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# 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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Keywords:	weight loss, weight loss maintenance, liraglutide, GLP-1 analog, exercise, obesity

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4 1 **Protocol for a randomized controlled trial of the combined effects of the GLP-1 receptor**  
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6 2 **agonist liraglutide and exercise on maintenance of weight loss and health after a very low-**  
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8 3 **calorie diet**

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4 25 **Abstract**

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6 26 Introduction: The success rate of weight loss maintenance is limited. Therefore, the purpose of this  
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9 27 study is to investigate the maintenance of weight loss and immunometabolic health outcomes after  
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11 28 diet-induced weight loss followed by one-year treatment with a GLP-1 receptor agonist  
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13 29 (liraglutide), physical exercise, or the combination of both treatments as compared with placebo in  
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16 30 individuals with obesity.

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18 31 Methods and analysis: This is an investigator-initiated, randomized, placebo-controlled, parallel  
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20 32 group trial. We will recruit expectedly 200 women and men (age 18 to 65 years) with obesity (BMI  
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22 33 32 to 43 kg/m<sup>2</sup>). Initially, participants will adhere to a very low-calorie diet (800 kcal/day) for eight  
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24 34 weeks in order to lose at least 5 % of body weight. Subsequently, participants will be randomized in  
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27 35 a 1:1:1:1 ratio to one of four study groups for 52 weeks: 1) placebo, 2) exercise 150 min/week +  
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29 36 placebo, 3) liraglutide 3.0 mg/day, and 4) exercise 150 min/week + liraglutide 3.0 mg/day. The  
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31 37 primary endpoints are changes in body weight and body composition from randomization to end-of-  
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34 38 treatment.

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36 39 Ethics and dissemination: The trial has been approved by the ethical committee of the Capital  
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39 40 Region of Denmark and the Danish Medicines Agency. The trial will be conducted in agreement  
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41 41 with the Declaration of Helsinki and monitored to follow the guidelines for good clinical practice.  
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43 42 Results will be submitted for publication in international peer-reviewed scientific journals.

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45 43 Trial Registration: EudraCT Nr.: 2015-005585-32  
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4 44 **Strengths and limitations of this study**

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7 45 • First randomized controlled trial investigating the combined and individual effects of liraglutide  
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9 46 and exercise to maintain diet-induced weight loss in individuals with obesity.  
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11 47 • Direct comparison of liraglutide and exercise on weight loss maintenance and immunometabolic  
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13 48 health.  
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16 49 • Applying state-of-the-art methodologies, the study may identify novel targets for sustainable  
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18 50 immunometabolic health-promoting weight loss strategies.  
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## 51 **Introduction**

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

61 Increasing energy expenditure by increasing physical activity is the first-line lifestyle modification  
62 in the treatment of obesity along with reducing food intake. For exercise interventions targeting  
63 general public health recommendations (at least 150 min/week of moderate intensity aerobic  
64 exercise), the associated weight loss is often modest (0-3 %) without concomitant calorie restriction  
65 [10–12]. However, independent of weight loss, increasing physical activity improves body  
66 composition, glycemic control, low grade inflammatory profile, and cardiorespiratory fitness in  
67 individuals with overweight and obesity [13–16]. In addition, exercise may preserve lean mass  
68 during weight loss [17] and thereby counteract the associated decrease in resting metabolic rate  
69 [18], which may explain the observation that individuals performing regular exercise have less body  
70 weight regain after weight loss compared to participants that do not exercise [6,19].

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

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4 75 weeks with the GLP-1 analogue, liraglutide (3.0 mg), as an adjunct to regular diet and physical  
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6 76 activity recommendations has been shown to improve glycemic control and induce moderate weight  
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8 77 loss of 4.0 % in type 2 diabetic [22] and 5.4 % in non-diabetic [23] individuals with overweight or  
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10 78 obesity compared to placebo. In addition, liraglutide has been shown to maintain a diet-induced  
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12 79 weight loss over 56 weeks [24] and maintain very low-calorie diet-induced improvements of fasting  
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14 80 plasma glucose and triglycerides over 52 weeks of weight loss maintenance superior to similar diet-  
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16 81 induced weight loss maintenance in obese nondiabetic individuals [25].  
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23 83 Obesity is associated with chronic low-grade inflammation [26,27] which is linked to the  
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25 84 development of atherosclerosis and insulin resistance [28–30]. Physically active individuals have  
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27 85 lower inflammatory biomarker concentrations than their inactive counterparts [15], possibly  
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29 86 explained by antiinflammatory effects of an acute bout of exercise [31] and lower levels of visceral  
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31 87 adipose tissue [32]. GLP-1 has also emerged as an immunomodulatory agent, as illustrated by GLP-  
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33 88 1 analogue administration exerting anti-inflammatory actions in various cells, including endothelial  
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35 89 cells, adipocytes, peripheral blood mononuclear cells, and in plasma [33–37].  
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41 91 Thus, both physical activity and GLP-1 analogue treatment seem to facilitate weight loss  
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43 92 maintenance, improve metabolic health, and reduce systemic inflammation. However, diet-induced  
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45 93 weight loss decreases energy expenditure and increases appetite. We hypothesize that the  
46  
47 94 combination of physical activity and liraglutide treatment improves weight loss maintenance and  
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49 95 immunometabolic health since the decreased energy expenditure is targeted with exercise and the  
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51 96 increased appetite with liraglutide.  
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## 55 97 56 57 98 **Objective** 58 59 60



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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*

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4 122 We will recruit expectedly 200 participants. Eligible participants are adults (age 18-65 years) with  
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6 123 obesity (BMI 32-43 kg/m<sup>2</sup>) and no known serious chronic illness (incl. type 1 and 2 diabetes).  
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9 124 Inclusion and exclusion criteria are listed in Table 1. Recruitment will be done via local  
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11 125 newspapers, online media, and flyers from Department of Endocrinology, Hvidovre University  
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13 126 Hospital, and Department of Biomedical Sciences, University of Copenhagen. Individuals who  
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16 127 agree to participate will be invited to a pre-screening that includes screening of the study eligibility  
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18 128 criteria before being finally included in the study. Withdrawn subjects will not be replaced. Re-  
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20 129 screening is allowed within the recruitment period.  
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### 25 131 *Participant involvement*

