Statistical Analysis Plan for clinical outcomes in the S-LiTE study

Section 1: Administrative information

Title: ***S*ynergy effect of the appetite hormone GLP-1 (*Li*raglu*T*ide) and *E*xercise on maintenance of weight loss and health after a low calorie diet – the *S-LiTE* randomized trial**

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This document is a supplement to the S-LiTE study protocol (1) and contains the statistical analysis plan for the article with the tentative title "A randomized controlled trial of the combined effects of the GLP-1 receptor agonist liraglutide and exercise on maintenance of weight loss, body composition and health after a very low-calorie diet" This document follows the guidelines for content of statistical analysis plans in clinical trials (2).

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Section 2: Introduction

7 Background and Rationale (adapted from published protocol(1))

Obesity is associated with increased risk of developing cardiovascular disease and type 2 diabetes (T2D), along with increased risk of all-cause mortality (3,4). 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 (5,6). However, weight regain reverse these health benefits (7,8). Furthermore, intentional weight loss is typically followed by a 30 to 50 % regain of lost weight within the first year (9–11). 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 (12,13) in combination with increased appetite in the weight-reduced state (14,15).

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 (16–18). However, with exercise almost exclusively fat mass is lost whereas lean mass is close to unchanged (19–22) thereby improving body composition. Furthermore, independent of weight loss, exercise improves glycemic control, low grade inflammatory profile and cardiorespiratory fitness in individuals with overweight and obesity (21,23–25).

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from 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 (26,27). 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 (28) and 5.4 % in non-diabetic individuals with overweight or obesity (29) compared to placebo. In addition, liraglutide has been shown to maintain a diet-induced weight loss over 56 weeks (30) 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 individuals (15). Weight loss with liraglutide is a result of both reduced fat and lean mass (31,32).

Obesity is associated with chronic low-grade inflammation (33,34) which is linked to the development of atherosclerosis and insulin resistance (35–37). Physically active individuals have lower inflammatory biomarker concentrations than their inactive counterparts (24), possibly explained by anti-inflammatory effects of an acute bout of exercise (38) and lower levels of visceral adipose tissue (39). 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 (40–44). Notably, in patients with T2D and high cardiovascular disease risk, GLP-1 RAs reduced the rate of occurrence of first major cardiovascular event (45,46). 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, body composition and immunometabolic health since the decreased energy expenditure is targeted with exercise and the increased appetite with liraglutide.

8 Objectives

The overall objectives of this study are to investigate the maintenance of weight loss, body composition 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.
The hypothesis hierarchy was not clearly specified in the protocol and we hereby define it as described below.

Hypothesis hierarchy for the primary outcome:

1. We hypothesize that the combined treatment (exercise+liraglutide) is superior to placebo for maintaining total body weight loss. Superiority is claimed if the estimated difference between the changes in total body weight for the two groups (delta for exercise+liraglutide from V1 to V3 – delta for placebo from V1 to V3) favorizes the exercise+liraglutide group and that the lower limit of the CI95% for the estimated difference exceeds 0.

2. We hypothesize that liraglutide is superior to placebo for maintaining total body weight loss. Superiority is claimed if the estimated difference between the changes in total body weight for the two groups (delta for liraglutide from V1 to V3 – delta for placebo from V1 to V3) favorizes the liraglutide group and that the lower limit of the CI95% for the estimated difference exceeds 0.

3. We hypothesize that the combined treatment (exercise+liraglutide) is non-inferior to liraglutide for maintaining total body weight loss.

4. We hypothesize that exercise is non-inferior to placebo for maintaining total body weight loss.

5. We hypothesize that the combined treatment (exercise+liraglutide) is superior to exercise for maintaining total body weight loss.

Secondary outcome:

6. We hypothesize that the combined treatment (liraglutide+exercise) is superior to liraglutide for body composition (total body fat percentage). Superiority is claimed if the estimated difference between the changes in total body fat percentage for the two groups (delta for liraglutide+exercise from V1 to V3 – delta for liraglutide from V1 to V3) favorizes the liraglutide+exercise group and that the lower limit of the CI95% for the estimated difference exceeds 0.

