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Protocol for a randomized controlled trial of the combined effects of the GLP-1 receptor agonist liraglutide and exercise on maintenance of weight loss and health after a very low-calorie diet

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Abstract

Introduction: The success rate of weight loss maintenance is limited. Therefore, the purpose of this study is to investigate the maintenance of weight loss and immunometabolic health outcomes after diet-induced weight loss followed by one-year treatment with a GLP-1 receptor agonist (liraglutide), physical exercise, or the combination of both treatments as compared with placebo in individuals with obesity.

Methods and analysis: This is an investigator-initiated, randomized, placebo-controlled, parallel group trial. We will recruit expectedly 200 women and men (age 18 to 65 years) with obesity (BMI 32 to 43 kg/m^2). Initially, participants will adhere to a very low-calorie diet (800 kcal/day) for eight weeks in order to lose at least 5 % of body weight. Subsequently, participants will be randomized in a 1:1:1:1 ratio to one of four study groups for 52 weeks: 1) placebo, 2) exercise 150 min/week + placebo, 3) liraglutide 3.0 mg/day, and 4) exercise 150 min/week + liraglutide 3.0 mg/day. The primary endpoints are changes in body weight and body composition from randomization to end-of-treatment.

Ethics and dissemination: The trial has been approved by the ethical committee of the Capital Region of Denmark and the Danish Medicines Agency. The trial will be conducted in agreement with the Declaration of Helsinki and monitored to follow the guidelines for good clinical practice. Results will be submitted for publication in international peer-reviewed scientific journals.

Trial Registration: EudraCT Nr.: 2015-005585-32

Strengths and limitations of this study

- First randomized controlled trial investigating the combined and individual effects of liraglutide and exercise to maintain diet-induced weight loss in individuals with obesity.
- Direct comparison of liraglutide and exercise on weight loss maintenance and immunometabolic health.
- Applying state-of-the-art methodologies, the study may identify novel targets for sustainable immunometabolic health-promoting weight loss strategies.

Introduction

Obesity is associated with increased risk of developing cardiovascular disease and type 2 diabetes, and carries increased risk of all-cause mortality [1,2]. In individuals with obesity, moderate weight loss of more than 5 % of initial body weight improves cardiometabolic risk factors, such as glycaemic control, blood pressure, and blood lipid profile [3–5]. However, the success rate of weight loss maintenance, defined as more than 10 % weight loss maintained for at least one year, is less than 20 % [6]. The main biological reasons for this low success-rate may be that weight loss causes a decrease in total energy expenditure to a degree that is greater than predicted from changes in fat and lean mass [7,8] in combination with increased appetite in the weight-reduced state [9].

Increasing energy expenditure by increasing physical activity is the first-line lifestyle modification in the treatment of obesity along with reducing food intake. For exercise interventions targeting general public health recommendations (at least 150 min/week of moderate intensity aerobic exercise), the associated weight loss is often modest (0-3 %) without concomitant calorie restriction [10–12]. However, independent of weight loss, increasing physical activity improves body composition, glycemic control, low grade inflammatory profile, and cardiorespiratory fitness in individuals with overweight and obesity [13–16]. In addition, exercise may preserve lean mass during weight loss [17] and thereby counteract the associated decrease in resting metabolic rate [18], which may explain the observation that individuals performing regular exercise have less body weight regain after weight loss compared to participants that do not exercise [6,19].

Glucagon-like peptide-1 (GLP-1) is an incretin hormone primarily secreted from enteroendocrine L-cells in the gut after food intake. GLP-1 stimulates glucose-dependent insulin secretion thereby lowering blood glucose and reduces appetite and thereby food intake [20,21]. Treatment for 56

weeks with the GLP-1 analogue, liraglutide (3.0 mg), as an adjunct to regular diet and physical activity recommendations has been shown to improve glycemic control and induce moderate weight loss of 4.0 % in type 2 diabetic [22] and 5.4 % in non-diabetic [23] individuals with overweight or obesity compared to placebo. In addition, liraglutide has been shown to maintain a diet-induced weight loss over 56 weeks [24] and maintain very low-calorie diet-induced improvements of fasting plasma glucose and triglycerides over 52 weeks of weight loss maintenance superior to similar diet-induced weight loss maintenance in obese nondiabetic individuals [25].

Obesity is associated with chronic low-grade inflammation [26,27] which is linked to the development of atherosclerosis and insulin resistance [28–30]. Physically active individuals have lower inflammatory biomarker concentrations than their inactive counterparts [15], possibly explained by antiinflammatory effects of an acute bout of exercise [31] and lower levels of visceral adipose tissue [32]. GLP-1 has also emerged as an immunomodulatory agent, as illustrated by GLP-1 analogue administration exerting anti-inflammatory actions in various cells, including endothelial cells, adipocytes, peripheral blood mononuclear cells, and in plasma [33–37].

Thus, both physical activity and GLP-1 analogue treatment seem to facilitate weight loss maintenance, improve metabolic health, and reduce systemic inflammation. However, diet-induced weight loss decreases energy expenditure and increases appetite. We hypothesize that the combination of physical activity and liraglutide treatment improves weight loss maintenance and immunometabolic health since the decreased energy expenditure is targeted with exercise and the increased appetite with liraglutide.

Objective

The objectives of this study are to investigate the maintenance of weight loss and immunometabolic health outcomes over 52 weeks with liraglutide treatment, physical exercise, and the combination in individuals with obesity, after a very low-calorie diet.

Methods and analysis

Participants, interventions, and endpoints

Trial design

This study protocol describes an investigator-initiated, randomized, placebo-controlled, parallel group trial, the S-LiTE trial (acronym for 'Synergy effect of the appetite hormone GLP-1 (LiragluTide) and Exercise on maintenance of weight loss and health after a low calorie diet'). The trial is double-blinded with regards to study medication. The study design is outlined in Figure 1. The study is registered at the European Clinical Trials Database (EudraCT Nr.: 2015-005585-32).

Study setting

All examinations in the trial will be carried out at Department of Endocrinology, Hvidovre University Hospital, University of Copenhagen and Department of Biomedical Sciences, University of Copenhagen.

Study status

Recruitment of participants was initiated in September 2016. Last participant last visit is planned for November 2020.

Participants and recruitment

We will recruit expectedly 200 participants. Eligible participants are adults (age 18-65 years) with obesity (BMI 32-43 kg/m^2) and no known serious chronic illness (incl. type 1 and 2 diabetes). Inclusion and exclusion criteria are listed in Table 1. Recruitment will be done via local newspapers, online media, and flyers from Department of Endocrinology, Hvidovre University Hospital, and Department of Biomedical Sciences, University of Copenhagen. Individuals who agree to participate will be invited to a pre-screening that includes screening of the study eligibility criteria before being finally included in the study. Withdrawn subjects will not be replaced. Re-screening is allowed within the recruitment period.

Participant involvement

The design of the study was partly inspired by qualitative interviews of a similar participant group performed by an anthropologist[38]. Upon completion of data analyses, results will be disseminated to all participants as a lay summary of the main findings.

Interventions

Diet-induced weight loss

Initially, all participants will undergo eight weeks with a very low-calorie diet (VLCD) (Cambridge Weight Plan, 800 kcal/day) with the objective to lose at least 5 % of body weight. Participants will be instructed to eat four meal replacements per day containing approximately 200 kcal and to only drink water and non-caloric beverages. Participants who have lost at least 5 % of body weight after the eight-week weight loss phase will be randomized to one of the four study groups: 52 weeks of treatment with 1) placebo, 2) exercise + placebo, 3) liraglutide, or 4) exercise + liraglutide. After randomization, participants will undergo a four-week phase-out plan with three daily Cambridge

meal products and one regular meal the first week and two daily Cambridge meal products and two regular meals the three subsequent weeks.

Liraglutide or placebo

The GLP-1 analogue, liraglutide (3.0 mg) (Saxenda, Novo Nordisk, Bagsværd, Denmark), or placebo will be administrated once daily as subcutaneous injections in the abdomen or thigh. The starting dose is 0.6 mg with weekly increments of 0.6 mg until 3.0 mg is achieved. The titration procedure will be prolonged for participants who do not tolerate fast up-titration. Participants who do not tolerate the 3.0 mg dose may in special circumstances stay at lower dose (2.4 mg). However, the aim is to reach 3.0 mg for all study participants.

