent will be obtained
24 from participants prior to participation in accordance with ICH-GCP guidelines. The
25 declaration of consent will emphasize that participation in the project is voluntary and
26 that participants may withdraw their consent to participate at any time without
27 providing a reason and without any consequences for the patient's current or future
28 treatment by the health service. The participants will receive a randomisation number
29 after the baseline OGTT. All data forms and blood samples will only be labelled with
30 the patient's initials and study number. The sponsor-investigator is responsible for
31 keeping a list separately for all randomised participants containing patient numbers,
32 full names and date of birth. Extra plasma, white blood cells, and urine will be stored
33 for up to 15 years after the end of the study for repeated measurements in case of
34 error analysis or the need for more analyses. The protocol is registered at the Danish
35 Data Protection Agency (01714 GEH-2012-024).
36
37
38
39
40
41
42
43
44
45
46

47 The advantage for the participant, of participating in this trial, is the initial screening
48 for diabetes and the subsequent monitoring of her blood glucose, which also allows
49 for detection of diabetes, and hence early treatment. Serious adverse events are
50 rare. Research will gain more understanding of the pathophysiology in the early
51 stages of T2D. This may provide new and effective interventions aiming at preventing
52 the development of the disease.
53
54
55
56
57
58
59
60

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

1
2
3
4 Gynaecology, Copenhagen University Hospital Herlev; and JJH is co-investigator at
5 Department of Biomedical Sciences, Faculty of Health Sciences, The NNF Center for
6 Basic Metabolic Research, University of Copenhagen, Denmark. All authors have
7 read and approved the final version of the manuscript.
8
9

10 11 12 **FUNDING**

13
14 This study was initiated by the authors and is supported as an investigator-initiated
15 study (IIS) by an unrestricted grant from Novo Nordisk.
16
17

18 19 **DECLARATION OF INTERESTS**

20 Louise Vedtofte and Jens A. Svare have no conflict of interests in relation to the
21 present paper. Signe Foghsgaard has received research support from Novo Nordisk.
22 Lise L. Gluud has participated in a trial sponsored by Merck. Filip K. Knop has
23 received lecture fees from AstraZeneca, Boehringer Ingelheim Pharmaceuticals,
24 Bristol-Myers Squibb, Eli Lilly and Company, Gilead Sciences, Merck Sharp &
25 Dohme, Novo Nordisk, Ono Pharmaceuticals, Sanofi, and Zealand Pharma, is a
26 member of the Advisory Boards of Eli Lilly, Bristol-Myers Squibb/AstraZeneca and
27 Zealand Pharma, and has consulted for AstraZeneca, Gilead Sciences, Ono
28 Pharmaceuticals and Zealand Pharma. Elisabeth R. Mathiesen and Peter Damm
29 have received lecture fees from Novo Nordisk and have been members of an
30 Advisory Board of Novo Nordisk, Jens J. Holst has consulted for Merck Sharp and
31 Dome, Novo Nordisk and Roche, Tina Vilsbøll has received lecture fees from
32 AstraZeneca, Boehringer Ingelheim Pharmaceuticals, Bristol-Myers Squibb, Eli Lilly
33 and Company, Merck Sharp & Dohme, Novo Nordisk, Novartis, Sanofi, and Zealand
34 Pharma, and is a member of the Advisory Boards of Novo Nordisk, Merck Sharp &
35 Dohme and Bristol-Myers Squibb/AstraZeneca.
36
37
38
39
40
41
42
43
44
45
46
47

48 49 **Figure legend**

50 **Figure 1.** Schematic illustration of study design. The first year is blinded and the remaining four years
51 are open-label. Arrow: screening visit; star: oral glucose tolerance tests (OGTT); triangle: isoglycaemic
52 intravenous glucose infusion, full body dual energy X-ray absorptiometry scanning, ultrasound
53 scanning of the liver, fibro-scanning, glucagon test, and ad libitum meal test; dot: OGTT and
54 subsequent glucagon test, light blue arrows: clinical control visits.
55
56
57
58
59
60