26  
27 132 The design of the study was partly inspired by qualitative interviews of a similar participant group  
28  
29 133 performed by an anthropologist[38]. Upon completion of data analyses, results will be disseminated  
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31 134 to all participants as a lay summary of the main findings.  
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### 36 136 *Interventions*

#### 37 137 Diet-induced weight loss

38  
39 138 Initially, all participants will undergo eight weeks with a very low-calorie diet (VLCD) (Cambridge  
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41 139 Weight Plan, 800 kcal/day) with the objective to lose at least 5 % of body weight. Participants will  
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44 140 be instructed to eat four meal replacements per day containing approximately 200 kcal and to only  
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46 141 drink water and non-caloric beverages. Participants who have lost at least 5 % of body weight after  
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48 142 the eight-week weight loss phase will be randomized to one of the four study groups: 52 weeks of  
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50 143 treatment with 1) placebo, 2) exercise + placebo, 3) liraglutide, or 4) exercise + liraglutide. After  
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53 144 randomization, participants will undergo a four-week phase-out plan with three daily Cambridge  
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145 meal products and one regular meal the first week and two daily Cambridge meal products and two  
146 regular meals the three subsequent weeks.

#### 148 Liraglutide or placebo

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

#### 156 Physical exercise

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

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4 169 randomized to an exercise group will undergo a 6-week ramp-up phase with one session in week 1  
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7 170 and 2, two sessions in week 3 and 4, and three sessions in week 5 and 6 before exercising four times  
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9 171 per week from week 7 to 52. If the planned ramp-up phase with exercise is not possible (e.g. due to  
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11 172 side effects of study medication or joint pain), ramp-up will proceed more slowly. Participants not  
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14 173 randomized to exercise will be instructed to maintain habitual physical activity according to level  
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16 174 before entering the trial.  
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### 18 175 19 20 176 Liraglutide and physical exercise

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23 177 Combination of the two interventions described above.  
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27 179 The trial will end at week 52 after randomization where liraglutide/placebo and exercise treatment  
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30 180 will be discontinued. One year after the intervention the participants will be invited for a follow-up  
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32 181 visit.  
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### 34 182 35 36 183 *Criteria for discontinuing/modifying allocated interventions*

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39 184 Participants may withdraw from the intervention at any time. Withdrawn participants will be invited  
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41 185 for the planned examinations at week 52 unless written consent is withdrawn. Participants may be  
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43 186 withdrawn from the trial at the discretion of the investigator due to a safety concern or a serious  
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46 187 violation of the protocol. Participants will be withdrawn in occurrence of pregnancy or at the  
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48 188 intention to become pregnant.  
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### 50 189 51 52 190 *Endpoints*

#### 53 54 55 191 Primary endpoints 56 57 58 59 60

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

#### 194 195 Secondary endpoint

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

#### 198 199 Explorative endpoints

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

#### 206 207 *Sample size calculation*

208 Sample size is calculated in relation to body weight. In our previous weight loss maintenance study  
209 [25], the response within each treatment group was normally distributed with standard deviation of  
210 5.5 kg. Thus, with expectedly 30 participants completing each study arm we will be able to detect a  
211 true difference of 4 kg between groups with a power of 0.8, assuming a two-sided  $\alpha$ -level of 0.05.

212 In our previous study, 10 % did not complete the initial weight loss phase [25]. Thus, with  
213 expectedly 200 recruited study participants and an expected dropout rate of 25 % after  
214 randomization, we expect to have at least 30 participants from each study arm to complete the trial.

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## 216 **Assignment of intervention**

### 217 *Treatment allocation*

218 Participants will be randomized to one of the four study groups in a 1:1:1:1 ratio in accordance with  
219 a subject randomization list (SRL) provided by Novo Nordisk (NN). An un-blinded study nurse (not  
220 otherwise associated with the trial) will allocate study participants according to the SRL.

221 Randomization will be stratified by sex (male/female) and age (below/above 40 years). NN will  
222 provide a total dispensing unit number list (TDL). The un-blinded study nurse will allocate trial  
223 medication using the TDL by matching a six digit Dispensing Unit Number (DUN) to the correct  
224 treatment. Each box of study medication will be labeled with a unique DUN. The DUN alone is not  
225 un-blinding. Thus, dispensing of trial medication to subjects can be carried out by blinded trial staff  
226 by selecting the DUN provided by the un-blinded study nurse. The SRL and the TDL are stored on  
227 site at Hvidovre Hospital with restricted access only to the designated un-blinded study nurse.

### 229 *Un-blinding*

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

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239 household, e.g. a child; for the submission of trial data to the Data Monitoring and Safety

240 Committee for the monitoring of safety and/or efficacy.

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## 242 **Data collection, management, and analysis**

### 243 *Study visits*

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

### 256 *Criteria for assessments*

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

### 262 *Assessments*

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4 263 Anthropometrics

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6 264 Body weight will be measured on a digital scale (TANITA WB-110MA, Tokyo, Japan) to the  
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9 265 nearest 0.1 kg without shoes and wearing light clothes. Waist circumference, the midpoint between  
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11 266 lowest rib and iliac crest, and hip circumference, the level of the great trochanters, will be measured  
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13 267 in duplicate to the nearest 0.1 cm after gentle expiration.  
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16 268  
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18 269 DXA scan

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20 270 Dual-energy X-ray absorptiometry (DXA) scans will be performed in fasting state to measure body  
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23 271 fat mass, fat free mass, and bone density (Hologic Discovery A, Hologic inc., Bedford, USA).  
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25 272  
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27 273 Blood pressure

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29 274 Blood pressure and resting heart rate will be measured in duplicate from the non-dominant arm with  
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32 275 a digital blood pressure monitor (Microlife BP A3 plus, Widnau, Switzerland) in sitting position  
33  
34 276 after at least 5 min of rest.  
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36 277  
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39 278 Fasting blood samples

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41 279 Fasting blood samples will be collected to measure circulating biomarkers of metabolic health,  
42  
43 280 appetite hormones, immune markers, plasma proteomics, and plasma metabolomics. Furthermore,  
44  
45  
46 281 peripheral blood mononuclear cells will be isolated and DNA will be collected. A set of standard  
47  
48 282 samples will be collected and analyzed on the same day for participants' safety, including:  
49  
50 283 hemoglobin, free calcium, creatinine and estimated glomerular filtration rate, potassium, sodium, C-  
51  
52 284 reactive protein, alanine aminotransferase, amylase, alkaline phosphatase, vitamin D, glycated  
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55 285 hemoglobin, parathyroid hormone, thyrotropin, and blood lipids.  
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4 287 Adipose tissue biopsy

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6 288 Subcutaneous abdominal adipose tissue biopsies (~1 g) will be obtained by needle aspiration under  
7  
8  
9 289 local anaesthesia using 5–10 ml 0.5% lidocaine. From adipose tissue biopsies, gene expression will  
10  
11 290 be determined from reverse transcription-qPCR: proinflammatory and antiinflammatory  
12  
13 291 adipocytokines, adipocyte differentiation markers, and markers of macrophages infiltration. The  
14  
15  
16 292 immune cells of the adipose tissue will be isolated to evaluate macrophage sup-populations and  
17  
18 293 activation status (single cell analysis).