7. We hypothesize that exercise is superior to placebo for body composition (total body fat percentage). Superiority is claimed if the estimated difference between the changes in total body fat percentage for the two groups (delta for exercise from V1 to V3 – delta for placebo from V1 to V3) favorizes the exercise group and that the lower limit of the CI95% for the estimated difference exceeds 0.

8. We hypothesize that the combined treatment (exercise+liraglutide) is non-inferior to exercise for body composition (total body fat percentage).

Section 3: Study Methods

9 Trial design (from published protocol(1))

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') is an investigator-initiated, randomized, placebo-controlled, parallel group trial. The trial is triple-blinded with regards to study medication but not exercise intervention. The trial is registered at the European Clinical Trials Database (EudraCT Nr.: 2015-005585-32).

Description of 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 benefits are evident already at modest weight loss of 2-3 % (e.g. triglycerides and HbA1c) (6), a ≥ 5 % cut-off after 8 weeks is chosen because fast weight losers have obtained a greater weight reduction and long-term maintenance, and were not more susceptible to weight regain than gradual weight losers (47). 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.

Liraglutide or placebo

The GLP-1 RA, liraglutide (3.0 mg), 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. Participants who do not tolerate the 3.0 mg dose may in special circumstances stay at a lower dose (2.4 mg). However, the aim is to reach 3.0 mg for all study participants.

Physical exercise

The exercise intervention follows WHO's global recommendations on physical activity for health 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 (48). The intervention aims for four sessions per week. Two sessions per week will be performed under supervision of the study staff and two sessions will be performed individually. 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 randomized to an exercise group will undergo a 6-week ramp-up phase with exercise before exercising four times per week from week 7 to 52. 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.

Study visits

Identical test days will take place before the initial weight loss phase (V0, week -8), after initial weight loss (V1, week 0) and after 52 weeks of treatment (V3). For details on test days, see study protocol (1).

Weight consultations will be performed weekly during initial VLCD phase and at week 1, 2, 3, 4, 9, 13, 17, 22, 26, 32, 39 and 46. Fasting blood samples will be collected at week 4, 13, 26 and 39.

Finally, participants will be invited to complete a post-trial unsupervised follow-up visit (V4) 1 year after intervention completion.

10 Randomization

After the initial eight-week VLCD phase, participants will be randomized after the test day (V1) 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. 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).

11 Sample size

Sample size calculation for total body weight:

Sample size for primary endpoint was calculated in relation to total body weight. Based on previous weight loss studies with liraglutide (15,49), we estimate the response within each treatment group to be normally distributed with a standard deviation of 5.5 kg. Thus, with 30 participants completing each study arm we will be able to detect a difference in delta of 4 kg between groups with a power of 0.8, assuming a two-sided α-level of 0.05. We will need 34 completers in each arm in order to attain a statistical power of 0.85 and 40 completers in each arm to attain a statistical power of 0.90. In our previous study, 10 % of participants who entered the initial VLCD phase did not complete this phase (15). With 222 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.

Sample size for total body fat percentage

Sample size for secondary endpoint body composition was calculated in relation to total body fat percentage. Based on previous exercise trials (20,50–52) the response within each treatment group was estimated to have a standard deviation of 2.1%. Thus, with 32 participants completing each study arm we will be able to detect a difference in delta body fat percentage of 1.5% between groups with a power of 0.8, assuming a two-sided α-level of 0.05. We will need 36 completers to attain a statistical power of 0.85. Sample size calculation for fat percentage was performed in relation to writing the SAP; after the intervention was started but prior to the termination of the intervention.

12 Framework

See point 8.

13 Statistical interim analyses and stopping guidance

No interim analyses were planned and no guidelines for terminating the trial early was made.

