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Efficacy and safety of the glucagon-like peptide-1 receptor agonist liraglutide added to insulin therapy in poorly regulated patients with type 1 diabetes – a protocol for a randomised, double-blind, placebo-controlled study: The Lira-1 Study

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Type 1 diabetes mellitus, liraglutide, Glucagon-like peptide-1 (GLP-1) receptor agonist, mixed meal test, gastric emptying.

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ABSTRACT

Introduction:

Intensive insulin therapy is recommended for the treatment of type 1 diabetes (T1D). Hypoglycaemia and weight gain are common side effects to insulin treatment and may reduce compliance. In patients with insulin treated type 2 diabetes, the addition of glucagon-like peptide-1 receptor agonist (GLP-1RA) therapy has proven effective in reducing weight gain and insulin dose. The efficacy and safety of adding a GLP-1RA to insulin treatment in patients with T1D have never been assessed in a randomised, double-blinded, controlled design. The present publication describes a protocol for such a study.

Methods and analysis:

In total 100 patients with type 1 diabetes, poor glycaemic control (glycated haemoglobin A1c (HbA1c) >8.0%) and overweight (body mass index (BMI) >25 kg/m^2) will be randomised to either liraglutide 1,8 mg once daily or placebo as add-on to intensive insulin therapy in this investigator initiated, double-blinded placebo-controlled parallel study. The primary endpoint is glycaemic control as measured by changes in HbA1c. Secondary endpoints include changes in insulin dose, hypoglycaemic events, body weight, lean body mass, fat mass, food preferences, and adverse events. Furthermore, glycaemic excursions, postprandial glucagon levels, gastric emptying rate during a standardised liquid meal test will be studied.

Ethics and dissemination:

The study is approved by the Danish Medicines Authority, the Regional Scientific-Ethical Committee of the Capital Region of Denmark and the Data Protection Agency. The study will be carried out under the surveillance and guidance of the Good Clinical Practice (GCP) unit at Copenhagen University Hospital Bispebjerg in accordance with ICH-GCP guidelines and the Helsinki Declaration.

Trial registration

ClinicalTrials.gov Identifier: NCT01612468

EudraCT: 2012-001150-26

Strengths and limitations of this study

- A randomised, double-blinded, placebo-controlled design.
- Length of the intervention is 24 weeks, which makes it possible to conclude on HbA1c, glycaemic variability and weight after a glycaemic steady state has been obtained.
- The study may not be powered to conclude on all secondary endpoints.
- The overweight and poorly controlled participants represent a sub-group of type 1 diabetic patients.

INTRODUCTION

Patients with type 1 diabetes (T1D) are most often treated either by insulin injection regimens including long and short acting insulin (basal/bolus therapy) or by insulin pump therapy[1]. Several studies have demonstrated, that an intensified insulin regimen in T1D can delay the onset and slow the progression of microvascular complications and potentially reduce the long term risk of macrovascular disease[2 3]. Based on this evidence, intensive insulin treatment with near-normalisation of blood glucose levels with an glycated haemoglobin A1c (HbA1c) of <7.0% is recommended[4].

Intensive insulin therapy with near normalised glycaemic control is associated with side effects such as weight gain and risk of hypoglycaemia[2]. Severe hypoglycaemic episodes are associated with serious physical and psychological morbidity and occasionally death[5]. Moreover, episodes of hypoglycaemia can introduce hypoglycaemia unawareness and have further been shown to cause significant anxiety and fear of future episodes, which may reduce compliance to treatment. Thus, hypoglycaemia, constitute a major limiting factor in obtaining strict glycaemic control[6]. Likewise, weight gain has been reported during intensive insulin treatment. This may potentially lead to increased risk of overweight related comorbidities[7] and furthermore induce non-compliance with the intensive insulin regimen resulting in poor glycaemic control. Presently, approximately 50% of patients with T1D in the developed world are overweight[8]. Basal insulin alone is insufficient in providing control of postprandial glucose (PPG) excursions. As a result, patients with T1D are depending on boluses of rapid-acting insulin at each meal, which match the carbohydrate content of meals and physical activity of the patient. For many patients this can be a complicated task and often associated with either hypoglycaemic or

hyperglycaemic postprandial glucose excursions resulting in poor glycaemic control. Taken all together, there is a need for a more convenient treatment regimen improving glycaemic control while at the same time mitigating the side effects of and problems associated with current insulin regimens; in particular hypoglycaemia, weight gain and exaggerated PPG excursions[9].

Risk of hypoglycaemia and weight gain is also evident in insulin-treated patients with type 2 diabetes (T2D)[10 11]. However, the combination of insulin with a glucagon-like peptide-1 receptor agonist (GLP-1RA) has proven effective in reducing the weight gain and insulin dose in insulin-treated patients with T2D - without deteriorating glycaemic control or exacerbating the risk of hypoglycaemia[12 13]. Few clinical trials have assessed the safety and efficacy of GLP-1RA treatment in patients with T1D. However, a few short-term studies have demonstrated weight loss in a range between 2.8 and 4.5 kg, reduced PPG excursions and reduced insulin requirements (0.07 to 0.19 U/kg/day) concomitant with improved or unaltered glycaemic control[14-21]. On this background it seems relevant to evaluate the efficacy and safety of long-term (24 weeks) GLP-1RA treatment in patients with T1D in a randomised, controlled and double-blinded study design. Such a protocol is described in the present publication.

HYPOTHESIS

Treatment with the GLP-1RA liraglutide as add-on to existing basal/bolus intensive insulin therapy in poorly regulated (HbA1c >8.0%) and overweight (body mass index (BMI) >25 kg/m^2) patients with T1D results in reductions in HbA1c, bodyweight, insulin dose, glycaemic variability, hypoglycaemic events and body fat mass.

OBJECTIVES AND ENDPOINTS

The objective of the present protocol is to evaluate the effect of liraglutide as an adjunctive therapy to intensive insulin treatment in overweight and poorly regulated patients with T1D.

The primary endpoint is metabolic control as measured by changes in HbA1c from baseline. Secondary endpoints include change in insulin dose, hypoglycaemic events, glycaemic variability measured by continuous glucose monitoring (CGM), body weight, lean body mass and fat mass as determined by dual-energy X-ray absorptiometry (DXA) scan, food preferences, appetite score, quality of life, treatment satisfaction, and adverse events. In addition, postprandial glucagon- and GLP-1 -levels, gastric emptying and glycaemic excursion will be estimated during a standardised liquid mixed meal test (MMT). Primary and secondary endpoints are shown in table 1.

METHODS AND ANALYSES

Research design

The study is an investigator-initiated, randomised, double-blinded, placebo-controlled intervention trial. In total 100 patients with T1D treated with basal/bolus insulin regimen will be randomised in a 1:1 ratio to liraglutide 1.8 mg once-daily or placebo as add on to insulin therapy. Treatment allocation will be generated through a web-based case report file (eCRF). If necessary, unblinding can be made individually so that treatment blinding of other patients in the study remains.

Study population

Patients will be recruited from outpatient clinics in the Capital Region of Denmark. All patients who meet the inclusion and exclusion criteria at screening will be enrolled for randomisation followed by a 24-week treatment period at Steno Diabetes Center, Gentofte, Denmark. The inclusion and exclusion criteria are listed in table 2.