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23 295 Meal test

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25 296 After fasting blood sampling, a liquid meal (Nutricia Nutridrink, 600 kcal, 49 E% from  
26  
27 297 carbohydrates, 35.2 E% from fat, and 15.8 E% from protein) will be ingested over 15 min, and  
28  
29  
30 298 blood samples will be collected continuously every 15 min for the first hour and every 30 min for  
31  
32 299 the next two hours to measure circulating biomarkers of metabolic health, appetite hormones,  
33  
34 300 immune markers, plasma proteomics and plasma metabolomics. During the meal test, appetite  
35  
36 301 sensation will be assessed after each blood sample using a visual analogue scale [40].

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39 302  
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41 303 Feces, urine, saliva, and semen

42  
43 304 Feces samples will be collected to investigate fecal bacterial composition. Semen will be collected  
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46 305 (if relevant) to investigate epigenetics of the spermatozoa. Additionally, urine and saliva samples  
47  
48 306 will be collected.

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50 307  
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52 308 Endothelial function

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55 309 Flow mediated dilation (FMD) of the brachial artery will be measured to assess endothelial function  
56  
57 310 [41]. FMD, also known as endothelium-dependent vasodilation, is the vasodilatory response of the  
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4 311 brachial artery to increased shear stress and reflects the ability of vascular endothelium to produce  
5  
6 312 nitric oxide. The brachial artery will be scanned with high resolution ultrasound imaging using a  
7  
8  
9 313 linear probe at rest and during hyperaemia. Hyperaemia will be induced by inflation and deflation  
10  
11 314 of a sphygmomanometer cuff around the forearm, distal to the site scanned with ultrasound. FMD is  
12  
13 315 calculated as the percentage change of the brachial artery diameter from rest to 60 seconds after the  
14  
15 316 cuff is released. To assess the endothelium-independent vasodilation, nitroglycerine is given  
16  
17  
18 317 sublingually (0.4 mg) with the diameter of the brachial artery measured before and 5 min after drug  
19  
20 318 administration. Carotid intima-media thickness will also be measured using ultrasound.  
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#### 24 25 320 Electrocardiography

26  
27 321 Electrocardiogram will performed to assess safety concerns related to study participation.  
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#### 31 32 323 Physical fitness

33  
34 324 Measurement of physical fitness will include three components: 1) Cardiorespiratory fitness (peak  
35  
36 325 oxygen consumption) will be assessed with an incremental maximal cycle protocol performed on an  
37  
38  
39 326 electromagnetically braked cycle ergometer (Corival, Lode Medical Technology, The Netherlands)  
40  
41 327 with continuously determined oxygen consumption and carbon dioxide production (MasterScreen  
42  
43 328 CPX, CareFusion, Germany). After a warm-up protocol, workload will be increased every minute  
44  
45  
46 329 (20 and 25 watt for females and males, respectively) until attainment of peak oxygen consumption  
47  
48 330 based on the following criteria: plateau in oxygen consumption, respiratory exchange ratio above  
49  
50 331 1.15 or attainment of age-predicted maximal heart rate (220 minus age), and voluntary exhaustion  
51  
52  
53 332 [42]. 2) Physical functioning will be measured as time to ascend and descend an 11-step stairway  
54  
55 333 twice. 3) Maximal strength will be measured as isometric maximal voluntary contraction force of  
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334 the dominant thigh using a dynamometer chair (Good Strength, Metitur Oy, Jyväskylä, Finland)

335 [43,44].

336

### 337 Questionnaires

338 Participants will answer five questionnaires to determine self-rated quality of life (The Short Form

339 (36) Health Survey[45]), eating habits (three-factor eating questionnaire[46]), physical activity

340 (International Physical Activity Questionnaire[47]), sleep quality (Pittsburgh Sleep Quality

341 Index[48]), and self-efficacy (General Self-Efficacy Scale[49]).

342

### 343 Food preferences

344 Food preferences and food reward responses will be measured in fasted state and postprandially

345 with the Leeds Food Preference Questionnaire [50]. This is a computerized task where standardized

346 pictures of 20 typical Danish food items are shown in the categories: high fat savoury, low fat

347 savoury, high fat sweet and low fat sweet. Participants are instructed to rate each individual food

348 item according to liking and to systematically choose the food items they prefer the most. Speed of

349 choices is covertly assessed as an index of implicit wanting.

350

### 351 Free-living assessment of physical activity and sleep

352 An accelerometer device (GENEActiv, ActivInsights Ltd, Cambridgeshire, UK) will be worn on the

353 wrist for seven consecutive days and nights with concomitant sleep registration (time for going to

354 and out of bed) to assess habitual physical activity and sleep duration.