14 Timing of final analyses

Results from V1 to V3 will be analyzed when the final participant completes the trial. Analyses including V4 will be performed when the final participant has completed V4.

15 Timing of outcome assessments

Body weight is measured at week -8 (V0), -7, -6, -5, -4, -3, -2, -1, 0 (V1), 1, 2, 4, 9, 13, 17, 22, 26, 39, 46, 52 (V3) and 104 (V4).

Body composition is measured at V0, V1, V3 and V4.
See point 26 for timing of descriptive/explorative outcome assessments.

Section 4: Statistical Principles

16-18 Confidence intervals and P values

P-values and 95% confidence intervals will be presented for comparisons (between and within group) and will not be adjusted for multiplicity. 95% confidence intervals will be presented for estimated levels and will not be adjusted for multiplicity. A hierarchical testing procedure will be used to control the type 1 error for tests with predefined hypotheses (see point 8); all subsequent tests will be regarded as descriptive/explorative if a test fails to confirm a given hypothesis. All non-hypothesis-based tests are per definition of a descriptive/exploratory nature. Statistical significance will be claimed if the null hypothesis is rejected at the alpha level of 0.05 (two-sided), i.e. the P-value of the null hypothesis test is $\leq$ 0.05. 95% confidence intervals will be required to support the tested hypothesis for hypothesis-based tests (see 8), and for exploratory/descriptive test on an absolute or relative scale not include 0 or 1 respectively.

19-20 Adherence and protocol deviations and Analysis populations

Intention-to-treat (ITT) analysis set:
All participants analyzed as randomized.

Per Protocol (PP) analysis set:
All participants who complete the 52-week randomized treatment period with sufficient compliance to study medication and/or exercise protocol as defined by:
Study medication: Having administered 2.4 or 3.0 mg subcutaneous (sc) liraglutide/placebo for at least 75% of the intervention period (measured by self-reporting during the 12 visits from week 1 to 52 after up-titration). Compliance to study medication will be summarized as number and percentage of participants equal to or above 75 %.
Exercise: Sports watches with heart rate monitors will be worn during all exercise sessions. For all participants in an exercise group, we will report duration of exercise (minutes per week), mean intensity during exercise (percentage of maximum heart rate) and exercise frequency (times per week). Exercise duration, intensity and frequency will be summarized as median plus 25^{th} and 75^{th} percentiles. Heart rate will be measured with a frequency of 1 Hz during all exercise. Relative exercise intensity for each heart rate measurement will be classified based on percentage of maximum heart rate (determined during an maximal incremental cycle ergometer test) in accordance with ACSM's position stand on prescribing exercise(53), i.e. very light intensity (<57% of HR_{max}), light intensity (57-63% of HR_{max}), moderate intensity (64-76% HR_{max}), vigorous intensity (77-95% HR_{max}) and near-maximal to maximal intensity ($\geq$ 96% HR_{max}).
For exercise sessions where heart rate data is missing (e.g. due to forgotten watch for supervised exercise), the exercise duration will be noted and the participants average intensity and time at different intensity zones will be imputed. Exercise will be averaged for all weeks after the ramp-up phase and until end-of-trial test day and exercise compliance will be calculated as percentage of WHO's global recommendations on physical activity for health (48): *Adults aged 18–64 should do at least 150 minutes of moderate-intensity aerobic physical activity throughout the week or do at least 75 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent combination of moderate- and vigorous-intensity*

activity. Thus, for example, one minute of moderate-intensity exercise will account for 1/150 and one minute of vigorous- or near-maximal to maximal intensity exercise will account for 1/75 of prescribed weekly exercise. Per protocol analysis set will be defined as meeting at least 75% of global recommendations.