Trial visits and examinations

Patients will receive written and oral information about the study. Sufficient time will be given before written informed consent is obtained. At screening (visit 0), demography, medical history and concomitant medication will be recorded. A physical examination including heart rate, blood pressure (BP) and electrocardiogram (ECG) will be performed together with blood samples and urine tests. Six days of CGM will be performed prior to randomisation (visit 1), visit 3 (week 14) and end-of-treatment (week 26). An overview of blood and urine samples is shown in table 3.

During the trial, insulin dose will be adjusted according to self-measured blood glucose (SMBG), CGM, and HbA1c. Treatment targets for BG will be preprandial values of 4-7 mmol/l and postprandial values of <10 mmol/l according to the Danish guidelines for the treatment of T1D[22]. At randomisation, insulin dose will be reduced by 33% for bolus and 25% for basal insulin before study drug initiation to reduce the risk of hypoglycaemia. Furthermore, patients will undergo physical examination, have blood and urine samples taken, have a DXA scan, and complete questionnaires concerning quality of life (WHO-5), problem areas in diabetes (PAID) and diabetes treatment and satisfaction questionnaire (DTSQ). Carotis intima media thickness (CIMT) and pulse wave velocity (PWV) scans will be determined together with a 24-

hour BP evaluation before and at end of treatment with the study drug.

A telephone contact to adjust insulin dosing and ensure compliance and evaluation of adverse effects will be performed between visit 1 and visit 2. Furthermore, the patients will be asked to contact the study team if they encounter any problems with the dosing of insulin and/or glucose control occurs.

At visit 1 (randomisation), visit 2 (clinical control) and visit 5 (end-of-treatment), the first 40 enrolled patients will undergo a 4-hour MMT measuring postprandial glucagon levels, endogenous GLP-1 and gastric emptying evaluated by paracetamol absorption test. Visual analogue scale (VAS) tests for appetite and satiety measures and a questionnaire concerning food preferences will also be performed during each MMT.

At all visits, adverse events, concomitant medication, body weight, basal and bolus insulin doses and hypoglycaemic events will be recorded. Additionally, BP and heart rate will be measured. Urinary human chorionic gonadotropin (HCG) will be measured in women of child-bearing potential.

A flow chart and an outline of the trial visits and examinations are shown in figure 1 and table 4.

INTERVENTION

Name: Victoza® (liraglutide) or matching placebo.

Pharmaceutical form: Liraglutide 6.0 mg/ml, 3 ml prefilled pen for subcutaneous injection. Placebo, 3 ml prefilled pen for subcutaneous injection.

Pharmaceutical dosage: Liraglutide or placebo will be initiated at a dose of 0.6

mg/day and gradually up escalated to 1.2 mg/day after one week and after another week to 1.8 mg/day. Intervals between dose increments can be extended based on subject's tolerance to trial product. Further, the trial drug dose can be reduced at any time during the trial if required. Injection can be done at any time during the day, but it is recommended that the time of injection is consistent from day to day.

Side effects: Common side effects (1-10%): Nausea, vomiting, diarrhoea, obstipation, hypoglycaemia and headache.

Shipping and packing: All trial products will be delivered, packed blinded and labelled by Novo Nordisk A/S.

SAMPLE SIZE

Sample size calculation is based on the ability to detect a difference in change from baseline in HbA1c (primary outcome) between study arms of 6 mmol/mol (0.6%) with 80% power, a 5%-significance level, and a presumed 9 mmol/mol (0.9%) standard deviation. This will require 35 patients included in each study arm (2-sided test). To allow for a 30% dropout rate, 100 patients in total will be included in the study, 50 in each study arm. The sample size calculation is based on data obtained from a randomised controlled trial in T1D patients treated with injection therapy vs. insulin pump-therapy[23]. Withdrawn subjects will not be substituted.

DATA ANALYSIS

The per protocol population will include all patients completing the study with a documented valid baseline and end-of-treatment (eventually last observation carried

forward) assessment of the primary endpoint without any major protocol violations. The intention-to-treat population will include all randomised patients.

Primary efficacy outcome parameter is change in HbA1c from baseline to end-of-treatment between the intervention and the control group. The absolute values and adjusted mean changes from baseline for each treatment group, the difference in adjusted mean change between the liraglutide group and the placebo group, and the 95% confidence interval will be presented. The primary analysis will be based on the per protocol population.

Comparisons between treatment groups will be performed by an unpaired two sample t test, Mann-Whitney test or χ^2 test as appropriate. Normally distributed variables will be presented as mean ± standard deviation (SD). Non-parametric statistics and appropriate log-transformation will be performed if assumption of normality is not met. A two-tailed p value of less than 0.05 will be considered statistically significant.

Additional analyses due to loss of follow-up include analysis of data from the intention-to-treat population to determine the validity of the conclusions of the per-protocol population and will include duration in study and reason for discontinuation as co-variables.

ETHICS AND PUBLICATION

It is expected, that the study will contribute with important and novel information about treatment of patients with T1D with liraglutide as add-on to insulin; and if liraglutide treatment improves glycamic control and is safe a large number of patients

worldwide will potentially benefit from this new treatment concept.

During the study, the patient will undergo frequent clinical check-ups by a physician. Thus, the patient will be carefully examined regarding safety and efficacy and glucose regulation will be optimised continuously, which will reduce the risk of adverse events. Liraglutide (Victoza®) is approved for treatment of T2D by Food and Drug Administration US (FDA), State Food and Drug Administration China (SFDA), Pharmaceuticals and Medical Devices Agency Japan (PMDA) and European Medicines Agency (EMA), and studies have shown limited adverse effects, i.e. nausea, vomiting, obstipation and headache. Nausea and vomiting are usually transient and mostly registered during the first weeks after initiation of treatment. Adverse effects are generally temporary and can be minimised by gradual dose escalation as planned in this study. In combination with insulin the risk of hypoglycaemia can be minimized by reducing insulin doses at start of treatment and carefully instructing patients in monitoring their blood glucose. For those receiving placebo there will be a modest risk of hyperglycaemia, but by frequent BG measurements the patient can titrate insulin doses as needed. Few cases of acute pancreatitis have been reported in relation to GLP-1 treatment, but overall the incidence did not different from that in T2D patients treated with other antidiabetic agents[24]. Thyroid C cell tumours have been seen in mice and rats treated with liraglutide, but have not been reported among subjects treated with liraglutide in clinical studies.

At the two DXA scans the patient will be exposed to a weak X-ray radiation; less than 1 mSv in total. For comparison, the background radiation in Denmark is about 3 mSv per year. The standardised meal test is preceded by 10 hours of fasting which may

cause slight discomfort. A volume of 200 ml blood is taken during each meal test, and a total of 750 ml blood is taken during the 26 week period.

The results from the study will be presented at national and international scientific meetings, and the manuscripts will be written in accordance with the CONSORT 2010 statement and submitted to peer-reviewed journals. Novo Nordisk A/S has been allowed to comment on the protocol before initiation of the study and will have 4 weeks to comment on the manuscripts before publication.

STUDY APPROVAL

The Lira-1 study is approved by the Danish Medicines Agency (EudraCT number: 2012-001150-26), the Scientific-Ethical Committee of the Capital Region of Denmark (H-1-2012-031) and the Danish Data Protection Agency (SDC-2012-001). It is registered at ClinicalTrials.gov (NCT01612468) and will be carried out under the surveillance and guidance of the good clinical practice (GCP) unit at Copenhagen University Hospital in compliance with the ICH-GCP guidelines. The study was initiated in June 2012 and expected to be reported in 2015. The study will be conducted in accordance with the Helsinki Declaration.