355

### 356 *Data management*

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4 357 Participants will be identified by study ID. Study data is collected and managed using the REDCap  
5  
6 358 secure web-based system [51] hosted by the Capital Region of Denmark, where electronic case  
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9 359 report forms (CRF) have been created. During the trial, data will be entered directly into REDCap  
10  
11 360 by study personnel. Extraction procedures will be performed by investigators or sponsor.  
12  
13 361 Laboratory data will be transferred electronically from the laboratory performing clinical analyses  
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16 362 and will be archived in secured hard drives with backup. All biological material (blood, adipose  
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18 363 tissue, faeces, urine, saliva, and semen) obtained from study participants will be kept in a research  
19  
20 364 bio-bank. Samples will be labelled with study ID. The bio-bank allows for analyses of samples to be  
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23 365 performed simultaneously to avoid large instrumental variations. The research bio-bank will be  
24  
25 366 terminated no later than August 1<sup>st</sup> 2036. After this date, the remains of the material will be  
26  
27 367 transferred to a regular bio-bank in Denmark. In order to access this material, a new protocol must  
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29  
30 368 be approved by an ethics committee. The Danish Data Protection Agency has been notified about  
31  
32 369 the trial and the trial adheres to the Data Protection Act, which requires data to be anonymized as  
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34 370 soon as it is practical to do so.  
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#### 37 371 38 39 372 *Data analysis plan*

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41 373 Analyses will be based on two defined analysis sets: an intention-to-treat analysis set (ITT) and a  
42  
43 374 per-protocol analysis set (PP). ITT includes all randomized participants exposed to at least one dose  
44  
45  
46 375 of trial product or exercise that have completed the visit at week 4. PP will include all participants  
47  
48 376 who complete the 52-week intervention with  $\geq 75\%$  compliance to the interventions. Safety  
49  
50 377 analysis will be performed on the ITT analysis set. Two-tailed tests will be performed and the  
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53 378 significance level will be set to  $\alpha = 0.05$ .

#### 54 55 379 56 57 380 Primary endpoints

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

#### 395 Secondary and exploratory endpoints

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

#### 402 *Auditing*

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4 403 The Good Clinical Practice (GCP) unit of the University Hospitals of Copenhagen will perform on-  
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7 404 site audits minimum once yearly to ensure that the trial adhere to the guidelines for good clinical  
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9 405 practice provided by the International Council for Harmonisation (ICH).  
10

## 11 406 12 13 407 **Ethics and dissemination**

### 14 15 16 408 *Harms*

17  
18 409 All adverse events (AE) will be collected from the first drug administration and in all following  
19  
20 410 contacts with participants throughout the trial. A serious adverse event (SAE) is defined as any  
21  
22 411 untoward medical occurrence that results in death, is life-threatening, requires hospitalization or  
23  
24 412 prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is  
25  
26 413 a congenial anomaly or birth defect. Once yearly, sponsor will send a report regarding SARs  
27  
28 414 occurring in the trial and safety of the study participants in regards to continuation of the trial to the  
29  
30 415 Danish Medicines Agency and the Ethics Committee. In case of any deadly or life-threatening  
31  
32 416 suspected unexpected serious adverse reaction (SUSAR), sponsor will immediately (and no later  
33  
34 417 than 7 days after becoming aware) notify the Danish Medicines Agency and the Ethics Committee.  
35  
36 418 No later than 8 days after reporting of a SUSAR, sponsor will notify the Danish Medicines Agency  
37  
38 419 and the Ethics Committee of all relevant information about sponsor's and investigator's follow-up  
39  
40 420 of the SUSAR. All other SUSARs will be reported to the Danish Medicines Agency and the Ethics  
41  
42 421 Committee no later than 15 days after sponsor becoming aware of this. All AEs and SAEs will be  
43  
44 422 noted in the CRF and recorded in the End-of-Trial Form to the Danish Medicines Agency and in the  
45  
46 423 report to the Ethics Committee (if requested) no later than 90 days after trial completion.  
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### 53 424 54 55 425 *Ethical considerations* 56 57 58 59 60

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4 426 Injection with liraglutide will be given with Saxenda injection pen. Saxenda is an approved drug  
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6  
7 427 and the dosage will be kept within the approved maximum (3.0 mg). Saxenda is safe but may cause  
8  
9 428 transient nausea during the first weeks. Other side effects include dizziness, insomnia (transient)  
10  
11 429 and gall stones. Uncommon/rare side effects include dehydration, inflamed gall bladder, allergic  
12  
13 430 reactions, and reduced kidney function. Placebo injections should not cause any discomfort. There  
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15  
16 431 should not be any discomfort to the injection if performed as prescribed. The Cambridge Weight  
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18 432 Plan is used in daily clinical practice and is considered safe but may cause constipation, fatigue,  
19  
20 433 headache, and dizziness. The exercise program does not exceed the recommendations from WHO  
21  
22  
23 434 [52]. However, participants are not habitual exercisers. Therefore, careful considerations regarding  
24  
25 435 the ramp-up of the exercise intervention will be given. Furthermore, most of the exercise are non-  
26  
27 436 weight bearing activity in an attempt to limit potential injuries associated with the exercise  
28  
29  
30 437 intervention. The discomfort associated with the planned examinations is considered minimal.  
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32 438 Applying a peripheral venous catheter for blood samples collection can cause transient discomfort,  
33  
34 439 irritation, and redness around the puncture site. Some discomfort might be experienced when  
35  
36 440 applying local anaesthesia for the adipose tissue biopsy. The biopsy itself should only cause  
37  
38  
39 441 minimal discomfort. DXA scans use radiation with a radiation dose of approximately 0.02 mSv per  
40  
41 442 examination. This dose is very low compared to the background radiation in Denmark  
42  
43 443 (approximately 3 mSv/year). The FMD and carotid intima-media thickness ultrasound scans do not  
44  
45  
46 444 use radiation. Applying nitroglycerine may cause transient headache, dizziness, decreased blood  
47  
48 445 pressure, and increased heart rate. Half-life of nitroglycerine is 1-3 min. Nitroglycerine will not be  
49  
50 446 used if systolic blood pressure is under 100 mmHg. The risks that are associated with this study are  
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52  
53 447 assessed as minimal. By participating in this project, the participants will contribute with new  
54  
55 448 important knowledge about the interaction between GLP-1 and exercise and their importance for  
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57 449 weight loss maintenance and metabolic health. Overall, we consider that any potential risks and side  
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4 450 effects are outweighed by the advantages of participation. The trial is approved by the ethical  
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7 451 committee of the Capital Region of Denmark (H-16027082) and the Danish Medicines Agency  
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9 452 EudraCT Nr.: 2015-005585-32. The trial will be carried out in accordance with the Declaration of  
10  
11 453 Helsinki, and adhere to the GCP-ICH guidelines. After careful written and oral information about  
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14 454 the trial and associated risks have been given, a written informed consent form will be obtained by  
15  
16 455 investigator or sub-investigators before any study-related activities are performed.  
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#### 20 457 *Dissemination plan*

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23 458 All study results (positive, negative, and inconclusive) will be presented at international scientific  
24  
25 459 conferences as oral presentations or poster presentations. Furthermore, results will be published in  
26  
27 460 international peer-reviewed scientific journals.  
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#### 32 462 *Protocol version*