Physical exercise

The exercise intervention follows the global recommendations from WHO of 150 minutes of moderate-intensity aerobic physical activity throughout the week or 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity activity [39]. The intervention consists of four sessions per week for a total of 150 min/week. Two sessions per week will be performed under supervision of the study staff and two sessions will be performed individually. A heart rate monitor (Polar A300, Polar Electro Oy, Kempele, Finland) will be worn during all planned exercise sessions. Supervised sessions will consist of structured exercise for a duration of 45 min. Of this, 30 min will be interval-based spinning and 15 min will be circuit training focusing on large muscle groups. Individual exercise sessions will include aerobic exercise such as cycling, rowing, or elliptical training as well as brisk walking or cycling to work. Participants will be advised to primarily perform non-weight bearing activities. The target aerobic exercise intensity is 80 % of maximal heart rate. Participants

randomized to an exercise group will undergo a 6-week ramp-up phase with one session in week 1 and 2, two sessions in week 3 and 4, and three sessions in week 5 and 6 before exercising four times per week from week 7 to 52. If the planned ramp-up phase with exercise is not possible (e.g. due to side effects of study medication or joint pain), ramp-up will proceed more slowly. Participants not randomized to exercise will be instructed to maintain habitual physical activity according to level before entering the trial.

Liraglutide and physical exercise

Combination of the two interventions described above.

The trial will end at week 52 after randomization where liraglutide/placebo and exercise treatment will be discontinued. One year after the intervention the participants will be invited for a follow-up visit.

Criteria for discontinuing/modifying allocated interventions

Participants may withdraw from the intervention at any time. Withdrawn participants will be invited for the planned examinations at week 52 unless written consent is withdrawn. Participants may be withdrawn from the trial at the discretion of the investigator due to a safety concern or a serious violation of the protocol. Participants will be withdrawn in occurrence of pregnancy or at the intention to become pregnant.

Endpoints

Primary endpoints

The primary endpoints are changes in body weight and body composition from after the initial weight loss phase (baseline/V1) to end of treatment after 52 weeks (end/V3).

Secondary endpoint

The secondary endpoint is change in metabolic health (glucose tolerance, lipid status, waist circumference, blood pressure) from V1 to V3.

Explorative endpoints

Explorative endpoints include changes from V1 to V3 in the following parameters: meal-related appetite hormone response; physical fitness and determination of daily physical activity and sleep; systemic markers of immunometabolism; endothelial function; immunometabolic changes in the subcutaneous adipose tissue; gene expression profile of circulating inflammatory cells; bone health; food preferences and subjective appetite sensation; faecal bacterial composition; plasma metabolomics and proteomics; epigenetics of spermatozoa.

Sample size calculation

Sample size is calculated in relation to body weight. In our previous weight loss maintenance study [25], the response within each treatment group was normally distributed with standard deviation of 5.5 kg. Thus, with expectedly 30 participants completing each study arm we will be able to detect a true difference of 4 kg between groups with a power of 0.8, assuming a two-sided α-level of 0.05. In our previous study, 10 % did not complete the initial weight loss phase [25]. Thus, with expectedly 200 recruited study participants and an expected dropout rate of 25 % after randomization, we expect to have at least 30 participants from each study arm to complete the trial.

Assignment of intervention

Treatment allocation

Participants will be randomized to one of the four study groups in a 1:1:1:1 ratio in accordance with a subject randomization list (SRL) provided by Novo Nordisk (NN). An un-blinded study nurse (not otherwise associated with the trial) will allocate study participants according to the SRL. Randomization will be stratified by sex (male/female) and age (below/above 40 years). NN will provide a total dispensing unit number list (TDL). The un-blinded study nurse will allocate trial medication using the TDL by matching a six digit Dispensing Unit Number (DUN) to the correct treatment. Each box of study medication will be labeled with a unique DUN. The DUN alone is not un-blinding. Thus, dispensing of trial medication to subjects can be carried out by blinded trial staff by selecting the DUN provided by the un-blinded study nurse. The SRL and the TDL are stored on site at Hvidovre Hospital with restricted access only to the designated un-blinded study nurse.

Un-blinding

The SRL and the TDL are stored on site at Hvidovre Hospital with restricted access only to the designated un-blinded study nurse. Study ID of the participant is matched with the SRL and TDL, which reveals if trial medication is liraglutide or placebo. Preferably, the un-blinded study nurse will perform any un-blinding of study participants. However, if needed, all trial staff (sponsor, investigator, and sub-investigators) can get access to the SRL and TDL and perform the un-blinding procedure. Un-blinding can be performed under the following circumstances: treatment of a participant in a medical emergency that requires knowledge of treatment allocation; treatment of a participant for an adverse event; in the event of a suspected unexpected serious adverse reaction; in the event that the participant's study medication is accidentally taken by a member of their

household, e.g. a child; for the submission of trial data to the Data Monitoring and Safety Committee for the monitoring of safety and/or efficacy.

Data collection, management, and analysis

Study visits

Identical test days will take place before the initial weight loss phase (screening/V0), after initial weight loss (baseline/V1), and after 52 weeks of treatment (end/V3) (Fig. 1). Furthermore, a visit will be performed after 26 weeks of treatment (mid/V2). An overview of performed assessments is provided in Table 2. During the weight loss phase, weekly consultations will be conducted to assess compliance to the VLCD, including measurement of body weight and handing out Cambridge meal products. During the 52-week weight maintenance phase, weight consultations, including assessment of adverse events, will be conducted at week 1, 2, 3, 4, 9, 13, 17, 22, 32, 39, and 46. Consultations at week 4, 13, and 39 will include collection of fasting blood samples, measurement of hip and waist circumference, blood pressure, and resting heart rate. Finally, participants will be invited to complete a post-trial unsupervised follow-up visit (V4) one year after intervention completion.

Criteria for assessments

- Participants must be fasting for minimum 10 hours prior to test days, including foods, liquids, and medication (except study medication)
- Study medication should be taken on the morning of the tests
- Exercise should not be performed the day before or in the morning before tests

Assessments

Anthropometrics

Body weight will be measured on a digital scale (TANITA WB-110MA, Tokyo, Japan) to the nearest 0.1 kg without shoes and wearing light clothes. Waist circumference, the midpoint between lowest rib and iliac crest, and hip circumference, the level of the great trochanters, will be measured in duplicate to the nearest 0.1 cm after gentle expiration.

DXA scan

Dual-energy X-ray absorptiometry (DXA) scans will be performed in fasting state to measure body fat mass, fat free mass, and bone density (Hologic Discovery A, Hologic inc., Bedford, USA).

Blood pressure

Blood pressure and resting heart rate will be measured in duplicate from the non-dominant arm with a digital blood pressure monitor (Microlife BP A3 plus, Widnau, Switzerland) in sitting position after at least 5 min of rest.

Fasting blood samples

Fasting blood samples will be collected to measure circulating biomarkers of metabolic health, appetite hormones, immune markers, plasma proteomics, and plasma metabolomics. Furthermore, peripheral blood mononuclear cells will be isolated and DNA will be collected. A set of standard samples will be collected and analyzed on the same day for participants' safety, including: hemoglobin, free calcium, creatinine and estimated glomerular filtration rate, potassium, sodium, C-reactive protein, alanine aminotransferase, amylase, alkaline phosphatase, vitamin D, glycated hemoglobin, parathyroid hormone, thyrotropin, and blood lipids.

Adipose tissue biopsy

Subcutaneous abdominal adipose tissue biopsies (~1 g) will be obtained by needle aspiration under local anaesthesia using 5–10 ml 0.5% lidocaine. From adipose tissue biopsies, gene expression will be determined from reverse transcription-qPCR: proinflammatory and antiinflammatory adipocytokines, adipocyte differentiation markers, and markers of macrophages infiltration. The immune cells of the adipose tissue will be isolated to evaluate macrophage sup-populations and activation status (single cell analysis).

Meal test

After fasting blood sampling, a liquid meal (Nutricia Nutridrink, 600 kcal, 49 E% from carbohydrates, 35.2 E% from fat, and 15.8 E% from protein) will be ingested over 15 min, and blood samples will be collected continuously every 15 min for the first hour and every 30 min for the next two hours to measure circulating biomarkers of metabolic health, appetite hormones, immune markers, plasma proteomics and plasma metabolomics. During the meal test, appetite sensation will be assessed after each blood sample using a visual analogue scale [40].

Feces, urine, saliva, and semen

Feces samples will be collected to investigate fecal bacterial composition. Semen will be collected (if relevant) to investigate epigenetics of the spermatozoa. Additionally, urine and saliva samples will be collected.

Endothelial function

Flow mediated dilation (FMD) of the brachial artery will be measured to assess endothelial function [41]. FMD, also known as endothelium-dependent vasodilation, is the vasodilatory response of the

brachial artery to increased shear stress and reflects the ability of vascular endothelium to produce nitric oxide. The brachial artery will be scanned with high resolution ultrasound imaging using a linear probe at rest and during hyperaemia. Hyperaemia will be induced by inflation and deflation of a sphygmomanometer cuff around the forearm, distal to the site scanned with ultrasound. FMD is calculated as the percentage change of the brachial artery diameter from rest to 60 seconds after the cuff is released. To assess the endothelium-independent vasodilation, nitroglycerine is given sublingually (0.4 mg) with the diameter of the brachial artery measured before and 5 min after drug administration. Carotid intima-media thickness will also be measured using ultrasound.