DISCUSSION

Insulin treatment of patients with T1D is often associated with weigh gain and risk of hypoglycaemia. Furthermore, many patients do not obtain the glycaemic goal despite basal/bolus insulin therapy in combination with intensive self-monitoring of blood glucose[8]. Novel treatments addressing the weight gain and improving glycaemic

control are warranted; GLP-1 RAs seem promising in this regard.

The present protocol describes the first randomised, double-blinded, placebo-controlled trial evaluating efficacy and safety of the long-acting GLP-1RA liraglutide as an add-on to insulin therapy in overweight and poorly controlled patients with T1D. The primary endpoint is changes in HbA1c from baseline, but the trial will also address changes in glycaemic variability, weight, cardiovascular risk factors and postprandial glucose control and hormonal changes during a MMT. Foremost, the trial will give information on efficacy and safety of combination therapy as compared with insulin treatment alone.

After years duration of diabetes endogenous insulin secretion is less important in patients with T1D than T2D, since no or very limited insulin secretion is present in T1D[25]. However, other hormones such as glucagon, GLP-1, glucagon-like peptide-2 (GLP-2), gastric inhibitory peptide (GIP), cholecystokinin, gastrin, and the fat derivated peptide adiponectin and leptin could be influenced by GLP-1RA and might play an important role in appetite regulation in overweight patients with T1D. Of interest, the lowering of glucagon during a meal may be important for regulation of PPG in patients with T1D[16 26]. Another intriguing mechanism of interest could be the effect of reduction in gastric emptying rate resulting in delayed intestinal absorption of carbohydrates resulting in a reduction in postprandial glucose hyperglycaemia[16 27]. All these outcomes will be addressed in the study as secondary endpoints.

Limitations of the trial are that the effects of combination therapy on weight and glycaemic control in normal weight patients with T1D are not addressed. Moreover, the effect of combination therapy on counterregulation during hypoglycaemia is not included in the protocol. A study investigating the effect on gastric emptying rate and on glucagon response during hypoglycaemia is of high priority, since both a delaying

in gastric emptying after food intake and an attenuated glucagon will compromise counterregulation[17].

In conclusion, the present study will elucidate many of the advantages and disadvantages of combination therapy with insulin and GLP-1RA in patients with T1D.

AUTHORS' CONTRIBUTIONS

TFD, FKK, TSH, LT, TA, JJH and HUA conceived and designed the study. HUA sponsors the trial. TFD is principal investigator. CSF is sub-investigator. TFD, CSF, HUA, FKK and SM have drafted the manuscript. All authors have read and approved the final version of the manuscript.

FUNDING

This work was initiated by the authors and is supported as an investigator-initiated study by an unrestricted grant from Novo Nordisk A/S.

COMPETING INTERESTS

TFD, CSF and TSH have received research support from Novo Nordisk. FKK 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, Novo Nordisk and Zealand Pharma, has consulted for AstraZeneca, Gilead Sciences, Ono

Pharmaceuticals, Novo Nordisk and Zealand Pharma and received research support from Sanofi. TA owns stocks in Novo Nordisk. LT owns stocks in Novo Nordisk, have received lecture fees and consulted for Novo Nordisk, JJH has consulted for Merck Sharp & Dome, Novo Nordisk and Roche. SM has participated in advisory boards for Novartis Pharma, Novo Nordisk, Merck Sharp & Dome, Sanofi, AstraZeneca, Johnson & Johnson, Roche, Mankind, Boehringer-Ingelheim, Zealand Pharma, Eli Lilly and Intarcia Therapeutics, and has received honoraria for lectures from Novo Nordisk, Merck Sharp & Dome, Astra-Zeneca, Johnson and Johnson, Roche, Shering-Ploug, Sanofi-Aventis, Novartis Pharma, Eli Lilly and Bristol-Meyer Squibb. HUA owns stocks in Novo Nordisk and participates in an advisory board for Abbott.

Table 1: Outcome measures

Primary endpoint

- HbA1c

Secondary endpoints

- Insulin dose
- Hypoglycaemic events
- Glycaemic excursions (time spent in hypo and hyperglycaemia as measured by continuous glucose monitoring)
- Body weight
- BMI
- Lean body mass and fat mass composition as determined by DXA scan
- Quality of life and treatment satisfaction
- Food preferences
- VAS score for appetite
- Postprandial glucagon, GLP-1, GLP-2, GIP, CCK, Gastrin, Adiponectin, Leptin levels, gastric emptying (paracetamol absorption test) and glycaemic excursion during standardised liquid MMT
- CIMT
- PWV
- 24 hour BP
- Heart rate
- Adverse events

HbA1c, glycated haemoglobin A1c; BMI, body mass index; DXA, dual-energy X-ray absorptiometry
VAS, visual analogue scale; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2
CCK, cholecystokinin; MMT, mixed meal test; CIMT, carotis intima media thickness
PWW, pulse wave velocity; BP, blood pressure

Table 2: Overview of inclusion and exclusion criteria

Inclusion criteria

- T1D (according to WHO criteria) ≥1 year
- Age ≥18 years
- BMI >25.0 kg/m^2
- HbA_{1c} >8.0% at visit 0
- Able to understand the written patient information and to give informed consent

Exclusion criteria

- Insulin pump treatment
- Hypoglycaemia unawareness
- Diabetic gastroparesis
- Impaired kidney function (eGFR <60 ml/min/1.73m^2), dialysis or kidney transplantation
- Liver disease with elevated plasma ALT >three times the upper normal limit.
- Acute or chronic pancreatitis
- Inflammatory bowel disease
- Cancer unless in complete remission for >5 years
- History of thyroid adenoma or carcinoma
- Other concomitant disease or treatment that according to the investigator's assessment makes the patient unsuitable for study participation
- Alcohol and/or drug abuse
- Fertile women not using chemical or mechanical contraceptives
- Pregnant or nursing women
- Known or suspected hypersensitivity to trial product or related products
- Receipt of an investigational drug within 30 days prior to visit 0
- Simultaneous participation in any other clinical intervention trial

T1D, type 1 diabetes; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase

Table 3: Blood samples

Sampling period	Analysis
Screening and control visits	➢ Glucose, HbA1c ➢ C-petide, GAD antibodies ➢ Haemoglobin, thrombocytes, leucocytes ➢ Albumin ➢ Creatinine, potassium, sodium ➢ TSH ➢ Cholesterol, triglycerides ➢ ALT, lipase, amylase ➢ Urine albumin-creatinine ratio, hCG
MMT	➢ Paracetamol ➢ Glucose, C-peptide ➢ Glucagon ➢ GLP-1, GLP-2, GIP ➢ Adiponectin, leptin ➢ CCK, Gastrin ➢ NT-proBNP ➢ hsCRP

HbA1c, glycated haemoglobin A1c; GAD, glutamic acid decarboxylase; TSH, thyroid-stimulating hormone; ALT, alanine aminotransferase; hCG, human chorionic gonadotrophin; MMT, mixed meal test; GLP, glucagon-like peptide; CCK, cholecystokinin; NT-proBNP, N-terminal prohormone brain natriuretic peptide; hsCRP, high-sensitive C-reactive protein