34 463 The study protocol was approved on June 30 2016. The present manuscript details the latest version  
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36 464 of the protocol (version 9) approved on October 6 2018.  
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## 41 466 **Discussion**

43 467 Obesity prevalence has increased dramatically in the past decades [53] and the high recidivism rates  
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45 468 after intentional weight loss [6,54] emphasize a strong need for new effective strategies to promote  
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48 469 sustainable weight loss maintenance in individuals with obesity. The present protocol describes the  
49  
50 470 first randomized controlled trial to investigate GLP-1 analogue treatment combined with physical  
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53 471 exercise in the context of long-term weight loss maintenance. The results have the potential to  
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55 472 reveal a novel approach of combined lifestyle and pharmacological intervention that may provide  
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57 473 substantial improvements of body weight, body composition, and immunometabolic health in  
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474 obesity. Additionally, by applying state-of-the-art methodologies, the study may identify novel  
475 targets for future immunometabolic health-promoting weight loss interventions.

### 477 **Contributors**

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

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### 492 **Competing interests**

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

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16 647 **Tables**

17  
18 Table 1. Eligibility criteria for participants in the S-LiTE trial

19  
20  
21 **Inclusion criteria**

- 22  
23 • BMI: 32-43 kg/m<sup>2</sup>  
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25 • Age: 18-65 years  
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27 • Safe contraceptive method or menopause for women  
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30 **Exclusion criteria**

- 31  
32 • Patients diagnosed with any known serious chronic illness, including type 1 or 2 diabetes  
33 (or a randomly measured fasting plasma glucose > 7 mmol/l)  
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35 • Angina pectoris, coronary heart disease, or congestive heart failure (NYHA III-IV)  
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37 • Severe renal impairment (creatinine clearance (GFR) <30 mL/min)  
38  
39 • Severe hepatic impairment  
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41 • Inflammatory bowel disease  
42  
43 • Diabetic gastroparesis  
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45 • Cancer  
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47 • Chronic obstructive lung disease  
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49 • Psychiatric disease, a history of major depressive, or other severe psychiatric disorders  
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51 • The use of medications that cause clinically significant weight gain or loss  
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- 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
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Table 2. Overview of study visits

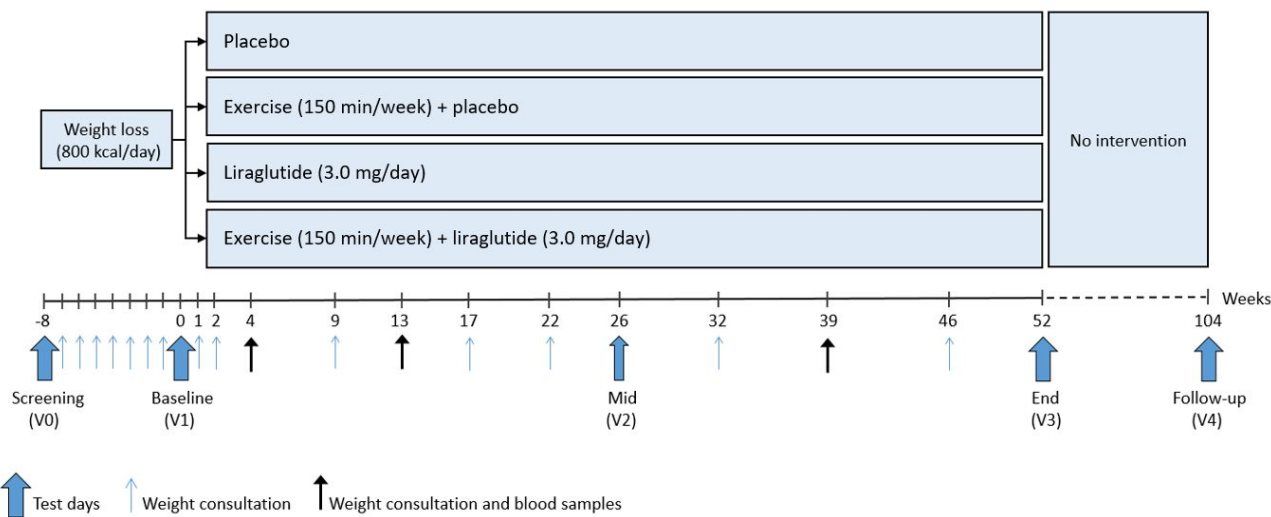
Visit	Pre screening	V0	V1	V2	V3	V4
Time point (week)	-	-8	0	26	52	104
Informed consent	X					
Anamnesis	X					
Inclusion/exclusion criteria	X					
Demographics	X					
Pregnancy test		X	X		X	
Adverse events		X	X	X	X	
Body weight	X	X	X	X	X	X
Waist and hip circumference		X	X	X	X	X
Blood pressure and heart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

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2					
3					
4	7-day accelerometry	X	X	X	X
5					
6	Adipose tissue biopsy	X	X	X	X
7					
8					
9	DXA scan	X	X		X
10					
11	Liquid meal test	X	X		X
12					
13	Fecal, urine, saliva, and semen	X	X		X
14					
15					
16	FMD	X	X		X
17					
18	ECG	X	X		X
19					
20	Physical fitness testing	X	X		X
21					
22					
23	Questionnaires	X	X		X
24					
25	Food preference test	X	X		X
26					

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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
<b>Administrative information</b>			
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

1	<b>Introduction</b>			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	4, 5
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	5
7				
8	Objectives	7	Specific objectives or hypotheses	5, 6
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	6
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	6
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	6, 7, Table 1
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	7, 8, 9
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	9
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	8
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	9, 10
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	12, Table 2
39			participants. A schematic diagram is highly recommended (see Figure)	
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1	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
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3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 7
5				

### 6 **Methods: Assignment of interventions (for controlled trials)**

#### 7 Allocation:

8				
9				
10	Sequence	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
11	generation			
12				
13				
14				
15				
16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
25				
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27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11, 12
28				
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### 31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
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39		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
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1	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
2				
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4				
5	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
6				
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17, 18
9				
10		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
11				
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13				
14	<b>Methods: Monitoring</b>			
15				
16	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
17				
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22		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
23				
24				
25	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
26				
27				
28	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
29				
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32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
35				
36				
37	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
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
5				
6				
7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22, 23
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
14				
15				
16	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
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20	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
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	22
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
32				
33				
34	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
35				
36				