For participants to be included in the per protocol analysis:
a) Liraglutide group: Having administered 2.4 or 3.0 mg sc liraglutide for at least 75% of the intervention period.
b) Placebo group: Having administered 2.4 or 3.0 mg sc placebo for at least 75% of the intervention period.
c) Exercise + placebo group: Having administered 2.4 or 3.0 mg sc placebo for at least 75% of the intervention period and having met at least 75% of global recommendations on physical activity.
d) Exercise + liraglutide group: Having administered 2.4 or 3.0 mg sc liraglutide for at least 75% of the intervention period and having met at least 75% of global recommendations on physical activity.

Section 5: Trial Population

21 Screening data

Screening data will not be reported.

22 Eligibility

Inclusion criteria:

- BMI > 32 and < 43 (kg/m^2)
- Age >18 and <65 years
- Safe contraceptive method

Exclusion criteria:

- Patients diagnosed with *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, 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. As intended per study design the intervention will include a 5% weight loss prior to randomization, thus it is

expected that possible participants with mild form of osteoarthritis will be able to manage exercise prescriptions.

- Pregnancy, expecting pregnancy or breast feeding. If a study participant is in doubt whether she could be pregnant, a urine pregnancy test is performed. Females of childbearing potential who are not using adequate contraceptive methods (as required by local law or practice). Adequate contraception must be used throughout the study period and at least 65 hours after discontinuation of trial medication (65 hours corresponds to 5 times the half-life of liraglutide).
- Allergy to any of the ingredients/excipients of the study medication: liraglutide, disodium phosphate dihydrate, propylene glycol, phenol, hydrochloric acid, sodium hydroxide.
- Regular exercise training at high intensity (e.g. spinning) >2 hours per week.

23 Recruitment

The flow chart of the trial will follow the CONSORT guidelines and will include the number of participants who a) received oral information, b) were assessed for eligibility at prescreening, c) were included in the trial, d) attended first test day and initiated VLCD, e) were withdrawn or excluded from the VLCD phase, f) were randomized, g) were allocated to the four intervention groups, h) lost to follow-up, i) discontinued the intervention, and j) were analyzed in the end.

24 Withdrawal/follow-up

If a randomized participant withdraws, we will offer to perform all or some of the investigations planned at V3. The number/frequency of participants lost to follow-up (those not attending a weight measurement at V3) will be provided for each group and for each time point. If possible, the reasons for participants not completing the trial will be given. Summary of baseline levels for variables reported in the baseline table will be provided for completers and non-completers. Spaghetti plots will be used to visualize levels of the main outcome for completers and non-completers.

25 Baseline participant characteristics

The distribution of all outcomes included in baseline characteristics will be visually inspected using QQ-plots and histograms; those with a Gaussian distribution will be presented as means and standard deviations and those with a non-Gaussian distribution will be presented as medians plus 25^{th} and 75^{th} percentiles.

The following outcomes will be included in the baseline participant characteristics table for all participants combined at V0, all participants combined at V1 and participants divided by randomization group at V1:

- Number of participants (men/women)
- Age (years)
- Weight (kg)
- BMI (kg/m^2)
- Waist circumference (cm)
- Total fat mass (kg)
- Total fat free mass (kg)
- Body fat percentage (%)

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- HbA1c (mmol/mol)
- Fasting glucose (mmol/L)
- Fasting insulin (pmol/L)
- Fasting C-peptide (pmol/L)
- Glucose AUC (mmol/L*min)
- Insulin AUC (pmol/L*min)
- Cholesterol: total, LDL and HDL (mmol/L)
- Triglycerides (mmol/L)
- Peak oxygen consumption (ml/min)
- Peak oxygen consumption (ml/min/kg)
- HOMA-IR (fasting insulin (pmol/L) * fasting glucose (mmol/L) / 22.5)
- Matsuda index (10000/sqrt(fasting glucose * fasting insulin * mean glucose * mean insulin)
- SF-36 (scoring of eight health concepts ranging from 0-100: physical functioning, role limitations due to physical health problems, role limitations due to personal or emotional problems, energy/fatigue, emotional well-being, social functioning, pain, and general health perception)
- ALAT (U/L)
- Amylase (U/L)
- CRP (mg/L)