Figure 1: Flowchart of the study

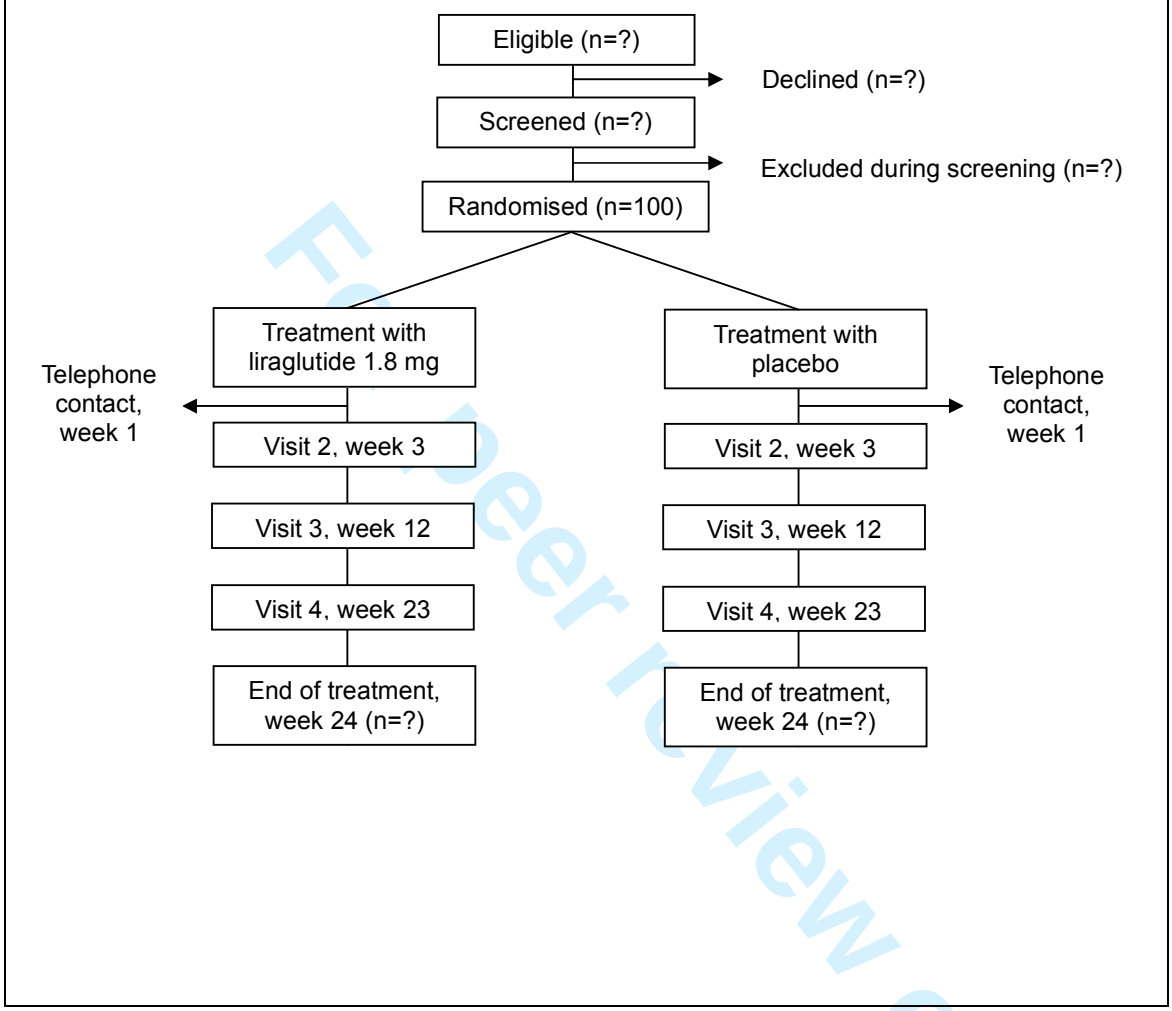

Table 4: Trial visits

	Visit 0 Screening	Visit 1 Randomi-sation	Telephone or e-mail contact	Visit 2 Clinical control	Visit 3 Clinical control	Visit 4 CGM and blood pressure	Visit 5 End of trial
Time (weeks)	**- 1 ± 1**	**0**	**-**	**3 ± 1**	**12 ± 1**	**23 ± 1**	**24 ± 1**
General							
Assessment of inclusion and exclusion criteria	X						
Demography	X						
Medical history	X						
Concomitant medication	X	X	X	X	X		X
Smoking/alcohol	X						
Endpoints							
HbA_{1c}	X	X			X		X
Weight – BMI	X	X		X	X		X
Insulin dose	X	X	X	X	X		X
Hypoglycaemia events		X	X	X	X		X
CGM	X				X	X	
DXA scan		X					X
Quality of life, problems in diabetes and treatment satisfaction		X		X			X
Food preference		X		X			X
VAS score for appetite[4]		X		X			X
Standardised meal test[4]		X		X			X
Carotis intima media thickness		X					X
Pulse wave velocity		X					X
24 hour blood pressure and pulse rate		X				X	
Clinical assessment							
Physical assessment incl. height	X						
Office blood pressure and heart rate	X	X		X	X		X
Electrocardiogram	X						X
Biosamples							
Biomarkers [2]		X			X		X
U-albumin / creatinine ratio		X					X
Biobank		X					X
Safety							
Adverse events		X	X	X	X		X
Blood tests[1]	X	X			X		X
Urinary HCG[3]	X	(X)		(X)	(X)		(X)
Study medication							
Drug accountability		X		X	X		X
Study drug dose titration			X				X

[1]Glucose, haemoglobin, leucocytes, thrombocytes, potassium, sodium, creatinine, albumin, lipids, alanine transferase, lipase, amylase
[2] Biomarkers: Leptin, GLP-2, Adiponectin, GAD antibodies, C-peptid, NT-proBNP, hsCRP
[3] Urinary HCG will be performed if menstruation is absent in a woman of childbearing potential
[4]The first 40 patients

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	SEE TITLE PAGE
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	SEE ABSTRACT
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	2-3
	2b	Specific objectives or hypotheses	3-4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	15
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	14
	6b	Any changes to trial outcomes after the trial commenced, with reasons	4, ~~14~~ & 18
Sample size	7a	How sample size was determined	NA
	7b	When applicable, explanation of any interim analyses and stopping guidelines	7
Randomisation:			NA
Sequence generation	8a	Method used to generate the random allocation sequence	4
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	NA
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	4
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	4

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	NA
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7 & 8
Results			8
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	NA
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	1, 11 & 12
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	NA
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	NA
Other information			
Registration	23	Registration number and name of trial registry	1 & 10
Protocol	24	Where the full trial protocol can be accessed, if available	7 & 12
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Efficacy and safety of the glucagon-like peptide-1 receptor agonist liraglutide added to insulin therapy in poorly regulated patients with type 1 diabetes – a protocol for a randomised, double-blind, placebo-controlled study: The Lira-1 Study

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Efficacy and safety of the glucagon-like peptide-1 receptor agonist liraglutide added to insulin therapy in poorly regulated patients with type 1 diabetes – a protocol for a randomised, double-blind, placebo-controlled study: The Lira-1 Study

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Keywords

Type 1 diabetes mellitus, liraglutide, Glucagon-like peptide-1 (GLP-1) receptor agonist, mixed meal test, gastric emptying.