Electrocardiography

Electrocardiogram will performed to assess safety concerns related to study participation.

Physical fitness

Measurement of physical fitness will include three components: 1) Cardiorespiratory fitness (peak oxygen consumption) will be assessed with an incremental maximal cycle protocol performed on an electromagnetically braked cycle ergometer (Corival, Lode Medical Technology, The Netherlands) with continuously determined oxygen consumption and carbon dioxide production (MasterScreen CPX, CareFusion, Germany). After a warm-up protocol, workload will be increased every minute (20 and 25 watt for females and males, respectively) until attainment of peak oxygen consumption based on the following criteria: plateau in oxygen consumption, respiratory exchange ratio above 1.15 or attainment of age-predicted maximal heart rate (220 minus age), and voluntary exhaustion [42]. 2) Physical functioning will be measured as time to ascend and descend an 11-step stairway twice. 3) Maximal strength will be measured as isometric maximal voluntary contraction force of

the dominant thigh using a dynamometer chair (Good Strength, Metitur Oy, Jyväskylä, Finland) [43,44].

Questionnaires

Participants will answer five questionnaires to determine self-rated quality of life (The Short Form (36) Health Survey[45]), eating habits (three-factor eating questionnaire[46]), physical activity (International Physical Activity Questionnaire[47]), sleep quality (Pittsburgh Sleep Quality Index[48]), and self-efficacy (General Self-Efficacy Scale[49]).

Food preferences

Food preferences and food reward responses will be measured in fasted state and postprandially with the Leeds Food Preference Questionnaire [50]. This is a computerized task where standardized pictures of 20 typical Danish food items are shown in the categories: high fat savoury, low fat savoury, high fat sweet and low fat sweet. Participants are instructed to rate each individual food item according to liking and to systematically choose the food items they prefer the most. Speed of choices is covertly assessed as an index of implicit wanting.

Free-living assessment of physical activity and sleep

An accelerometer device (GENEActiv, ActivInsights Ltd, Cambridgeshire, UK) will be worn on the wrist for seven consecutive days and nights with concomitant sleep registration (time for going to and out of bed) to assess habitual physical activity and sleep duration.

Data management

Participants will be identified by study ID. Study data is collected and managed using the REDCap secure web-based system [51] hosted by the Capital Region of Denmark, where electronic case report forms (CRF) have been created. During the trial, data will be entered directly into REDCap by study personnel. Extraction procedures will be performed by investigators or sponsor. Laboratory data will be transferred electronically from the laboratory performing clinical analyses and will be archived in secured hard drives with backup. All biological material (blood, adipose tissue, faeces, urine, saliva, and semen) obtained from study participants will be kept in a research bio-bank. Samples will be labelled with study ID. The bio-bank allows for analyses of samples to be performed simultaneously to avoid large instrumental variations. The research bio-bank will be terminated no later than August 1st 2036. After this date, the remains of the material will be transferred to a regular bio-bank in Denmark. In order to access this material, a new protocol must be approved by an ethics committee. The Danish Data Protection Agency has been notified about the trial and the trial adheres to the Data Protection Act, which requires data to be anonymized as soon as it is practical to do so.

Data analysis plan

Analyses will be based on two defined analysis sets: an intention-to-treat analysis set (ITT) and a per-protocol analysis set (PP). ITT includes all randomized participants exposed to at least one dose of trial product or exercise that have completed the visit at week 4. PP will include all participants who complete the 52-week intervention with ≥ 75 % compliance to the interventions. Safety analysis will be performed on the ITT analysis set. Two-tailed tests will be performed and the significance level will be set to $\alpha = 0.05$.

Primary endpoints

The changes from V1 to V3 in body weight (kg) and body composition (lean/fat mass ratio) will be analysed using a general linear model with treatment group as explanatory variable and baseline weight or BMI, age, sex, and initial weight loss (if relevant) as covariates. We will use drug treatment (liraglutide, placebo), exercise (yes, no) and their interaction to investigate the effects of drug and exercise. Adequacy of model assumptions will be assessed using graphical models, and outcome variables may be logarithmically transformed if considered necessary to meet the assumptions of linearity, variance homogeneity, and/or normality of residuals. The objective of the analyses is to determine whether the effect on body weight and body composition of 3.0 mg liraglutide in combination with exercise program is superior to 1) placebo and 2) to either treatments alone, and to quantify the extend of this superiority. Weight change over time will also be assessed by repeated measures analyses as appropriate (linear mixed model with an appropriate covariance structure and a suitably described effect over time). Blinding of study medication allocation will be kept for investigators until analysis of the primary endpoints has been completed.

Secondary and exploratory endpoints

For secondary and exploratory endpoints, general linear models as described above will be used to test for superiority. The change from V1 to V3 will be analysed using general linear models with treatment group as explanatory variable and baseline weight or BMI, initial weight loss (if relevant), sex as covariates. Longitudinal data will also be analysed using linear mixed models as described above.

Auditing

The Good Clinical Practice (GCP) unit of the University Hospitals of Copenhagen will perform on-site audits minimum once yearly to ensure that the trial adhere to the guidelines for good clinical practice provided by the International Council for Harmonisation (ICH).

Ethics and dissemination

Harms

All adverse events (AE) will be collected from the first drug administration and in all following contacts with participants throughout the trial. A serious adverse event (SAE) is defined as any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenial anomaly or birth defect. Once yearly, sponsor will send a report regarding SARs occurring in the trial and safety of the study participants in regards to continuation of the trial to the Danish Medicines Agency and the Ethics Committee. In case of any deadly or life-threatening suspected unexpected serious adverse reaction (SUSAR), sponsor will immediately (and no later than 7 days after becoming aware) notify the Danish Medicines Agency and the Ethics Committee. No later than 8 days after reporting of a SUSAR, sponsor will notify the Danish Medicines Agency and the Ethics Committee of all relevant information about sponsor's and investigator's follow-up of the SUSAR. All other SUSARs will be reported to the Danish Medicines Agency and the Ethics Committee no later than 15 days after sponsor becoming aware of this. All AEs and SAEs will be noted in the CRF and recorded in the End-of-Trial Form to the Danish Medicines Agency and in the report to the Ethics Committee (if requested) no later than 90 days after trial completion.

Ethical considerations

Injection with liraglutide will be given with Saxenda injection pen. Saxenda is an approved drug and the dosage will be kept within the approved maximum (3.0 mg). Saxenda is safe but may cause transient nausea during the first weeks. Other side effects include dizziness, insomnia (transient) and gall stones. Uncommon/rare side effects include dehydration, inflamed gall bladder, allergic reactions, and reduced kidney function. Placebo injections should not cause any discomfort. There should not be any discomfort to the injection if performed as prescribed. The Cambridge Weight Plan is used in daily clinical practice and is considered safe but may cause constipation, fatigue, headache, and dizziness. The exercise program does not exceed the recommendations from WHO [52]. However, participants are not habitual exercisers. Therefore, careful considerations regarding the ramp-up of the exercise intervention will be given. Furthermore, most of the exercise are non-weight bearing activity in an attempt to limit potential injuries associated with the exercise intervention. The discomfort associated with the planned examinations is considered minimal. Applying a peripheral venous catheter for blood samples collection can cause transient discomfort, irritation, and redness around the puncture site. Some discomfort might be experienced when applying local anaesthesia for the adipose tissue biopsy. The biopsy itself should only cause minimal discomfort. DXA scans use radiation with a radiation dose of approximately 0.02 mSv per examination. This dose is very low compared to the background radiation in Denmark (approximately 3 mSv/year). The FMD and carotid intima-media thickness ultrasound scans do not use radiation. Applying nitroglycerine may cause transient headache, dizziness, decreased blood pressure, and increased heart rate. Half-life of nitroglycerine is 1-3 min. Nitroglycerine will not be used if systolic blood pressure is under 100 mmHg. The risks that are associated with this study are assessed as minimal. By participating in this project, the participants will contribute with new important knowledge about the interaction between GLP-1 and exercise and their importance for weight loss maintenance and metabolic health. Overall, we consider that any potential risks and side

effects are outweighed by the advantages of participation. The trial is approved by the ethical committee of the Capital Region of Denmark (H-16027082) and the Danish Medicines Agency EudraCT Nr.: 2015-005585-32. The trial will be carried out in accordance with the Declaration of Helsinki, and adhere to the GCP-ICH guidelines. After careful written and oral information about the trial and associated risks have been given, a written informed consent form will be obtained by investigator or sub-investigators before any study-related activities are performed.