REFERENCES

1. Catalano PM, Tyzbir ED, Wolfe RR, et al. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. *Am J Physiol.* 1993 Jan;264(1 Pt 1):E60–67.
2. Damm P, Vestergaard H, Kühl C, et al. Impaired insulin-stimulated nonoxidative glucose metabolism in glucose-tolerant women with previous gestational diabetes. *Am J Obstet Gynecol.* 1996 Feb;174(2):722–9.
3. Lauenborg J, Hansen T, Jensen DM, et al. Increasing incidence of diabetes after gestational diabetes: a long-term follow-up in a Danish population. *Diabetes Care.* 2004 May;27(5):1194–9.
4. Xiang AH, Kjos SL, Takayanagi M, et al. Detailed physiological characterization of the development of type 2 diabetes in Hispanic women with prior gestational diabetes mellitus. *Diabetes.* 2010 Oct;59(10):2625–30.
5. Bellamy L, Casas J-P, Hingorani AD, et al. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet.* 2009 May 23;373(9677):1773–9.
6. Bian X, Gao P, Xiong X, et al. Risk factors for development of diabetes mellitus in women with a history of gestational diabetes mellitus. *Chin Med J (Engl).* 2000 Aug;113(8):759–62.
7. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998 Sep 12;352(9131):837–53.
8. Porte D Jr. Mechanisms for hyperglycemia in the metabolic syndrome. The key role of beta-cell dysfunction. *Ann N Y Acad Sci.* 1999 Nov 18;892:73–83.
9. Warram JH, Martin BC, Krolewski AS, et al. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med.* 1990 Dec 15;113(12):909–15.
10. Leslie RD, Volkmann HP, Poncher M, et al. Metabolic abnormalities in children of non-insulin dependent diabetics. *Br Med J Clin Res Ed.* 1986 Oct 4;293(6551):840–2.
11. Ishikawa M, Pruneda ML, Adams-Huet B, et al. Obesity-independent hyperinsulinemia in nondiabetic first-degree relatives of individuals with type 2 diabetes. *Diabetes.* 1998 May;47(5):788–92.
12. Gulli G, Ferrannini E, Stern M, et al. The metabolic profile of NIDDM is fully

1
2
3
4 established in glucose-tolerant offspring of two Mexican-American NIDDM
5 parents. *Diabetes*. 1992 Dec;41(12):1575–86.
6

- 7
8 13. O’Rahilly SP, Nugent Z, Rudenski AS, et al. Beta-cell dysfunction, rather
9 than insulin insensitivity, is the primary defect in familial type 2 diabetes.
10 *Lancet*. 1986 Aug 16;2(8503):360–4.
11
12 14. Van Haeften TW, Dubbeldam S, Zonderland ML, et al. Insulin secretion in
13 normal glucose-tolerant relatives of type 2 diabetic subjects. Assessments
14 using hyperglycemic glucose clamps and oral glucose tolerance tests.
15 *Diabetes Care*. 1998 Feb;21(2):278–82.
16
17 15. Alford FP, Henriksen JE, Rantza C, et al. Impact of family history of
18 diabetes on the assessment of beta-cell function. *Metabolism*. 1998
19 May;47(5):522–8.
20
21 16. Fernández-Castañer M, Biarnés J, Camps I, et al. Beta-cell dysfunction in
22 first-degree relatives of patients with non-insulin-dependent diabetes
23 mellitus. *Diabet Med J Br Diabet Assoc*. 1996 Nov;13(11):953–9.
24
25 17. Pimenta W, Korytkowski M, Mitrakou A, et al. Pancreatic beta-cell
26 dysfunction as the primary genetic lesion in NIDDM. Evidence from studies
27 in normal glucose-tolerant individuals with a first-degree NIDDM relative.
28 *Jama J Am Med Assoc*. 1995 Jun 21;273(23):1855–61.
29
30 18. Vilsbøll T, Krarup T, Madsbad S, et al. Both GLP-1 and GIP are
31 insulinotropic at basal and postprandial glucose levels and contribute
32 nearly equally to the incretin effect of a meal in healthy subjects. *Regul*
33 *Pept*. 2003 Jul 15;114(2-3):115–21.
34
35 19. Deane AM, Nguyen NQ, Stevens JE, et al. Endogenous glucagon-like
36 peptide-1 slows gastric emptying in healthy subjects, attenuating
37 postprandial glycemia. *J Clin Endocrinol Metab*. 2010;95(1):215–21.
38
39 20. Rijkkelijkhuizen JM, McQuarrie K, Girman CJ, et al. Effects of meal size and
40 composition on incretin, α -cell, and β -cell responses. *Metabolism*. 2010
41 Apr;59(4):502–11.
42
43 21. Vilsbøll T, Holst JJ. Incretins, insulin secretion and Type 2 diabetes
44 mellitus. *Diabetologia*. 2004 Mar;47(3):357–66.
45
46 22. Vilsbøll T. On the role of the incretin hormones GIP and GLP-1 in the
47 pathogenesis of Type 2 diabetes mellitus. *Dan Med Bull*. 2004
48 Nov;51(4):364–70.
49
50 23. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev*. 2007
51 Oct;87(4):1409–39.
52
53 24. Astrup A, Rössner S, Van Gaal L, et al. Effects of liraglutide in the
54 treatment of obesity: a randomised, double-blind, placebo-controlled
55
56
57
58
59
60