Word count

Abstract: 251 words

Body of manuscript (excl. title page, abstract, summary and references) 2.860 words

ABSTRACT

Introduction:

Intensive insulin therapy is recommended for the treatment of type 1 diabetes (T1D). Hypoglycemia and weight gain are common side effects to insulin treatment and may reduce compliance. In patients with insulin treated type 2 diabetes, the addition of glucagon-like peptide-1 receptor agonist (GLP-1RA) therapy has proven effective in reducing weight gain and insulin dose. The present publication describes a protocol for a study evaluating the efficacy and safety of adding a GLP-1RA to insulin treatment in overweight patients with T1D in a randomised, double-blinded, controlled design.

Methods and analysis:

In total 100 patients with type 1 diabetes, poor glycemic control (glycated haemoglobin A1c (HbA1c) >8.0%) and overweight (body mass index (BMI) >25 kg/m^2) will be randomised to either liraglutide 1,8 mg once daily or placebo as add-on to intensive insulin therapy in this investigator initiated, double-blinded placebo-controlled parallel study. The primary endpoint is glycemic control as measured by changes in HbA1c. Secondary endpoints include changes in insulin dose, hypoglycemic events, body weight, lean body mass, fat mass, food preferences, and adverse events.Glycemic excursions, postprandial glucagon levels, gastric emptying rate during a standardised liquid meal test will also be studied.

Ethics and dissemination:

The study is approved by the Danish Medicines Authority, the Regional Scientific-Ethical Committee of the Capital Region of Denmark and the Data Protection Agency. The study will be carried out under the surveillance and guidance of the Good Clinical Practice (GCP) unit at Copenhagen University Hospital Bispebjerg in accordance with ICH-GCP guidelines and the Helsinki Declaration.

Trial registration

ClinicalTrials.gov Identifier: NCT01612468

EudraCT: 2012-001150-26

Protocol version

Version 10, 5th April 2013

Strengths and limitations of this study

- A randomised, double-blinded, placebo-controlled design.
- Length of the intervention is 24 weeks, which makes it possible to draw conclusions HbA1c, glycemic variability and weight, after a glycemic steady state has been obtained.
- The study may not be sufficiently powered to draw on all secondary endpoints.
- These overweight and poorly controlled participants represent a sub-group of type 1 diabetic patients.

INTRODUCTION

Patients with type 1 diabetes (T1D) are most often treated either by insulin injection regimens, including long- and short-acting insulin (basal/bolus therapy) or by insulin pump therapy[1]. Several studies have demonstrated that an intensified insulin regimen in T1D can delay the onset and slow the progression of microvascular complications and may potentially reduce the long-term risk of macrovascular disease[2 3]. Based on this evidence, intensive insulin treatment aiming for near-normalisation of blood glucose levels with a glycated haemoglobin A1c (HbA1c) of <7.0% is recommended[4].

Intensive insulin therapy with near-normalised glycemic control is, however, associated with side effects such as weight gain and risk of hypoglycemia[2]. Severe hypoglycemic episodes are associated with serious physical and psychological morbidity and, occasionally, death[5].Episodes of hypoglycemia can also result in hypoglycemia unawareness and have been shown to cause significant anxiety and fear of future hypoglycemic episodes, which may result in reduced compliance with treatment. Therfore, hypoglycemia, constitutes a major limiting factor in obtaining strict glycemic control[6]. Weight gain has also been reported during intensive insulin treatment. This may potentially lead to increased risk of overweight-related comorbidities[7] and result in non-compliance with intensive insulin treatment resulting in poor glycemic control. Presently, approximately 50% of patients with T1D in high-income countries are overweight[8]. Basal insulin alone cannot controlpostprandial glucose (PPG) excursions. As a result, patients with T1D are dependent on boluses of rapid-acting insulin at each meal. The aim of this is tocorrect any premeal hyperglycemia and match the estimated carbohydrate content of the meal also taking into accountphysical activity. t. For many patients, this can be a complicated task and

is often associated with either hypoglycemic or hyperglycemic excursions, which may be accompanied by resulting emotional effects, resulting in both poor glycemic control and overall quality of life. Given these limiting factors, , there is a need for a more convenient treatment regimen, which can improve glycemic control, yet limitthe side-effects of, and problems associated with, current insulin regimens; namely, hypoglycemia, weight gain and exaggerated PPG excursions[9].Risk of hypoglycemia and weight gain is also evident in insulin-treated patients with type 2 diabetes (T2D)[10] [11]. However, the combination of insulin with a glucagon-like peptide-1 receptor agonist (GLP-1RA) has proven effective in reducing the weight gain and insulin dose in insulin-treated patients with T2D without a deterioration in glycemic control or increased risk of hypoglycemia[12 13].

In mechanistic studies, GLP-1RA improved glycemic control by delayed gastric emptying[14 15], supressed postprandial glucagon[14 15], enhanced insulin secretion both in T2D[16] and C-peptide positive patients with T1D[14], and reduced postprandial glycemic excursions[16 17].

Few clinical trials have assessed the safety and efficacy of GLP-1RA treatment in patients with T1D. However, a few short-term studies have demonstrated weight loss ranging between 2.8 and 4.5 kg, fewer PPG excursions, and reduced insulin requirements (0.07 to 0.19 U/kg/day), concomitant with improved or unaltered glycemic control[14 15 18-23]. The dose-response relationship has been evaluated, and a preliminary interim analysis showed that both the addition of 1.2 mg and 1.8 mg liraglutide significantly reduced HbA1c, mean blood glucose, and body weight in overweight people with T1D compared to placebo[24]. On this background, it would seem relevant to evaluate the efficacy and safety of long-term (24 weeks) GLP-1RA treatment with 1.8 mg liraglutide in patients with T1D in a randomised, controlled and

double-blinded study design. The protocol for this is described in this paper..

HYPOTHESIS

Treatment with the GLP-1RA liraglutide as add-on to existing basal/bolus intensive insulin therapy in poorly regulated (HbA1c >8.0%) and overweight (body mass index (BMI) >25 kg/m^2) patients with T1D results in reductions in HbA1c, bodyweight, insulin dose, glycemic variability, hypoglycemic events and body fat mass.

OBJECTIVES AND ENDPOINTS

The objective of the present protocol is to evaluate the effect of liraglutide as an adjunctive therapy to intensive insulin treatment in overweight and poorly-regulated patients with T1D.

The primary endpoint is metabolic control as measured by changes in HbA1c from baseline. Secondary endpoints include change in insulin dose, hypoglycemic events, glycemic variability measured by continuous glucose monitoring (CGM), body weight, lean body mass and fat mass as determined by dual-energy X-ray absorptiometry (DXA) scan, food preferences, appetite score, quality of life, treatment satisfaction, and adverse events. In addition, postprandial glucagon- and GLP-1 -levels, gastric emptying and glycemic excursion will be estimated during a standardised liquid mixed meal test (MMT). Primary and secondary endpoints are shown in table 1.