\*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”](https://creativecommons.org/licenses/by-nc-nd/3.0/) 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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-031431.R1
Article Type:	Protocol
Date Submitted by the Author:	12-Sep-2019
Complete List of Authors:	Jensen, Simon; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Lundgren, Julie; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Janus, Charlotte; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Juhl, Christian; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Olsen, Lisa; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Rosenkilde, Mads; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Holst, Jens; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research Stallknecht, Bente; University of Copenhagen, Department of Biomedical Sciences Madsbad, Sten; Hvidovre Hospital, Department of Endocrinology Torekov, Signe; University of Copenhagen, Department of Biomedical Sciences; University of Copenhagen, Novo Nordisk Foundation Center for Basic Metabolic Research
<b>Primary Subject Heading</b>:	Diabetes and endocrinology
Secondary Subject Heading:	Sports and exercise medicine
Keywords:	weight loss, weight loss maintenance, liraglutide, exercise, obesity, GLP-1 receptor agonist

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4 1 **Protocol for a randomized controlled trial of the combined effects of the GLP-1 receptor**  
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8 3 **calorie diet**

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13 5 **Authors**

14  
15 6 Simon Jensen<sup>1,2\*</sup>, Julie Lundgren<sup>1,2\*</sup>, Charlotte Janus<sup>1,2\*</sup>, Christian Rimer Juhl<sup>1,2</sup>, Lisa Olsen<sup>1,2</sup>,  
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4 25 **Abstract**

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6 26 Introduction: The success rate of weight loss maintenance is limited. Therefore, the purpose of this  
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9 27 study is to investigate the maintenance of weight loss and immunometabolic health outcomes after  
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11 28 diet-induced weight loss followed by one-year treatment with a glucagon-like peptide-1 receptor  
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13 29 agonist (liraglutide), physical exercise, or the combination of both treatments as compared with  
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16 30 placebo in individuals with obesity.

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18 31 Methods and analysis: This is an investigator-initiated, randomized, placebo-controlled, parallel  
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20 32 group trial. We will enroll expectedly 200 women and men (age 18 to 65 years) with obesity (body  
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22 33 mass index 32 to 43 kg/m<sup>2</sup>) to adhere to a very low-calorie diet (800 kcal/day) for eight weeks in  
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24 34 order to lose at least 5 % of body weight. Subsequently, participants will be randomized in a 1:1:1:1  
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26 35 ratio to one of four study groups for 52 weeks: 1) placebo, 2) exercise 150 min/week + placebo, 3)  
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28 36 liraglutide 3.0 mg/day, and 4) exercise 150 min/week + liraglutide 3.0 mg/day. The primary  
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30 37 endpoint is change in body weight from randomization to end-of-treatment.  
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34 38 Ethics and dissemination: The trial has been approved by the ethical committee of the Capital  
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36 39 Region of Denmark and the Danish Medicines Agency. The trial will be conducted in agreement  
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38 40 with the Declaration of Helsinki and monitored to follow the guidelines for good clinical practice.  
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40 41 Results will be submitted for publication in international peer-reviewed scientific journals.  
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43 42 Trial Registration: EudraCT Nr.: 2015-005585-32  
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4 43 **Strengths and limitations of this study**

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7 44 • First randomized controlled trial investigating the combined and individual effects of liraglutide  
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9 45 and exercise to maintain diet-induced weight loss in individuals with obesity.  
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11 46 • Direct comparison of liraglutide and exercise on weight loss maintenance and immunometabolic  
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13 47 health.  
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16 48 • Applying state-of-the-art methodologies, the study may identify novel targets for sustainable  
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18 49 immunometabolic health-promoting weight loss strategies.  
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## 50 **Introduction**

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

64 Increasing energy expenditure by increasing physical activity is the first-line lifestyle modification  
65 in the treatment of obesity along with reducing food intake. For exercise interventions targeting  
66 general public health recommendations (at least 150 min/week of moderate intensity aerobic  
67 exercise), the associated weight loss is often modest (0-3 %) without concomitant calorie restriction  
68 [19–21]. However, independent of weight loss, increasing physical activity improves body  
69 composition, glycemic control, low grade inflammatory profile, and cardiorespiratory fitness in  
70 individuals with overweight and obesity [22–25]. In addition, exercise may preserve lean mass  
71 during weight loss [26] and thereby counteract the associated decrease in resting metabolic rate  
72 [27], which may explain the observation that individuals performing regular exercise have less body  
73 weight regain after weight loss compared to participants that do not exercise [28,29].

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

86

87 Obesity is associated with chronic low-grade inflammation [35,36] which is linked to the  
88 development of atherosclerosis and insulin resistance [37–39]. Physically active individuals have  
89 lower inflammatory biomarker concentrations than their inactive counterparts [24], possibly  
90 explained by antiinflammatory effects of an acute bout of exercise [40] and lower levels of visceral  
91 adipose tissue [41]. GLP-1 has also emerged as an immunomodulatory agent [42,43]. In mice, GLP-  
92 1 RA administration reduces macrophage accumulation and inflammatory markers in the arterial  
93 wall [44], adipose tissue [45], and heart [46]. Similarly, GLP-1 RAs have shown antiinflammatory  
94 effects in human coronary artery endothelial cells and aortic endothelial cells [47]. In humans with  
95 T2D, short term GLP-1 RA treatment exert antiinflammatory actions, reflected in reduced levels of  
96 the macrophage activation molecule sCD163 [48] and reduced production of several  
97 proinflammatory markers, such as TNF- $\alpha$ , IL1 $\beta$ , and IL-6 in peripheral blood mononuclear cells

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4 98 [48,49]. Another study showed no improvement of obesity-associated adipose tissue dysfunction in  
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6 99 T2D patients after GLP-1RA treatment [50]. One year treatment with GLP-1 RAs reduce the  
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9 100 inflammation marker, high-sensitivity C-reactive protein, in overweight and obese individuals [33]  
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11 101 and T2D patients [51]. Notably, in patients with T2D and high cardiovascular risk, GLP-1 RAs  
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13 102 reduced the rate of occurrence of first major cardiovascular event [52,53].  
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18 104 Thus, both physical activity and GLP-1 RA treatment seem to facilitate weight loss maintenance,  
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20 105 improve metabolic health, and reduce systemic inflammation. However, diet-induced weight loss  
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23 106 decreases energy expenditure and increases appetite. We hypothesize that the combination of  
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25 107 physical activity and liraglutide treatment improves weight loss maintenance and immunometabolic  
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27 108 health since the decreased energy expenditure is targeted with exercise and the increased appetite  
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30 109 with liraglutide.  
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## 32 110