Dissemination plan

All study results (positive, negative, and inconclusive) will be presented at international scientific conferences as oral presentations or poster presentations. Furthermore, results will be published in international peer-reviewed scientific journals.

Protocol version

The study protocol was approved on June 30 2016. The present manuscript details the latest version of the protocol (version 9) approved on October 6 2018.

Discussion

Obesity prevalence has increased dramatically in the past decades [53] and the high recidivism rates after intentional weight loss [6,54] emphasize a strong need for new effective strategies to promote sustainable weight loss maintenance in individuals with obesity. The present protocol describes the first randomized controlled trial to investigate GLP-1 analogue treatment combined with physical exercise in the context of long-term weight loss maintenance. The results have the potential to reveal a novel approach of combined lifestyle and pharmacological intervention that may provide substantial improvements of body weight, body composition, and immunometabolic health in

obesity. Additionally, by applying state-of-the-art methodologies, the study may identify novel targets for future immunometabolic health-promoting weight loss interventions.

Contributors

SST formulated, initiated, and designed the study. BS, JJH, SM, MR, CJ and JL contributed to the study design. SJ, JL and SST drafted the manuscript. All authors have contributed to and approved the final version of the manuscript. Authorship eligibility will follow the Vancouver guidelines.

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Competing interests

SST has received research grant from Novo Nordisk. SM has received research grants from Novo Nordisk and Boehringer Ingelheim, lecture fees from AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli Lilly, Merck Sharp & Dohme, Novartis, Novo Nordisk, and Sanofi Aventis, and is a member of advisory boards of AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novartis; Novo Nordisk,

and Sanofi Aventis. JJH has served on advisory panels for GlaxoSmithKline, Novo Nordisk, Zealand Pharma, AstraZeneca, MSD, Intarcia and Hanmi and as a consultant for Novo Nordisk, and has received research support from Merck, Sharp & Dohme.

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Tables

Table 1. Eligibility criteria for participants in the S-LiTE trial

Inclusion criteria

- BMI: 32-43 kg/m^2
- Age: 18-65 years
- Safe contraceptive method or menopause for women

Exclusion criteria

- Patients diagnosed with any known serious chronic illness, including type 1 or 2 diabetes (or a randomly measured fasting plasma glucose > 7 mmol/l)
- Angina pectoris, coronary heart disease, or congestive heart failure (NYHA III-IV)
- Severe renal impairment (creatinine clearance (GFR) <30 mL/min)
- Severe hepatic impairment
- Inflammatory bowel disease
- Diabetic gastroparesis
- Cancer
- Chronic obstructive lung disease
- Psychiatric disease, a history of major depressive, or other severe psychiatric disorders
- The use of medications that cause clinically significant weight gain or loss

- Previous bariatric surgery
- A history of idiopathic acute pancreatitis
- A family or personal history of multiple endocrine neoplasia type 2 or familial medullary thyroid carcinoma
- Osteoarthritis, which is judged to be too severe to manage the exercise programme
- Pregnancy, expecting pregnancy, or breast feeding
- Allergy to any of the ingredients of the study medication: liraglutide, disodium phosphate dihydrate, propylene glycol, phenol, hydrochloric acid, and sodium hydroxide
- Regular exercise training at high intensity (e.g. spinning) >2 hours per week

Table 2. Overview of study visits

Visit	**Pre screening**	**V0**	**V1**	**V2**	**V3**	**V4**
Time point (week)	**-**	**-8**	**0**	**26**	**52**	**104**
Informed consent	X					
Anamnesis	X					
Inclusion/exclusion criteria	X					
Demographics	X					
Pregnancy test		X	X		X	
Adverse events		X	X	X	X	
Body weight	X	X	X	X	X	X
Waist and hip circumference		X	X	X	X	X
Blood pressure and heart rate		X	X	X	X	X
Fasting blood samples		X	X	X	X	X

7-day accelerometry	X	X	X	X	X
Adipose tissue biopsy	X	X	X	X	
DXA scan	X	X		X	X
Liquid meal test	X	X		X	
Fecal, urine, saliva, and semen	X	X		X	
FMD	X	X		X	
ECG	X	X		X	
Physical fitness testing	X	X		X	
Questionnaires	X	X		X	X
Food preference test	X	X		X	X

DXA, Dual-energy X-ray absorptiometry; FMD, flow-mediated dilation; ECG, electrocardiogram.

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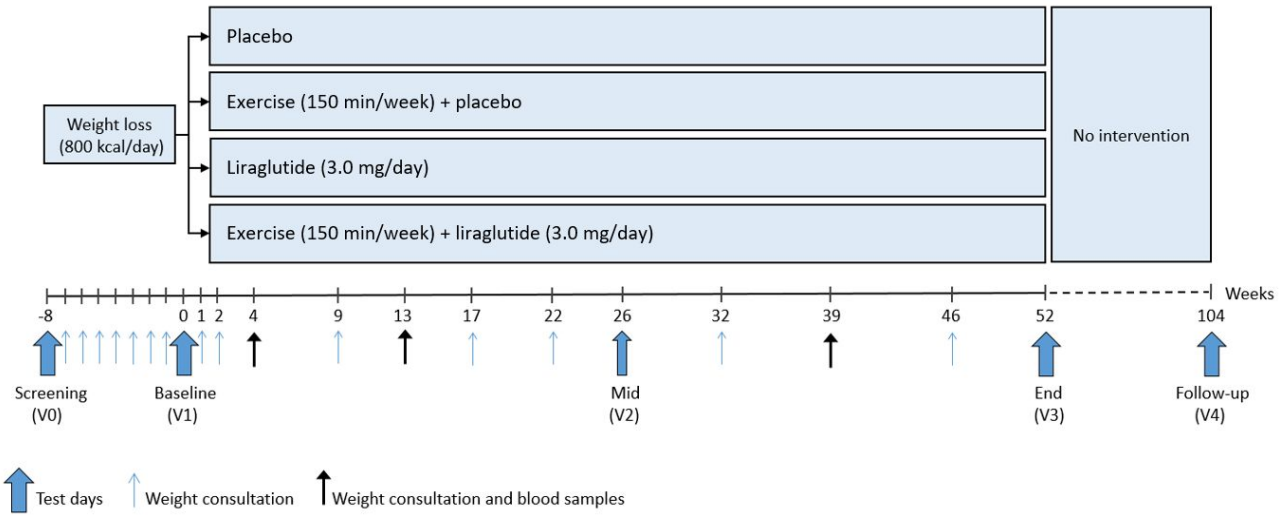

SPIRIT 2013 Checklist for the S-LiTE randomised trial: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Yes, at EudraCT
Protocol version	3	Date and version identifier	21
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 21, 22
	5b	Name and contact information for the trial sponsor	1
	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	22
	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)	n/a

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	4, 5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5, 6
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)	6
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	6
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)	6, 7, Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7, 8, 9
	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)	9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
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	9, 10
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)	12, Table 2

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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 7
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	11
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	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11, 12
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	12-16, Table 2
	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	9

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	16, 17
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	17, 18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17, 18
	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)	17
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	18, 19
	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	n/a
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	19
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18, 19
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
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)	21

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	16, 17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22, 23
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	22
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	n/a
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	21
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
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	16, 17

*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.

BMJ Open

Protocol for a randomized controlled trial of the combined effects of the GLP-1 receptor agonist liraglutide and exercise on maintenance of weight loss and health after a very low-calorie diet

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Protocol for a randomized controlled trial of the combined effects of the GLP-1 receptor agonist liraglutide and exercise on maintenance of weight loss and health after a very low-calorie diet

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Abstract

Introduction: The success rate of weight loss maintenance is limited. Therefore, the purpose of this study is to investigate the maintenance of weight loss and immunometabolic health outcomes after diet-induced weight loss followed by one-year treatment with a glucagon-like peptide-1 receptor agonist (liraglutide), physical exercise, or the combination of both treatments as compared with placebo in individuals with obesity.

Methods and analysis: This is an investigator-initiated, randomized, placebo-controlled, parallel group trial. We will enroll expectedly 200 women and men (age 18 to 65 years) with obesity (body mass index 32 to 43 kg/m^2) to adhere to a very low-calorie diet (800 kcal/day) for eight weeks in order to lose at least 5 % of body weight. Subsequently, participants will be randomized in a 1:1:1:1 ratio to one of four study groups for 52 weeks: 1) placebo, 2) exercise 150 min/week + placebo, 3) liraglutide 3.0 mg/day, and 4) exercise 150 min/week + liraglutide 3.0 mg/day. The primary endpoint is change in body weight from randomization to end-of-treatment.