METHODS AND ANALYSES

Research design

The study is an investigator-initiated, randomised, double-blinded, placebo-controlled intervention trial. In total, 100 patients with T1D treated with basal/bolus insulin regimen will be randomised in a 1:1 ratio to liraglutide 1.8 mg once-daily, or placebo as add-on to insulin therapy. Treatment allocation will be generated and data maintained in a web-based case report file (eCRF). If necessary, unblinding can be made individually so that treatment blinding of other patients in the study remains.

Study population

Patients will be recruited from outpatient clinics in the Capital Region of Denmark by the investigator. All patients who meet the inclusion and exclusion criteria at screening will be enrolled for randomisation followed by a 24-week treatment period at Steno Diabetes Center, Gentofte, Denmark. The inclusion and exclusion criteria are listed in table 2.

Trial visits and examinations

Patients will receive written and oral information about the study. Sufficient time will be given before written informed consent is obtained by the investigator. At screening (visit 0), demography, medical history and concomitant medication will be recorded. A physical examination including heart rate, blood pressure (BP) and electrocardiogram (ECG) will be performed together with blood samples and urine tests. Six days of

CGM will be performed prior to randomisation (visit 1), visit 3 (week 14) and end-of-treatment (week 26). An overview of blood and urine samples is shown in table 3.

During the trial, insulin dose will be adjusted according to self-measured blood glucose (SMBG), CGM, and HbA1c. Treatment targets for BG will be preprandial values of 4-7 mmol/l and postprandial values of <10 mmol/l according to the Danish guidelines for the treatment of T1D[25]. At randomisation, insulin dose will be reduced by 33% for bolus and 25% for basal insulin before study drug initiation to reduce the risk of hypoglycemia. Furthermore, patients will undergo physical examination, have blood and urine samples taken, have a DXA scan, and complete questionnaires concerning quality of life (WHO-5), problem areas in diabetes (PAID), and diabetes treatment and satisfaction questionnaire (DTSQ). Carotid intima media thickness (CIMT) and pulse wave velocity (PWV) scans will be determined together with a 24-hour BP evaluation before and at end of treatment with the study drug.

A telephone contact to adjust insulin dosing and ensure compliance and evaluation of adverse effects will be performed between visit 1 and visit 2. The patients will be advisedto contact the study team if they encounter any problems with the dosing of insulin and/or glucose control occurs.

At visit 1 (randomisation), visit 2 (clinical control) and visit 5 (end-of-treatment), the first 40 enrolled patients will undergo a 4-hour MMT measuring postprandial glucagon levels, endogenous GLP-1 and gastric emptying evaluated by paracetamol absorption test. Visual analogue scale (VAS) tests for appetite and satiety measures and a questionnaire concerning food preferences will also be performed during each MMT.

At all visits, adverse events, currentmedication, body weight, basal and bolus insulin

doses and hypoglycemic events will be recorded. Additionally, BP and heart rate will be measured. Urinary human chorionic gonadotropin (HCG) will be measured in women of child-bearing potential.

A flow chart and an outline of the trial visits and examinations are shown in figure 1 and table 4.

INTERVENTION

Name: Victoza® (liraglutide) or matching placebo.

Pharmaceutical form: Liraglutide 6.0 mg/ml, 3 ml prefilled pen for subcutaneous injection. Placebo, 3 ml prefilled pen for subcutaneous injection.

Pharmaceutical dosage: Liraglutide or placebo will be initiated at a dose of 0.6 mg/day and gradually up escalated to 1.2 mg/day after one week and after another week to 1.8 mg/day. Intervals between dose increments can be extended based on subject's tolerance to trial product. Further, the trial drug dose can be reduced at any time during the trial if required. Injection can be done at any time during the day, but it is recommended that the time of injection is consistent from day to day.

Side effects: Common side effects (1-10%): Nausea, vomiting, diarrhoea, obstipation, hypoglycemia and headache.

Shipping and packing: All trial products will be delivered, packed blinded and labelled by Novo Nordisk A/S.

SAMPLE SIZE

Sample size calculation is based on the ability to detect a difference in change from baseline in HbA1c (primary outcome) between study arms of 6 mmol/mol (0.6%) with 80% power, a 5%-significance level, and a presumed 9 mmol/mol (0.9%) standard deviation. This will require 35 patients included in each study arm (2-sided test). To allow for a 30% dropout rate, 100 patients in total will be included in the study, 50 in each study arm. The sample size calculation is based on data obtained from a randomised controlled trial in T1D patients treated with injection therapy vs. insulin pump-therapy[26]. Withdrawn subjects will not be substituted.

DATA ANALYSIS

The per-protocol population will include all patients completing the study with a documented valid baseline and end-of-treatment (eventually last observation carried forward) assessment of the primary endpoint without any major protocol violations. The intention-to-treat population will include all randomised patients.

The primary efficacy outcome parameter is change in HbA1c from baseline to end-of-treatment between the intervention and the control group. The absolute values and adjusted mean changes from baseline for each treatment group, the difference in adjusted mean change between the liraglutide group and the placebo group, and the 95% confidence interval will also be reported. The primary analysis will be based on the per-protocol population.

Comparisons between treatment groups will be performed by an unpaired two-sample t test, Mann-Whitney test or χ^2 test as appropriate. Normally distributed variables will be presented as mean ± standard deviation (SD). Non-parametric

statistics and appropriate log-transformation will be performed if assumption of normality is not met. A two-tailed p value of less than 0.05 will be considered statistically significant.

Additional analyses due to loss of follow-up include analysis of data from the intention-to-treat population to determine the validity of the conclusions of the per-protocol population and will include duration in study and the reason for discontinuation as co-variables.

ETHICS AND PUBLICATION

It is expected that the study will contribute important and novel information about; treatment of patients with T1D with liraglutide as add-on to insulin therapy; whether liraglutide treatment improves glycemic control, and important safety information. A large number of patients worldwide could potentially benefit from this new treatment concept.

During the study, the patient will undergo frequent clinical check-ups by a physician. Therefore, the patient will be carefully evaluated with regard to safety and efficacy, and glucose regulation will be optimised continuously, which should reduce the risk of adverse events. Liraglutide (Victoza®) is approved for treatment of T2D by Food and Drug Administration US (FDA), State Food and Drug Administration China (SFDA), Pharmaceuticals and Medical Devices Agency Japan (PMDA) and European Medicines Agency (EMA), and studies have shown limited adverse effects, i.e. nausea, vomiting, obstipation and headache. Nausea and vomiting are usually transient and occur mostly during the first weeks after initiation of treatment. Adverse effects are generally temporary and can be minimised by gradual dose escalation, as

planned in this study. In combination with insulin, the risk of hypoglycemia can be minimized by reducing insulin doses at start of treatment and carefully instructing patients to monitor their blood glucose. For those receiving placebo, there will be a modest risk of hyperglycemia, but patients can titrate insulin doses as needed, using frequent bood glucose measurements. Few cases of acute pancreatitis have been reported in relation to GLP-1 treatment, but overall the incidence did not differ from that in T2D patients treated with other antidiabetic agents[27]. Thyroid C-cell tumours have been seen in mice and rats treated with liraglutide, but have not been reported among subjects treated with liraglutide in clinical studies.

At the two DXA scans the patient will be exposed to a weak X-ray radiation; less than 1 mSv in total. For comparison, the background radiation in Denmark is about 3 mSv per year. The standardised meal test is preceded by 10 hours of fasting which may cause slight discomfort. A volume of 200 ml blood is taken during each meal test, and a total of 750 ml blood is taken during the 26 week period.