### 34 111 **Objective**

35  
36 112 The objectives of this study are to investigate the maintenance of weight loss and immunometabolic  
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39 113 health outcomes over 52 weeks with liraglutide treatment, physical exercise, and the combination in  
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41 114 individuals with obesity, after a very low-calorie diet.  
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### 46 116 **Methods and analysis**

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#### 48 117 **Participants, interventions, and endpoints**

##### 49

##### 50 118 *Trial design*

51  
52 119 This study protocol describes an investigator-initiated, randomized, placebo-controlled, parallel  
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55 120 group trial, the S-LiTE trial (acronym for ‘Synergy effect of the appetite hormone GLP-1  
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57 121 (LiragluTide) and Exercise on maintenance of weight loss and health after a low calorie diet’). The  
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4 122 trial is double-blinded with regards to study medication but not exercise intervention. The study  
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7 123 design is outlined in Figure 1. The study is registered at the European Clinical Trials Database  
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9 124 (EudraCT Nr.: 2015-005585-32).  
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#### 11 125 12 13 14 126 *Study setting*

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16 127 All examinations in the trial will be carried out at Department of Endocrinology, Hvidovre  
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18 128 University Hospital, University of Copenhagen and Department of Biomedical Sciences, University  
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20 129 of Copenhagen.  
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#### 23 130 24 25 131 *Study status*

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27 132 Recruitment of participants was initiated in September 2016. Last participant last visit is planned  
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30 133 for November 2020.  
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#### 32 134 33 34 135 *Participants and recruitment*

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36 136 We will enroll expectedly 200 participants. Eligible participants are adults (age 18-65 years) with  
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39 137 obesity (body mass index (BMI) 32-43 kg/m<sup>2</sup>) and no known serious chronic illness (including type  
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41 138 1 and 2 diabetes). Inclusion and exclusion criteria are listed in Table 1. Recruitment will be done  
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43 139 via local newspapers, online media, and flyers from Department of Endocrinology, Hvidovre  
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45 140 University Hospital, and Department of Biomedical Sciences, University of Copenhagen.  
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48 141 Individuals who agree to participate will be invited to a pre-screening that includes screening of the  
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50 142 study eligibility criteria before being finally included in the study. Withdrawn subjects will not be  
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53 143 replaced. Re-screening is allowed within the recruitment period.  
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#### 55 144 56 57 145 *Participant involvement* 58 59 60

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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  $\geq 5$  % 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 administered 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

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4 170 do not tolerate the 3.0 mg dose may in special circumstances stay at lower dose (2.4 mg). However,  
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7 171 the aim is to reach 3.0 mg for all study participants.  
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9 172  
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11 173 Physical exercise  
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13 174 The exercise intervention follows the global recommendations from the World Health Organization  
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15 (WHO) of 150 minutes of moderate-intensity aerobic physical activity throughout the week or 75  
16 175 minutes of vigorous-intensity aerobic physical activity throughout the week or an equivalent  
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18 176 combination of moderate- and vigorous-intensity activity [58]. The intervention consists of four  
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20 177 sessions per week for a total of 150 min/week. Two sessions per week will be performed under  
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22 178 supervision of the study staff and two sessions will be performed individually. A heart rate monitor  
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24 (Polar A300, Polar Electro Oy, Kempele, Finland) will be worn during all planned exercise  
25 179 sessions. Supervised sessions will consist of structured exercise for a duration of 45 min. Of this, 30  
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27 180 min will be interval-based spinning and 15 min will be circuit training focusing on large muscle  
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29 181 groups. Individual exercise sessions will include aerobic exercise such as cycling, rowing, or  
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31 182 elliptical training as well as brisk walking or cycling to work. Participants will be advised to  
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33 183 primarily perform non-weight bearing activities. The target aerobic exercise intensity is 80 % of  
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35 184 maximal heart rate. Participants randomized to an exercise group will undergo a 6-week ramp-up  
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37 185 phase with one session in week 1 and 2, two sessions in week 3 and 4, and three sessions in week 5  
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39 186 and 6 before exercising four times per week from week 7 to 52. If the planned ramp-up phase with  
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41 187 exercise is not possible (e.g. due to side effects of study medication or joint pain), ramp-up will  
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43 188 proceed more slowly. Participants not randomized to exercise will be instructed to maintain habitual  
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45 189 physical activity according to level before entering the trial.  
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57 193 Liraglutide and physical exercise  
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194 Combination of the two interventions described above.

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

199

#### 200 *Criteria for discontinuing/modifying allocated interventions*

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

206

#### 207 *Endpoints*

##### 208 Primary endpoint

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

211

##### 212 Secondary endpoint

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

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##### 216 Explorative endpoints

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4 217 Explorative endpoints include changes from V1 to V3 in the following parameters: meal-related  
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6 218 appetite hormone response; physical fitness and determination of daily physical activity and sleep;  
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9 219 systemic markers of immunometabolism; endothelial function; immunometabolic changes in the  
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11 220 subcutaneous adipose tissue; gene expression profile of circulating inflammatory cells; bone health;  
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13 221 food preferences and subjective appetite sensation; faecal bacterial composition; plasma  
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16 222 metabolomics and proteomics; epigenetics of spermatozoa.  
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### 20 224 *Sample size calculation*

22 225 Sample size is calculated in relation to body weight. In our previous weight loss maintenance study  
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25 226 [18], the response within each treatment group was normally distributed with standard deviation of  
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27 227 5.5 kg. Thus, with expectedly 30 participants completing each study arm we will be able to detect a  
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30 228 true difference of 4 kg between groups with a power of 0.8, assuming a two-sided  $\alpha$ -level of 0.05.