Ethics and dissemination: The trial has been approved by the ethical committee of the Capital Region of Denmark and the Danish Medicines Agency. The trial will be conducted in agreement with the Declaration of Helsinki and monitored to follow the guidelines for good clinical practice. Results will be submitted for publication in international peer-reviewed scientific journals.

Trial Registration: EudraCT Nr.: 2015-005585-32

Strengths and limitations of this study

- First randomized controlled trial investigating the combined and individual effects of liraglutide and exercise to maintain diet-induced weight loss in individuals with obesity.
- Direct comparison of liraglutide and exercise on weight loss maintenance and immunometabolic health.
- Applying state-of-the-art methodologies, the study may identify novel targets for sustainable immunometabolic health-promoting weight loss strategies.

Introduction

Obesity is associated with increased risk of developing cardiovascular disease and type 2 diabetes (T2D), along with increased risk of all-cause mortality [1,2]. Obesity management guidelines recommends weight loss of more than 5 % of initial body weight to improve cardiometabolic risk factors, with greater weight loss producing greater benefits [3,4]. A 5 to 10 % weight loss improves lipid profile (~20% reduction in triglycerides, ~15 % reduction in LDL-cholesterol, ~8 % increase in HDL-cholesterol levels) [1,4,5], reduces systolic and diastolic blood pressure (~5 and ~4 mmHg, respectively) [3,6], reduces HbA1c [3,4], and improves insulin sensitivity [7–9]. However, weight regain reverse these health benefits [10,11]. Furthermore, intentional weight loss is typically followed by a 30 to 50 % regain of lost weight within the first year [12–14]. The main biological reasons for the rapid weight regain may be that weight loss causes a decrease in total energy expenditure to a degree that is greater than predicted from the decrease in fat and lean mass [15,16] in combination with increased appetite in the weight-reduced state [17,18].

Increasing energy expenditure by increasing physical activity is the first-line lifestyle modification in the treatment of obesity along with reducing food intake. For exercise interventions targeting general public health recommendations (at least 150 min/week of moderate intensity aerobic exercise), the associated weight loss is often modest (0-3 %) without concomitant calorie restriction [19–21]. However, independent of weight loss, increasing physical activity improves body composition, glycemic control, low grade inflammatory profile, and cardiorespiratory fitness in individuals with overweight and obesity [22–25]. In addition, exercise may preserve lean mass during weight loss [26] and thereby counteract the associated decrease in resting metabolic rate [27], which may explain the observation that individuals performing regular exercise have less body weight regain after weight loss compared to participants that do not exercise [28,29].

Glucagon-like peptide-1 (GLP-1) is an incretin hormone primarily secreted from enteroendocrine L-cells in the gut after food intake. GLP-1 stimulates glucose-dependent insulin secretion thereby lowering blood glucose and reduces appetite and thereby food intake [30,31]. Treatment for 56 weeks with the GLP-1 receptor agonist (GLP-1 RA), liraglutide (3.0 mg), as an adjunct to regular diet and physical activity recommendations has been shown to improve glycemic control and induce moderate weight loss of 4.0 % in patients with T2D [32] and 5.4 % in non-diabetic individuals with overweight or obesity [33] compared to placebo. In addition, liraglutide has been shown to maintain a diet-induced weight loss over 56 weeks [34] and maintain very low-calorie diet-induced improvements of fasting plasma glucose and triglycerides over 52 weeks of weight loss maintenance superior to similar diet-induced weight loss maintenance in obese nondiabetic individuals [18].

Obesity is associated with chronic low-grade inflammation [35,36] which is linked to the development of atherosclerosis and insulin resistance [37–39]. Physically active individuals have lower inflammatory biomarker concentrations than their inactive counterparts [24], possibly explained by antiinflammatory effects of an acute bout of exercise [40] and lower levels of visceral adipose tissue [41]. GLP-1 has also emerged as an immunomodulatory agent [42,43]. In mice, GLP-1 RA administration reduces macrophage accumulation and inflammatory markers in the arterial wall [44], adipose tissue [45], and heart [46]. Similarly, GLP-1 RAs have shown antiinflammatory effects in human coronary artery endothelial cells and aortic endothelial cells [47]. In humans with T2D, short term GLP-1 RA treatment exert antiinflammatory actions, reflected in reduced levels of the macrophage activation molecule sCD163 [48] and reduced production of several proinflammatory markers, such as TNF-α, IL1β, and IL-6 in peripheral blood mononuclear cells

[48,49]. Another study showed no improvement of obesity-associated adipose tissue dysfunction in T2D patients after GLP-1RA treatment [50]. One year treatment with GLP-1 RAs reduce the inflammation marker, high-sensitivity C-reactive protein, in overweight and obese individuals [33] and T2D patients [51]. Notably, in patients with T2D and high cardiovascular risk, GLP-1 RAs reduced the rate of occurrence of first major cardiovascular event [52,53].

Thus, both physical activity and GLP-1 RA treatment seem to facilitate weight loss maintenance, improve metabolic health, and reduce systemic inflammation. However, diet-induced weight loss decreases energy expenditure and increases appetite. We hypothesize that the combination of physical activity and liraglutide treatment improves weight loss maintenance and immunometabolic health since the decreased energy expenditure is targeted with exercise and the increased appetite with liraglutide.

Objective

The objectives of this study are to investigate the maintenance of weight loss and immunometabolic health outcomes over 52 weeks with liraglutide treatment, physical exercise, and the combination in individuals with obesity, after a very low-calorie diet.

Methods and analysis

Participants, interventions, and endpoints

Trial design

This study protocol describes an investigator-initiated, randomized, placebo-controlled, parallel group trial, the S-LiTE trial (acronym for 'Synergy effect of the appetite hormone GLP-1 (LiragluTide) and Exercise on maintenance of weight loss and health after a low calorie diet'). The

trial is double-blinded with regards to study medication but not exercise intervention. The study design is outlined in Figure 1. The study is registered at the European Clinical Trials Database (EudraCT Nr.: 2015-005585-32).

Study setting

All examinations in the trial will be carried out at Department of Endocrinology, Hvidovre University Hospital, University of Copenhagen and Department of Biomedical Sciences, University of Copenhagen.

Study status

Recruitment of participants was initiated in September 2016. Last participant last visit is planned for November 2020.

Participants and recruitment

We will enroll expectedly 200 participants. Eligible participants are adults (age 18-65 years) with obesity (body mass index (BMI) 32-43 kg/m^2) and no known serious chronic illness (including type 1 and 2 diabetes). Inclusion and exclusion criteria are listed in Table 1. Recruitment will be done via local newspapers, online media, and flyers from Department of Endocrinology, Hvidovre University Hospital, and Department of Biomedical Sciences, University of Copenhagen. Individuals who agree to participate will be invited to a pre-screening that includes screening of the study eligibility criteria before being finally included in the study. Withdrawn subjects will not be replaced. Re-screening is allowed within the recruitment period.

Participant involvement

The design of the study was partly inspired by qualitative interviews of a similar participant group performed by an anthropologist[54]. Upon completion of data analyses, results will be disseminated to all participants as a lay summary of the main findings.

Interventions

Diet-induced weight loss

Initially, all participants will undergo eight weeks with a very low-calorie diet (VLCD) (Cambridge Weight Plan, 800 kcal/day) with the objective to lose at least 5 % of body weight. Although some benefits may be evident already at modest weight loss of 2-3 % (e.g. triglycerides and HbA1c) [3], a ≥ 5 % cut-off is chosen because it is associated with improved cardiovascular disease risk factors [55] and T2D prevention [56] and thus generally considered a clinically meaningful weight loss [3,20,57]. Participants will be instructed to eat four meal replacements per day containing approximately 200 kcal and to only drink water and non-caloric beverages. Participants who have lost at least 5 % of body weight after the eight-week weight loss phase will be randomized to one of the four study groups: 52 weeks of treatment with 1) placebo, 2) exercise + placebo, 3) liraglutide, or 4) exercise + liraglutide. After randomization, participants will undergo a four-week phase-out plan with three daily Cambridge meal products and one regular meal the first week and two daily Cambridge meal products and two regular meals the three subsequent weeks.

Liraglutide or placebo

The GLP-1 RA, liraglutide (3.0 mg) (Saxenda®, Novo Nordisk A/S, Bagsværd, Denmark), or placebo will be administrated once daily as subcutaneous injections in the abdomen or thigh. The starting dose is 0.6 mg with weekly increments of 0.6 mg until 3.0 mg is achieved. The titration procedure will be prolonged for participants who do not tolerate fast up-titration. Participants who

do not tolerate the 3.0 mg dose may in special circumstances stay at lower dose (2.4 mg). However, the aim is to reach 3.0 mg for all study participants.