The results from the study will be presented at national and international scientific meetings, and the manuscripts will be written in accordance with the CONSORT 2010 statement and submitted to peer-reviewed journals. Novo Nordisk A/S has been allowed to comment on the protocol before initiation of the study and will have 4 weeks to comment on the manuscripts before publication.

STUDY APPROVAL

The Lira-1 study is approved by the Danish Medicines Agency (EudraCT number: 2012-001150-26), the Scientific-Ethical Committee of the Capital Region of Denmark

(H-1-2012-031) and the Danish Data Protection Agency (SDC-2012-001). It is registered at ClinicalTrials.gov (NCT01612468) and will be carried out under the surveillance and guidance of the good clinical practice (GCP) unit at Copenhagen University Hospital in compliance with the ICH-GCP guidelines. The study was initiated in June 2012 and expected to be reported in 2015. The study will be conducted in accordance with the Helsinki Declaration.

DISCUSSION

Insulin treatment of patients with T1D is often associated with weight gain and risk of hypoglycemia. Many patients do not obtain the glycemic goal despite basal/bolus insulin therapy in combination with intensive self-monitoring of blood glucose[8]. Novel treatments addressing weight gain while improving glycemic control are warranted; GLP-1 RAs appear promising in this regard.

The present protocol describes a randomised, double-blinded, placebo-controlled trial evaluating the efficacy and safety of the long-acting GLP-1RA liraglutide as an add-on to insulin therapy in overweight and poorly-controlled patients with T1D. The primary endpoint is change in HbA1c from baseline, but the trial will also address changes in glycemic variability, weight, cardiovascular risk factors and postprandial glucose control and hormonal changes during a MMT. Foremost, the trial will give information on efficacy and safety of combination therapy as compared with insulin treatment alone.

As duration of diabetes increases, endogenous insulin secretion is less important in patients with T1D than T2D, since no or very limited insulin secretion is present in T1D[28]. However, other hormones such as glucagon, GLP-1, glucagon-like peptide-2

(GLP-2), gastric inhibitory peptide (GIP), cholecystokinin, gastrin, and the peptide adiponectin and leptin could be influenced by GLP-1RA and might play an important role in appetite regulation in overweight patients with T1D. Of interest, the lowering of glucagon during a meal may be important for the regulation of PPG in patients with T1D[14 29]. Another mechanism of interest could be the effect of the reduction in gastric emptying rate, resulting in delayed intestinal absorption of carbohydrates, and therefore, a reduction in postprandial glucose hyperglycemia[14 30]. All of these outcomes will be addressed in the study as secondary endpoints.

The limitations of this trial are that the effects of combination therapy on weight and glycemic control in normal weight patients with T1D are not addressed. Also, , the effect of combination therapy on counterregulation during hypoglycemia is not included in this protocol. A study investigating the effect on gastric emptying rate and on glucagon response during hypoglycemia is of high priority, since both a delaying in gastric emptying after food intake and an attenuated glucagon will compromise counterregulation[19].

In conclusion, the present study will elucidate many of the advantages and disadvantages associated with combination therapy with insulin and GLP-1RA in patients with T1D.

AUTHORS' CONTRIBUTIONS

TFD, FKK, TSH, LT, TA, JJH and HUA conceived and designed the study. HUA sponsors the trial and owns the data. TFD is principal investigator. CSF is sub-investigator. TFD, CSF, HUA, FKK and SM have drafted the manuscript. All authors have read and approved the final version of the manuscript.

FUNDING

This work was initiated by the authors and is supported as an investigator-initiated study by an unrestricted grant from Novo Nordisk A/S.

COMPETING INTERESTS

TFD, CSF and TSH have received research support from Novo Nordisk. FKK 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, Novo Nordisk and Zealand Pharma, has consulted for AstraZeneca, Gilead Sciences, Ono Pharmaceuticals, Novo Nordisk and Zealand Pharma and received research support from Sanofi. TA owns stocks in Novo Nordisk. LT owns stocks in Novo Nordisk, have received lecture fees and consulted for Novo Nordisk, JJH has consulted for Merck Sharp & Dome, Novo Nordisk and Roche. SM has participated in advisory boards for Novartis Pharma, Novo Nordisk, Merck Sharp & Dome, Sanofi, AstraZeneca, Johnson & Johnson, Roche, Mankind, Boehringer-Ingelheim, Zealand Pharma, Eli Lilly and Intarcia Therapeutics, and has received honoraria for lectures from Novo Nordisk, Merck Sharp & Dome, Astra-Zeneca, Johnson and Johnson, Roche, Shering-Ploug, Sanofi-Aventis, Novartis Pharma, Eli Lilly and Bristol-Meyer Squibb. HUA owns stocks in Novo Nordisk and participates in an advisory board for Abbott.

Table 1: Outcome measures

Primary endpoint

- HbA1c

Secondary endpoints

- Insulin dose
- Hypoglycemic events
- Glycemic excursions (time spent in hypo and hyperglycemia as measured by continuous glucose monitoring)
- Body weight
- BMI
- Lean body mass and fat mass composition as determined by DXA scan
- Quality of life and treatment satisfaction
- Food preferences
- VAS score for appetite
- Postprandial glucagon, GLP-1, GLP-2, GIP, CCK, Gastrin, Adiponectin, Leptin levels, gastric emptying (paracetamol absorption test) and glycemic excursion during standardised liquid MMT
- CIMT
- PWV
- 24 hour BP
- Heart rate
- Adverse events

HbA1c, glycated haemoglobin A1c; BMI, body mass index; DXA, dual-energy X-ray absorptiometry
VAS, visual analogue scale; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2
CCK, cholecystokinin; MMT, mixed meal test; CIMT, carotis intima media thickness
PWW, pulse wave velocity; BP, blood pressure

Table 2: Overview of inclusion and exclusion criteria

Inclusion criteria

- T1D (according to WHO criteria) ≥1 year
- Age ≥18 years
- BMI >25.0 kg/m^2
- HbA_{1c} >8.0% at visit 0
- Able to understand the written patient information and to give informed consent

Exclusion criteria

- Insulin pump treatment
- Hypoglycemia unawareness
- Diabetic gastroparesis
- Impaired kidney function (eGFR <60 ml/min/1.73m^2), dialysis or kidney transplantation
- Liver disease with elevated plasma ALT >three times the upper normal limit.
- Acute or chronic pancreatitis
- Inflammatory bowel disease
- Cancer unless in complete remission for >5 years
- History of thyroid adenoma or carcinoma
- Other concomitant disease or treatment that according to the investigator's assessment makes the patient unsuitable for study participation
- Alcohol and/or drug abuse
- Fertile women not using chemical or mechanical contraceptives
- Pregnant or nursing women
- Known or suspected hypersensitivity to trial product or related products
- Receipt of an investigational drug within 30 days prior to visit 0
- Simultaneous participation in any other clinical intervention trial

T1D, type 1 diabetes; eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase

Table 3: Blood samples

Sampling period	Analysis
Screening and control visits	➢ Glucose, HbA1c ➢ C-peptide, GAD antibodies ➢ Hemoglobin, thrombocytes, leucocytes ➢ Albumin ➢ Creatinine, potassium, sodium ➢ TSH ➢ Cholesterol, triglycerides ➢ ALT, lipase, amylase ➢ Urine albumin-creatinine ratio, hCG
MMT	➢ Paracetamol ➢ Glucose, C-peptide ➢ Glucagon ➢ GLP-1, GLP-2, GIP ➢ Adiponectin, leptin ➢ CCK, Gastrin ➢ NT-proBNP ➢ hsCRP