Section 6: Analysis

26 Outcome definitions

Primary outcome: Primary outcome is the change in total body weight (measured to the nearest 0.1 kg) from baseline (V1) to end-of-treatment (V3). A weight difference of 2.5-4.5 % body weight, obtained with liraglutide or semaglutide compared to placebo, have been associated with beneficial cardiovascular outcomes (45,46). A weight difference of 1-5% of body weight obtained with exercise has been associated with beneficial cardiovascular disease risk factors (17,18) and prevention of diabetes (54).
Thus, a difference in delta of 3-5% of total body weight has been defined as the minimal important difference. With a study population consisting of men and women (expected mean height of 170-174 cm based on previous lifestyle trials performed in Denmark (15,55,56) and a BMI range of 32-43 kg /m^2, a difference of 3-5% of total body weight will correspond to 4 kg.

Secondary outcome: Secondary outcome body composition is defined as change in total body fat percentage (measured to the nearest 0.01 % in fasted state with dual-energy X-ray absorptiometry (DXA) scans) from V1 to V3. In most exercise interventions with aerobic exercise, almost exclusively fat mass is lost whereas lean mass is close to unchanged (19–22). In weight loss studies with liraglutide, a combination of fat and lean mass is lost (31,32). The clinically relevant effect size for changes in fat percentage is not well described or investigated. However, based on the results of previous exercise interventions (20,57), a decrease in fat percentage equivalent of approximately 1.5 % seems to be physiologically relevant.

Descriptive/explorative outcomes:

Change from V1 to V3:

- Body composition (fat free mass (kg) and fat mass (kg))
- Circulating biomarkers of metabolic regulation to evaluate metabolic health (fasting and 3h AUC glucose (mmol/L and mmol/L*min) and fasting and 3h AUC insulin (pmol/L and pmol/L*min) for HOMA-IR and Matsuda index, HbA1c (mmol/mol), waist circumference (cm), systolic and diastolic blood pressure (mmHg), lipids (total cholesterol, HDL, LDL, and triglycerides (mmol/L))
- Safety (adverse events). Adverse events with an incident of ≥ 5 % in any treatment group and all serious adverse events will be reported by system organ class and preferred term for participants in all four treatment groups separately and for liraglutide pooled (exercise and non-exercise) and placebo pooled (exercise and non-exercise). Events will be reported as percentage of individuals experiencing an adverse event.
- Self-rated quality of life will be measured with the SF-36 questionnaire. Eight health concepts ranging from 0-100 will be scored: physical functioning, role limitations due to physical health problems, role limitations due to personal or emotional problems, energy/fatigue, emotional well-being, social functioning, pain, and general health perception.
- Peak oxygen uptake (absolute (ml/min) and relative (ml/min/kg))
- Stair test (S) and maximal isometric strenght test (N)
- Use of medication (n, frequency)
- Meal related appetite response
- Determination of daily physical activity and sleep
- Systemic markers of immune-metabolism and oxidation
- Endothelial function
- Immuno-metabolic changes in the subcutaneous adipose tissue
- Gene expression profile of circulating inflammatory cells
- Bone health
- Food preference questionnaire (LFPQ) and subjective appetite sensation
- Faecal bacterial composition
- Plasma metabolomics and proteomics
- Epigenetics of spermatozoa
- Questionnaires (TFEQ, IPAQ, G-SES, PSQI)

Outcomes measured at other time points:

- Body weight (week -7, -6, -5, -4, -3, -2, -1, 1, 2, 4, 9, 13, 17, 22, 26, 32, 39, 46 and V4)
- Number/proportion of participants that have reduced total body weight by ≥5%, ≥10% and ≥15% at V3 compared with V0
- Body composition (V0 and V4)
- Metabolic health (week 4, 13, 26, 39 and V4)
- Safety (adverse events) (week 1, 2, 3, 4, 9, 13, 17, 22, 26, 32, 39 and 46)
- Meal related appetite response (V0)
- Peak oxygen uptake, stair test and maximal isometric strenght test (V0)
- Systemic markers of immune-metabolism and oxidation (V0)