Physical exercise

The exercise intervention follows the global recommendations from the World Health Organization (WHO) of 150 minutes of moderate-intensity aerobic physical activity throughout the week or 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity activity [58]. The intervention consists of four sessions per week for a total of 150 min/week. Two sessions per week will be performed under supervision of the study staff and two sessions will be performed individually. A heart rate monitor (Polar A300, Polar Electro Oy, Kempele, Finland) will be worn during all planned exercise sessions. Supervised sessions will consist of structured exercise for a duration of 45 min. Of this, 30 min will be interval-based spinning and 15 min will be circuit training focusing on large muscle groups. Individual exercise sessions will include aerobic exercise such as cycling, rowing, or elliptical training as well as brisk walking or cycling to work. Participants will be advised to primarily perform non-weight bearing activities. The target aerobic exercise intensity is 80 % of maximal heart rate. Participants randomized to an exercise group will undergo a 6-week ramp-up phase with one session in week 1 and 2, two sessions in week 3 and 4, and three sessions in week 5 and 6 before exercising four times per week from week 7 to 52. If the planned ramp-up phase with exercise is not possible (e.g. due to side effects of study medication or joint pain), ramp-up will proceed more slowly. Participants not randomized to exercise will be instructed to maintain habitual physical activity according to level before entering the trial.

Liraglutide and physical exercise

Combination of the two interventions described above.

The trial will end at week 52 after randomization where liraglutide 3.0 mg/placebo and exercise treatment will be discontinued. One year after the intervention the participants will be invited for a follow-up visit.

Criteria for discontinuing/modifying allocated interventions

Participants may withdraw from the intervention at any time. Withdrawn participants will be invited for the planned examinations at week 52 unless written consent is withdrawn. Participants may be withdrawn from the trial at the discretion of the investigator due to a safety concern or a serious violation of the protocol. Participants will be withdrawn in occurrence of pregnancy or at the intention to become pregnant.

Endpoints

Primary endpoint

The primary endpoint is change in body weight from after the initial weight loss phase (baseline/V1) to end of treatment after 52 weeks (end/V3).

Secondary endpoint

The secondary endpoints are changes in body composition (lean/fat mass ratio) and metabolic health (glucose tolerance, lipid status, waist circumference, blood pressure) from V1 to V3.

Explorative endpoints

Explorative endpoints include changes from V1 to V3 in the following parameters: meal-related appetite hormone response; physical fitness and determination of daily physical activity and sleep; systemic markers of immunometabolism; endothelial function; immunometabolic changes in the subcutaneous adipose tissue; gene expression profile of circulating inflammatory cells; bone health; food preferences and subjective appetite sensation; faecal bacterial composition; plasma metabolomics and proteomics; epigenetics of spermatozoa.

Sample size calculation

Sample size is calculated in relation to body weight. In our previous weight loss maintenance study [18], the response within each treatment group was normally distributed with standard deviation of 5.5 kg. Thus, with expectedly 30 participants completing each study arm we will be able to detect a true difference of 4 kg between groups with a power of 0.8, assuming a two-sided α-level of 0.05. In our previous study, 10 % did not complete the initial weight loss phase [18]. Thus, with expectedly 200 enrolled study participants and an expected dropout rate of 25 % after randomization, we expect to have at least 30 participants from each study arm to complete the trial.

Assignment of intervention

Treatment allocation

After the initial eight-week VLCD phase, participants will be randomized after the test day to one of the four study groups in a 1:1:1:1 ratio in accordance with a subject randomization list (SRL) provided by Novo Nordisk (NN). An un-blinded study nurse (not otherwise associated with the trial) will allocate study participants according to the SRL. Randomization will be stratified by sex (male/female) and age (below/above 40 years). NN will provide a total dispensing unit number list (TDL). The un-blinded study nurse will allocate trial medication using the TDL by matching a six

digit Dispensing Unit Number (DUN) to the correct treatment. Each box of study medication will be labeled with a unique DUN. The DUN alone is not un-blinding. Thus, dispensing of trial medication to subjects can be carried out by blinded trial staff by selecting the DUN provided by the un-blinded study nurse.

Un-blinding

The SRL and the TDL are stored on site at Hvidovre Hospital with restricted access only to the designated un-blinded study nurse. Study ID of the participant is matched with the SRL and TDL, which reveals if trial medication is liraglutide or placebo. Preferably, the un-blinded study nurse will perform any un-blinding of study participants. However, if needed, all trial staff (sponsor, investigator, and sub-investigators) can get access to the SRL and TDL and perform the un-blinding procedure. Un-blinding can be performed under the following circumstances: treatment of a participant in a medical emergency that requires knowledge of treatment allocation; treatment of a participant for an adverse event; in the event of a suspected unexpected serious adverse reaction; in the event that the participant's study medication is accidentally taken by a member of their household, e.g. a child; for the submission of trial data to the Data Monitoring and Safety Committee for the monitoring of safety and/or efficacy.

Data collection, management, and analysis

Study visits

Identical test days will take place before the initial weight loss phase (screening/V0), after initial weight loss (baseline/V1), and after 52 weeks of treatment (end/V3) (Figure 1). Furthermore, a visit will be performed after 26 weeks of treatment (mid/V2). An overview of performed assessments is provided in Table 2. During the weight loss phase, weekly consultations will be conducted to assess

compliance to the VLCD, including measurement of body weight and handing out Cambridge meal products. During the 52-week weight maintenance phase, weight consultations, including assessment of adverse events, will be conducted at week 1, 2, 3, 4, 9, 13, 17, 22, 32, 39, and 46. Consultations at week 4, 13, and 39 will include collection of fasting blood samples, measurement of hip and waist circumference, blood pressure, and resting heart rate. Finally, participants will be invited to complete a post-trial unsupervised follow-up visit (V4) one year after intervention completion.

Criteria for assessments

- Participants must be fasting for minimum 10 hours prior to test days, including foods, liquids, and medication (except study medication)
- Study medication should preferably be taken on the morning of the tests
- Exercise should not be performed the day before or in the morning before tests

Assessments

Anthropometrics

Body weight will be measured on a digital scale (TANITA WB-110MA, Tokyo, Japan) to the nearest 0.1 kg without shoes and wearing light clothes. Waist circumference, the midpoint between lowest rib and iliac crest, and hip circumference, the level of the great trochanters, will be measured in duplicate to the nearest 0.1 cm after gentle expiration.

DXA scan

Dual-energy X-ray absorptiometry (DXA) scans will be performed in fasting state to measure body fat mass, fat free mass, and bone density (Hologic Discovery A, Hologic inc., Bedford, USA).

Blood pressure

Blood pressure and resting heart rate will be measured in duplicate from the non-dominant arm with a digital blood pressure monitor (Microlife BP A3 plus, Widnau, Switzerland) in sitting position after at least 5 min of rest.

Fasting blood samples

Fasting blood samples will be collected to measure circulating biomarkers of metabolic health, appetite hormones, immune markers, plasma proteomics, and plasma metabolomics. Furthermore, peripheral blood mononuclear cells will be isolated and DNA will be collected. A set of standard samples will be collected and analyzed on the same day for participants' safety, including: hemoglobin, free calcium, creatinine and estimated glomerular filtration rate, potassium, sodium, C-reactive protein, alanine aminotransferase, amylase, alkaline phosphatase, vitamin D, glycated hemoglobin, parathyroid hormone, thyrotropin, and blood lipids.

Adipose tissue biopsy

Subcutaneous abdominal adipose tissue biopsies (~1 g) will be obtained by needle aspiration under local anaesthesia using 5–10 ml 0.5% lidocaine. From adipose tissue biopsies, gene expression will be determined from reverse transcription-qPCR: proinflammatory and antiinflammatory adipocytokines, adipocyte differentiation markers, and markers of macrophages infiltration. The immune cells of the adipose tissue will be isolated to evaluate macrophage sup-populations and activation status (single cell analysis).

Meal test

After fasting blood sampling, a liquid meal (Nutricia Nutridrink, 600 kcal, 49 E% from carbohydrates, 35.2 E% from fat, and 15.8 E% from protein) will be ingested over 15 min, and blood samples will be collected continuously every 15 min for the first hour and every 30 min for the next two hours to measure circulating biomarkers of metabolic health, appetite hormones, immune markers, plasma proteomics and plasma metabolomics. During the meal test, appetite sensation will be assessed after each blood sample using a visual analogue scale [59].

Feces, urine, saliva, and semen

Feces samples will be collected to investigate fecal bacterial composition. Semen will be collected (if relevant) to investigate epigenetics of the spermatozoa. Additionally, urine and saliva samples will be collected.