HbA1c, glycated haemoglobin A1c; GAD, glutamic acid decarboxylase; TSH, thyroid-stimulating hormone; ALT, alanine aminotransferase; hCG, human chorionic gonadotrophin; MMT, mixed meal test; GLP, glucagon-like peptide; CCK, cholecystokinin; NT-proBNP, N-terminal prohormone brain natriuretic peptide; hsCRP, high-sensitive C-reactive protein

Table 4: Trial visits

	Visit 0 Screening	Visit 1 Randomi-sation	Telephone or e-mail contact	Visit 2 Clinical control	Visit 3 Clinical control	Visit 4 CGM and blood pressure	Visit 5 End of trial
Time (weeks)	**- 1 ± 1**	**0**	**-**	**3 ± 1**	**12 ± 1**	**23 ± 1**	**24 ± 1**
General							
Assessment of inclusion and exclusion criteria	X						
Demography	X						
Medical history	X						
Concomitant medication	X	X	X	X	X		X
Smoking/alcohol	X						
Endpoints							
HbA_{1c}	X	X			X		X
Weight – BMI	X	X		X	X		X
Insulin dose	X	X	X	X	X		X
Hypoglycemia events		X	X	X	X		X
CGM	X				X	X	
DXA scan		X					X
Quality of life, problems in diabetes and treatment satisfaction		X		X			X
Food preference		X		X			X
VAS score for appetite[4]		X		X			X
Standardised meal test[4]		X		X			X
Carotid intima media thickness		X					X
Pulse wave velocity		X					X
24-hour blood pressure and pulse rate		X				X	
Clinical assessment							
Physical assessment incl. height	X						
Office blood pressure and heart rate	X	X		X	X		X
Electrocardiogram	X						X
Biosamples							
Biomarkers [2]		X			X		X
U-albumin / creatinine ratio		X					X
Biobank		X					X
Safety							
Adverse events		X	X	X	X		X
Blood tests[1]	X	X			X		X
Urinary HCG[3]	X	(X)		(X)	(X)		(X)
Study medication							
Drug accountability		X		X	X		X
Study drug dose titration			X				X

[1]Glucose, haemoglobin, leucocytes, thrombocytes, potassium, sodium, creatinine, albumin, lipids, alanine transferase, lipase, amylase
[2] Biomarkers: Leptin, GLP-2, Adiponectin, GAD antibodies, C-peptid, NT-proBNP, hsCRP
[3] Urinary HCG will be performed if menstruation is absent in a woman of childbearing potential
[4]The first 40 patients

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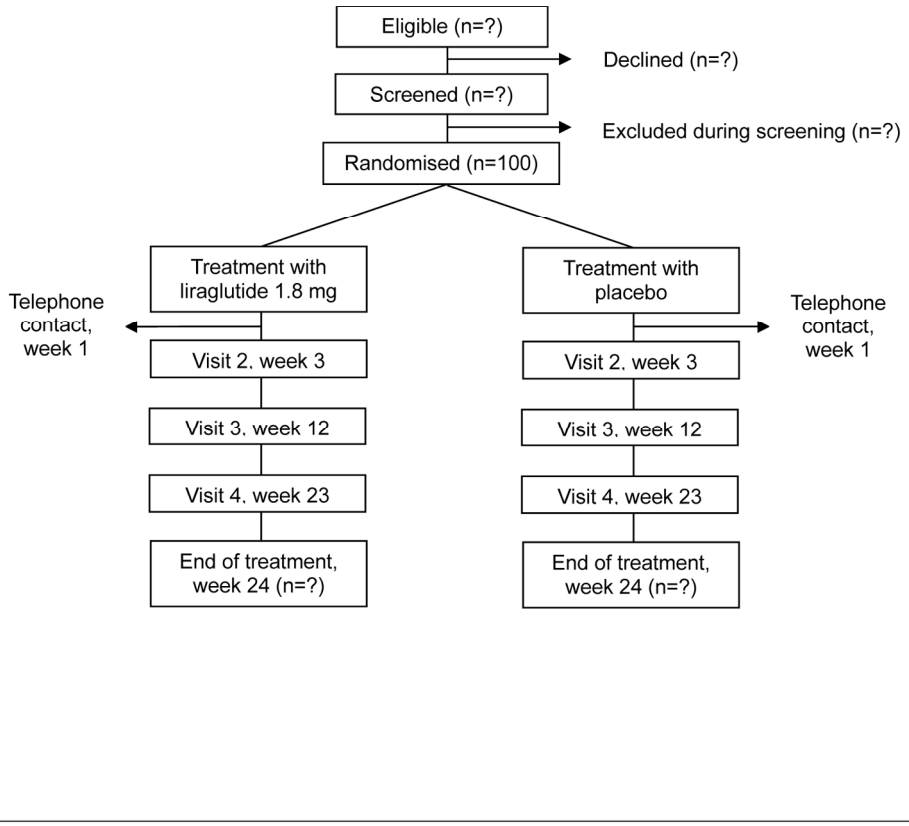
Figure 1: Flowchart of the study



Flowchart of the study
155x147mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P.1 Ln. 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P.4 Ln. 1-11
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	P.4 Ln. 13
Funding	4	Sources and types of financial, material, and other support	P.16 Ln. 11
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P.1 Ln. 6-15 AND P.16 Ln. 5-9
	5b	Name and contact information for the trial sponsor	
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	P.16 Ln. 5-9
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P.5 Ln. 1 – P.7 Ln. 9
	6b	Explanation for choice of comparators	P.6 Ln. 24 – P.7 Ln. 9
Objectives	7	Specific objectives or hypotheses	P.7 Ln. 11 – P.9 Ln. 7

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P.8 Ln. 11-17
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P.8 Ln. 19-23
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P.19 Table 2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P.10 Ln. 14 – P.11 Ln.4
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	P.10 Ln. 14 – P.11 Ln.4
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	P.10 Ln. 14 – P.11 Ln.4
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P.10 Ln. 14 – P.11 Ln.4
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P.7 Ln. 18 – P.8 Ln. 7 AND P.18 Table 1
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P.21 Figure 1 AND P.22 Table 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P.11 Ln, 6-14
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	P.8 Ln. 19
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P.8 Ln. 15
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P.8 Ln. 15
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P.8 Ln. 11-24
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P.8 Ln. 12-17
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	P.8 Ln. 16-17
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P.9 Ln. 1-P10 Ln. 12
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P.8 Ln. 15
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P.11 Ln. 16 - P.12 Ln. 13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P.12 Ln. 10-13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P.12 Ln. 10-13

Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P.9 Ln.9-11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P.22 Table 4
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P.9 Ln.9-11
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P.14 Ln.5
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P.9 Ln.3
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P.8 Ln.15-17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P.16 Ln.9-13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P.16 Ln.9-13
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases or other data sharing arrangements), including any publication restrictions	P.13 Ln.3-7
	31b	Authorship eligibility guidelines and any intended use of professional writers	P.13 Ln.3-7
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	P.22 Table 4

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.