1
2
3
4 study. *Lancet*. 2009 Nov 7;374(9701):1606–16.
5

- 6 25. Astrup A, Carraro R, Finer N, et al. Safety, tolerability and sustained weight
7 loss over 2 years with the once-daily human GLP-1 analog, liraglutide. *Int J*
8 *Obes* 2005. 2012 Jun;36(6):843–54.
9
- 10 26. Myers RP, Pollett A, Kirsch R, et al. Controlled Attenuation Parameter
11 (CAP): a noninvasive method for the detection of hepatic steatosis based
12 on transient elastography. *Liver Int Off J Int Assoc Study Liver*. 2012
13 Jul;32(6):902–10.
14
- 15 27. Bagger JI, Knop FK, Lund A, et al. Impaired regulation of the incretin effect
16 in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2011
17 Mar;96(3):737–45.
18
- 19 28. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated
20 guidelines for reporting parallel group randomised trials. *BMJ*.
21 2010;340:c332.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

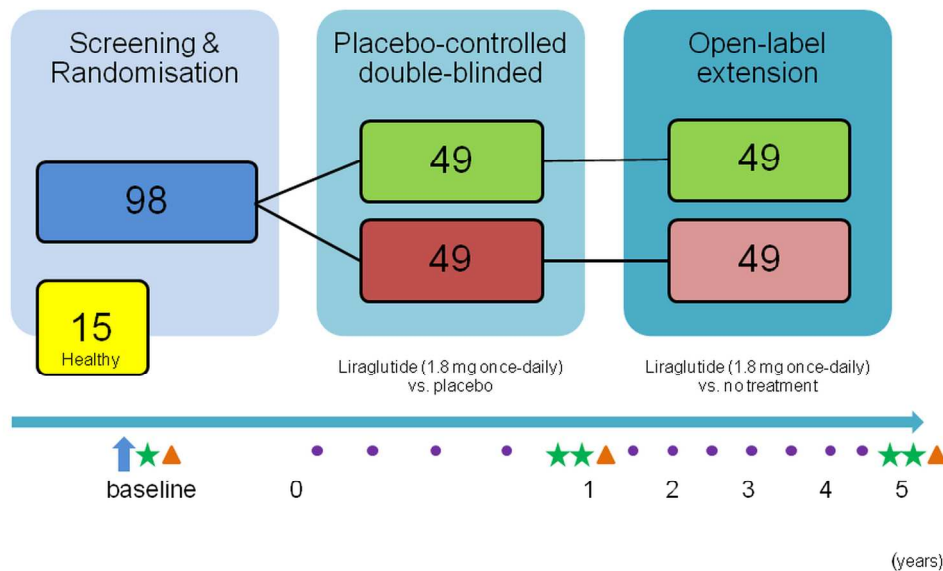


Figure 1. Schematic illustration of study design. The first year is blinded and the remaining four years are open-label. Arrow: screening visit; star: oral glucose tolerance tests (OGTT); triangle: isoglycaemic intravenous glucose infusion, full body dual energy X-ray absorptiometry scanning, ultrasound 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.
254x190mm (300 x 300 DPI)