Endothelial function

Flow mediated dilation (FMD) of the brachial artery will be measured to assess endothelial function [60]. FMD, also known as endothelium-dependent vasodilation, is the vasodilatory response of the brachial artery to increased shear stress and reflects the ability of vascular endothelium to produce nitric oxide. The brachial artery will be scanned with high resolution ultrasound imaging using a linear probe at rest and during hyperaemia. Hyperaemia will be induced by inflation and deflation of a sphygmomanometer cuff around the forearm, distal to the site scanned with ultrasound. FMD is calculated as the percentage change of the brachial artery diameter from rest to 60 seconds after the cuff is released. To assess the endothelium-independent vasodilation, nitroglycerine is given sublingually (0.4 mg) with the diameter of the brachial artery measured before and 5 min after drug administration. Carotid intima-media thickness will also be measured using ultrasound.

Electrocardiography

Electrocardiogram will performed to assess safety concerns related to study participation.

Physical fitness

Measurement of physical fitness will include three components: 1) Cardiorespiratory fitness (peak oxygen consumption) will be assessed with an incremental maximal cycle protocol performed on an electromagnetically braked cycle ergometer (Corival, Lode Medical Technology, The Netherlands) with continuously determined oxygen consumption and carbon dioxide production (MasterScreen CPX, CareFusion, Germany). After a warm-up protocol, workload will be increased every minute (20 and 25 watt for females and males, respectively) until attainment of peak oxygen consumption based on the following criteria: plateau in oxygen consumption, respiratory exchange ratio above 1.15 or attainment of age-predicted maximal heart rate (220 minus age), and voluntary exhaustion [61]. 2) Physical functioning will be measured as time to ascend and descend an 11-step stairway twice. 3) Maximal strength will be measured as isometric maximal voluntary contraction force of the dominant thigh using a dynamometer chair (Good Strength, Metitur Oy, Jyväskylä, Finland) [62,63].

Questionnaires

Participants will answer five questionnaires to determine self-rated quality of life (The Short Form (36) Health Survey[64]), eating habits (three-factor eating questionnaire[65]), physical activity (International Physical Activity Questionnaire[66]), sleep quality (Pittsburgh Sleep Quality Index[67]), and self-efficacy (General Self-Efficacy Scale[68]).

Food preferences

Food preferences and food reward responses will be measured in fasted state and postprandially with the Leeds Food Preference Questionnaire [69]. This is a computerized task where standardized pictures of 20 typical Danish food items are shown in the categories: high fat savoury, low fat savoury, high fat sweet and low fat sweet. Participants are instructed to rate each individual food item according to liking and to systematically choose the food items they prefer the most. Speed of choices is covertly assessed as an index of implicit wanting.

Free-living assessment of physical activity and sleep

An accelerometer device (GENEActiv, ActivInsights Ltd, Cambridgeshire, UK) will be worn on the wrist for seven consecutive days and nights with concomitant sleep registration (time for going to and out of bed) to assess habitual physical activity and sleep duration.

Data management

Participants will be identified by study ID. Study data is collected and managed using the REDCap secure web-based system [70] hosted by the Capital Region of Denmark, where electronic case report forms (CRF) have been created. During the trial, data will be entered directly into REDCap by study personnel. Extraction procedures will be performed by investigators or sponsor. Laboratory data will be transferred electronically from the laboratory performing clinical analyses and will be archived in secured hard drives with backup. All biological material (blood, adipose tissue, faeces, urine, saliva, and semen) obtained from study participants will be kept in a research bio-bank. Samples will be labelled with study ID. The bio-bank allows for analyses of samples to be performed simultaneously to avoid large instrumental variations. The research bio-bank will be terminated no later than August 1st 2036. After this date, the remains of the material will be transferred to a regular bio-bank in Denmark. In order to access this material, a new protocol must

be approved by an ethics committee. The Danish Data Protection Agency has been notified about the trial and the trial adheres to the Data Protection Act, which requires data to be anonymized as soon as it is practical to do so.

Data analysis plan

Analyses will be based on two defined analysis sets: an intention-to-treat analysis set (ITT) and a per-protocol analysis set (PP). ITT includes all randomized participants exposed to at least one dose of trial product or exercise that have completed the visit at week 4. PP will include all participants who complete the 52-week intervention with ≥ 75 % compliance to the interventions. Safety analysis will be performed on the ITT analysis set. Two-tailed tests will be performed and the significance level will be set to $\alpha = 0.05$.

Primary endpoint

The change from V1 to V3 in body weight (kg) will be analysed using a general linear model with treatment group as explanatory variable and baseline weight or BMI, age, sex, and initial weight loss (if relevant) as covariates. We will use drug treatment (liraglutide, placebo), exercise (yes, no) and their interaction to investigate the effects of drug and exercise. Adequacy of model assumptions will be assessed using graphical models, and outcome variables may be logarithmically transformed if considered necessary to meet the assumptions of linearity, variance homogeneity, and/or normality of residuals. The objective of the analysis is to determine whether the effect on body weight of liraglutide 3.0 mg in combination with exercise program is different from 1) placebo and 2) to either treatments alone, and to quantify the extend of this difference. Weight change over time will also be assessed by repeated measures analyses as appropriate (linear mixed model with an appropriate covariance structure and a suitably described effect over time). Blinding of study

medication allocation will be kept for investigators until analysis of the primary endpoint has been completed.

Secondary and exploratory endpoints

For secondary and exploratory endpoints, general linear models will be used to test for differences. The change from V1 to V3 will be analysed using general linear models with treatment group as explanatory variable and baseline weight or BMI, initial weight loss (if relevant), sex as covariates. Longitudinal data will also be analysed using linear mixed models as described above.

Auditing

The Good Clinical Practice (GCP) unit of the University Hospitals of Copenhagen will perform on-site audits minimum once yearly to ensure that the trial adhere to the guidelines for good clinical practice provided by the International Council for Harmonisation (ICH).

Ethics and dissemination

Harms

All adverse events (AE) will be collected from the first drug administration and in all following contacts with participants throughout the trial. A serious adverse event (SAE) is defined as any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenial anomaly or birth defect. Once yearly, sponsor will send a report regarding SARs occurring in the trial and safety of the study participants in regards to continuation of the trial to the Danish Medicines Agency and the Ethics Committee. In case of any deadly or life-threatening suspected unexpected serious adverse reaction (SUSAR), sponsor will immediately (and no later

than 7 days after becoming aware) notify the Danish Medicines Agency and the Ethics Committee. No later than 8 days after reporting of a SUSAR, sponsor will notify the Danish Medicines Agency and the Ethics Committee of all relevant information about sponsor's and investigator's follow-up of the SUSAR. All other SUSARs will be reported to the Danish Medicines Agency and the Ethics Committee no later than 15 days after sponsor becoming aware of this. All AEs and SAEs will be noted in the CRF and recorded in the End-of-Trial Form to the Danish Medicines Agency and in the report to the Ethics Committee (if requested) no later than 90 days after trial completion.

Ethical considerations

Liraglutide 3.0 mg will be given with injection pen. Liraglutide 3.0 mg is an approved drug and the dosage will be kept within the approved maximum (3.0 mg). Liraglutide 3.0 mg is safe but may cause transient nausea during the first weeks. Other side effects include dizziness, insomnia (transient), and gall stones. Uncommon/rare side effects include dehydration, inflamed gall bladder, allergic reactions, and reduced kidney function. Placebo injections should not cause any discomfort. There should not be any discomfort to the injection if performed as prescribed. The Cambridge Weight Plan is used in daily clinical practice and is considered safe but may cause constipation, fatigue, headache, and dizziness. The exercise program does not exceed the recommendations from WHO [71]. However, participants are not habitual exercisers. Therefore, careful considerations regarding the ramp-up of the exercise intervention will be given. Furthermore, most of the exercise are non-weight bearing activity in an attempt to limit potential injuries associated with the exercise intervention. The discomfort associated with the planned assessments is considered minimal. Applying a peripheral venous catheter for blood samples collection can cause transient discomfort, irritation, and redness around the puncture site. Some discomfort might be experienced when applying local anaesthesia for the adipose tissue biopsy. The biopsy itself should only cause

minimal discomfort. DXA scans use radiation with a radiation dose of approximately 0.02 mSv per examination. This dose is very low compared to the background radiation in Denmark (approximately 3 mSv/year). The FMD and carotid intima-media thickness ultrasound scans do not use radiation. Applying nitroglycerine may cause transient headache, dizziness, decreased blood pressure, and increased heart rate. Half-life of nitroglycerine is 1-3 min. Nitroglycerine will not be used if systolic blood pressure is under 100 mmHg. The risks that are associated with this study are assessed as minimal. By participating in this project, the participants will contribute with new important knowledge about the interaction between GLP-1 and exercise and their importance for weight loss maintenance and metabolic health. Overall, we consider that any potential risks and side effects are outweighed by the advantages of participation. The trial is approved by the ethical committee of the Capital Region of Denmark (H-16027082) and the Danish Medicines Agency EudraCT Nr.: 2015-005585-32. The trial will be carried out in accordance with the Declaration of Helsinki, and adhere to the GCP-ICH guidelines. After careful written and oral information about the trial and associated risks have been given, a written informed consent form will be obtained by investigator or sub-investigators before any study-related activities are performed. An English translation of the informed consent form is provided in supplementary file 1.