- Endothelial function (V0)
- Immuno-metabolic changes in the subcutaneous adipose tissue (V0 and week 26)
- Gene expression profile of circulating inflammatory cells (V0)
- Bone health (V0 and V4)
- Food preference questionnaire (LFPQ) (V0 and V4) and subjective appetite sensation (V0)
- Faecal bacterial composition (V0)
- Plasma metabolomics and proteomics (V0, week 4, 13, 26, 39 and V4)
- Epigenetics of spermatozoa (V0)
- Questionnaries (SF-36, TFEQ, IPAQ, G-SES, PSQI) (V0 and V4)
- Measurement of sleep and physical activity levels (V0, week 13, 26 and V4)

27 Analysis methods

Analyses of primary and secondary outcome (except for the pre-planned sensitivity analyses) will be performed based on the intention-to-treat (ITT) principle. All continuous endpoints/outcomes will be modelled using linear mixed effects models with the following fixed effects and interactions: Time (factorial), Treatment, Time (factorial)*Treatment, sex (female, male) and age group (<40 years, >40 years). The models will be specified with a restricted maximum likelihood estimation method, the Kenward-Roger degrees of freedom method, an unstructured covariance structure and a random intercept on participant level. Model fit will be evaluated using graphical methods and if necessary, outcomes will be log-transformed. Estimated mean differences (CI95%) for changes between groups (main study effects), conditional means (CI95%), and within group changes (CI95%) will be extracted from the model. For log-transformed outcomes the results will be back-transformed and be presented as the ratio between estimated relative changes (CI95%), estimated conditional geometric means (CI95%) and within group relative changes (CI95%), respectively. Between group differences in changes will be null-hypothesis tested and presented with P-values.
The following predefined sensitivity analyses will be performed for the primary and secondary outcome; an additional ITT analysis with adjustment for initial weight loss, an ITT analysis using multiple imputation to assess effects of missing data (see also 28), and finally an analysis of per protocol completers.

The analysis of the primary and secondary outcome will be performed blinded to group allocation by a researcher (Martin Bæk Blond) that have not been involved in the execution of the trial. The statistical and clinical/physiological implications of the results will be evaluated by the research team before un-blinding.

28 Missing data

The number/frequency of missing values for the primary and secondary outcome in each group at each time point will be provided. For the main analysis, the results will be based on likelihood inference and missing data will be assumed to be missing completely at random or missing at random. An additional analysis based on multiple imputation of missing values will be performed (see also 27). The dataset used for the imputations will include all observations for total body weight measured at or between V1-V3, and the auxiliary variables Sex (male or female) and Age (continuous, years). Under the assumption that weight changes in participants after loss to follow-up would resemble the development in the placebo groups rather than the development in the group to which they were originally randomized, the participants with

missing values at V3 will be pooled with the placebo group and using a Markov chain Monte Carlo method all missing values will be imputed, assuming a multivariate normal distribution for the data, to create 1000 new datasets. Subsequently, the imputed datasets will be analyzed using the same mixed linear model used for the main analysis and averaged estimates calculated. If the main outcome has been transformed to fit the statistical model used for the main analysis this transformation will be applied in the imputation procedure.

29 Additional analyses

Not relevant

30 Harms

Adverse events (defined in the trial protocol) with an incident of ≥ 5 % in any treatment group and all serious adverse events will be reported by system organ class and preferred term for participants in all four treatment groups separately and for liraglutide pooled (exercise and non-exercise) and placebo pooled (exercise and non-exercise). Events will be reported as the number of and percentage of individuals experiencing an adverse event. Rates of adverse events will not be compared by null-hypothesis testing.

31 Statistical software

R version 3.6.0 or newer version (The R Foundation for Statistical Computing, www.R-project.org) and SAS version 9.4 or newer version (SAS Institute, Cary, NC, USA).

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