Dissemination plan

All study results (positive, negative, and inconclusive) will be presented at international scientific conferences as oral presentations or poster presentations. Furthermore, results will be published in international peer-reviewed scientific journals.

Protocol version

The study protocol was approved on June 30 2016. The present manuscript details the latest version of the protocol (version 9) approved on October 6 2018.

Discussion

Obesity prevalence has increased dramatically in the past decades [72] and the high recidivism rates after intentional weight loss [13,29] emphasize a strong need for new effective strategies to promote sustainable weight loss maintenance in individuals with obesity. The present protocol describes the first randomized controlled trial to investigate GLP-1 RA treatment combined with physical exercise in the context of long-term weight loss maintenance. The results have the potential to reveal a novel approach of combined lifestyle and pharmacological intervention that may provide substantial improvements of body weight, body composition, and immunometabolic health in obesity. Additionally, by applying state-of-the-art methodologies, the study may identify novel targets for future immunometabolic health-promoting weight loss interventions.

Contributors

SST formulated, initiated, and designed the study. BS, JJH, SM, MR, CJ, and JL contributed to the overall study design. SST, SJ, JL, CJ, CRJ, and LO contributed to detailed description of interventions, assessments and/or data analysis plan. SJ, JL, CJ, and SST drafted the manuscript. All authors have contributed to and approved the final version of the manuscript. Authorship eligibility will follow the Vancouver guidelines.

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grant, and a grant from Helsefonden. The GLP-1 RA (liraglutide 3.0 mg) and placebo are provided by Novo Nordisk. Cambridge weight plan diet products for the initial 8 weeks weight loss program are provided by Cambridge weight plan. JL is funded by a PhD grant from Faculty of Health and Medical Sciences, University of Copenhagen. CJ is funded by a PhD grant from Danish Diabetes Academy. The planning and conduct of the study, interpretation of data, and writing of manuscripts are completely independent of the funders.

Competing interests

SST has received research grant from Novo Nordisk. SM has received research grants from Novo Nordisk and Boehringer Ingelheim, lecture fees from AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli Lilly, Merck Sharp & Dohme, Novartis, Novo Nordisk, and Sanofi Aventis, and is a member of advisory boards of AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novartis; Novo Nordisk, and Sanofi Aventis. JJH has served on advisory panels for GlaxoSmithKline, Novo Nordisk, Zealand Pharma, AstraZeneca, MSD, Intarcia and Hanmi and as a consultant for Novo Nordisk, and has received research support from Merck, Sharp & Dohme.

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Figure 1 legend

Study design

Tables

Table 1. Eligibility criteria for participants in the S-LiTE trial

Inclusion criteria

- BMI: 32-43 kg/m^2
- Age: 18-65 years
- Safe contraceptive method or menopause for women

Exclusion criteria

- Patients diagnosed with any known serious chronic illness, including type 1 or 2 diabetes (or a randomly measured fasting plasma glucose > 7 mmol/l)
- Angina pectoris, coronary heart disease, or congestive heart failure (NYHA III-IV)
- Severe renal impairment (creatinine clearance (GFR) <30 mL/min)
- Severe hepatic impairment
- Inflammatory bowel disease
- Gastroparesis
- Cancer
- Chronic obstructive lung disease
- Psychiatric disease, a history of major depressive, or other severe psychiatric disorders
- The use of medications that cause clinically significant weight gain or loss
- Previous bariatric surgery
- A history of idiopathic acute pancreatitis
- A family or personal history of multiple endocrine neoplasia type 2 or familial medullary thyroid carcinoma
- Osteoarthritis, which is judged to be too severe to manage the exercise programme
- Pregnancy, expecting pregnancy, or breast feeding

- Allergy to any of the ingredients of the study medication: liraglutide, disodium phosphate dihydrate, propylene glycol, phenol, hydrochloric acid, and sodium hydroxide
- Regular exercise training at high intensity (e.g. spinning) >2 hours per week

Table 2. Overview of study visits

Visit	**Pre screening**	**V0**	**V1**	**V2**	**V3**	**V4**
Time point (week)	**-**	**-8**	**0**	**26**	**52**	**104**
Informed consent	X					
Anamnesis	X					
Inclusion/exclusion criteria	X					
Demographics	X					
Pregnancy test		X	X		X	
Adverse events		X	X	X	X	
Body weight	X	X	X	X	X	X
Waist and hip circumference		X	X	X	X	X
Blood pressure and heart rate		X	X	X	X	X
Fasting blood samples		X	X	X	X	X
7-day accelerometry		X	X	X	X	X
Adipose tissue biopsy		X	X	X	X	
DXA scan		X	X		X	X
Liquid meal test		X	X		X	
Fecal, urine, saliva, and semen		X	X		X	
FMD		X	X		X	

ECG	X	X	X	
Physical fitness testing	X	X	X	
Questionnaires	X	X	X	X
Food preference test	X	X	X	X

DXA, Dual-energy X-ray absorptiometry; FMD, flow-mediated dilation; ECG, electrocardiogram.

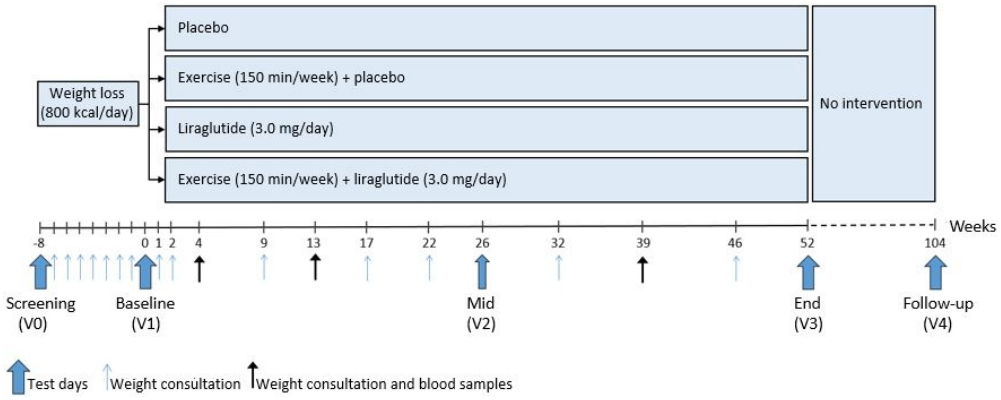

Figure 1. Study design

187x73mm (120 x 120 DPI)

English translation of informed consent form

Project titel: Synergy effect of the appetite hormone GLP-1 (LiragluTide) and Exercise on maintenance of weight loss and health after a low calorie diet – the S-LiTE randomized trial

Statement by study participant:

I have received written and oral information about the study and have sufficient knowledge about objectives, methods, advantages, risks and disadvantages to participate.

I know that participation is voluntary and that I can withdraw consent without losing my present or future rights to treatment.

I consent to participate in the trial and to have biological material collected and stored in a research biobank. I have received a copy of this consent form and a copy of the written information about the study.

Name of participant: _____

Date: _____ Signature: _____

If new information about your state of health appears in the study, you will be informed. If you wish to **decline knowledge** of new information about your state of health, please mark here: _____ (mark with X).

Do you wish to be informed about the results of the study and potential implications for you?

Yes_____ (mark with X) No_____ (mark with X)

Statement by researcher

I confirm that the participant has received oral and written information about the study.

To the best of my belief, sufficient information has been given in order for the participant to make a decision about participation in the study.

Name of person taking the consent:

Date: _____ Signature: _____

Project identification:

EudraCT nr. 2015-005585-32

SPIRIT 2013 Checklist for the S-LiTE randomised trial: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	Yes, at EudraCT
Protocol version	3	Date and version identifier	21
Funding	4	Sources and types of financial, material, and other support	22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 21, 22
	5b	Name and contact information for the trial sponsor	1
	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	22
	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)	n/a

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	4, 5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	5, 6
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)	6
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	6
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)	6, 7, Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7, 8, 9
	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)	9
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
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	9, 10
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)	12, Table 2

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	10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 7
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	11
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	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11, 12
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	12-16, Table 2
	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	9

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	16, 17
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	17, 18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17, 18
	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)	17
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	18, 19
	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	n/a
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	19
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	18, 19
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
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)	21

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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	16, 17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22, 23
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	22
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	n/a
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	21
	31b	Authorship eligibility guidelines and any intended use of professional writers	22
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
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	16, 17

*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.