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32 229 In our previous study, 10 % did not complete the initial weight loss phase [18]. Thus, with  
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34 230 expectedly 200 enrolled study participants and an expected dropout rate of 25 % after  
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36 231 randomization, we expect to have at least 30 participants from each study arm to complete the trial.  
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### 41 233 **Assignment of intervention**

#### 43 234 *Treatment allocation*

45 235 After the initial eight-week VLCD phase, participants will be randomized after the test day to one  
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48 236 of the four study groups in a 1:1:1:1 ratio in accordance with a subject randomization list (SRL)  
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50 237 provided by Novo Nordisk (NN). An un-blinded study nurse (not otherwise associated with the  
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52 238 trial) will allocate study participants according to the SRL. Randomization will be stratified by sex  
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55 239 (male/female) and age (below/above 40 years). NN will provide a total dispensing unit number list  
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57 240 (TDL). The un-blinded study nurse will allocate trial medication using the TDL by matching a six  
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241 digit Dispensing Unit Number (DUN) to the correct treatment. Each box of study medication will  
242 be labeled with a unique DUN. The DUN alone is not un-blinding. Thus, dispensing of trial  
243 medication to subjects can be carried out by blinded trial staff by selecting the DUN provided by  
244 the un-blinded study nurse.

245

### 246 *Un-blinding*

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

258

### 259 **Data collection, management, and analysis**

#### 260 *Study visits*

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

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4 265 compliance to the VLCD, including measurement of body weight and handing out Cambridge meal  
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6 266 products. During the 52-week weight maintenance phase, weight consultations, including  
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9 267 assessment of adverse events, will be conducted at week 1, 2, 3, 4, 9, 13, 17, 22, 32, 39, and 46.  
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11 268 Consultations at week 4, 13, and 39 will include collection of fasting blood samples, measurement  
12  
13 269 of hip and waist circumference, blood pressure, and resting heart rate. Finally, participants will be  
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16 270 invited to complete a post-trial unsupervised follow-up visit (V4) one year after intervention  
17  
18 271 completion.  
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### 22 23 273 *Criteria for assessments*

- 25 274 • Participants must be fasting for minimum 10 hours prior to test days, including foods, liquids,  
26  
27 275 and medication (except study medication)
- 29  
30 276 • Study medication should preferably be taken on the morning of the tests
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32 277 • Exercise should not be performed the day before or in the morning before tests
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### 34 278 35 36 37 279 *Assessments*

#### 38 39 280 Anthropometrics

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41 281 Body weight will be measured on a digital scale (TANITA WB-110MA, Tokyo, Japan) to the  
42  
43 282 nearest 0.1 kg without shoes and wearing light clothes. Waist circumference, the midpoint between  
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46 283 lowest rib and iliac crest, and hip circumference, the level of the great trochanters, will be measured  
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48 284 in duplicate to the nearest 0.1 cm after gentle expiration.  
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#### 50 285 51 52 53 286 DXA scan

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55 287 Dual-energy X-ray absorptiometry (DXA) scans will be performed in fasting state to measure body  
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57 288 fat mass, fat free mass, and bone density (Hologic Discovery A, Hologic inc., Bedford, USA).  
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### 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



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4 313 After fasting blood sampling, a liquid meal (Nutricia Nutridrink, 600 kcal, 49 E% from  
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6 314 carbohydrates, 35.2 E% from fat, and 15.8 E% from protein) will be ingested over 15 min, and  
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9 315 blood samples will be collected continuously every 15 min for the first hour and every 30 min for  
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11 316 the next two hours to measure circulating biomarkers of metabolic health, appetite hormones,  
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13 317 immune markers, plasma proteomics and plasma metabolomics. During the meal test, appetite  
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16 318 sensation will be assessed after each blood sample using a visual analogue scale [59].  
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#### 18 319 19 20 320 Feces, urine, saliva, and semen

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23 321 Feces samples will be collected to investigate fecal bacterial composition. Semen will be collected  
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25 322 (if relevant) to investigate epigenetics of the spermatozoa. Additionally, urine and saliva samples  
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27 323 will be collected.  
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#### 30 324 31 32 325 Endothelial function

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34 326 Flow mediated dilation (FMD) of the brachial artery will be measured to assess endothelial function  
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36 327 [60]. FMD, also known as endothelium-dependent vasodilation, is the vasodilatory response of the  
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39 328 brachial artery to increased shear stress and reflects the ability of vascular endothelium to produce  
40  
41 329 nitric oxide. The brachial artery will be scanned with high resolution ultrasound imaging using a  
42  
43 330 linear probe at rest and during hyperaemia. Hyperaemia will be induced by inflation and deflation  
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45  
46 331 of a sphygmomanometer cuff around the forearm, distal to the site scanned with ultrasound. FMD is  
47  
48 332 calculated as the percentage change of the brachial artery diameter from rest to 60 seconds after the  
49  
50 333 cuff is released. To assess the endothelium-independent vasodilation, nitroglycerine is given  
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53 334 sublingually (0.4 mg) with the diameter of the brachial artery measured before and 5 min after drug  
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55 335 administration. Carotid intima-media thickness will also be measured using ultrasound.  
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4 337 Electrocardiography

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7 338 Electrocardiogram will performed to assess safety concerns related to study participation.

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11 340 Physical fitness

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13 341 Measurement of physical fitness will include three components: 1) Cardiorespiratory fitness (peak  
14 342 oxygen consumption) will be assessed with an incremental maximal cycle protocol performed on an  
15  
16 343 electromagnetically braked cycle ergometer (Corival, Lode Medical Technology, The Netherlands)  
17  
18 344 with continuously determined oxygen consumption and carbon dioxide production (MasterScreen  
19  
20 345 CPX, CareFusion, Germany). After a warm-up protocol, workload will be increased every minute  
21  
22 346 (20 and 25 watt for females and males, respectively) until attainment of peak oxygen consumption  
23  
24 347 based on the following criteria: plateau in oxygen consumption, respiratory exchange ratio above  
25  
26 348 1.15 or attainment of age-predicted maximal heart rate (220 minus age), and voluntary exhaustion  
27  
28 349 [61]. 2) Physical functioning will be measured as time to ascend and descend an 11-step stairway  
29  
30 350 twice. 3) Maximal strength will be measured as isometric maximal voluntary contraction force of  
31  
32 351 the dominant thigh using a dynamometer chair (Good Strength, Metitur Oy, Jyväskylä, Finland)  
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34 352 [62,63].

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39 354 Questionnaires

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41 355 Participants will answer five questionnaires to determine self-rated quality of life (The Short Form  
42  
43 356 (36) Health Survey[64]), eating habits (three-factor eating questionnaire[65]), physical activity  
44  
45 357 (International Physical Activity Questionnaire[66]), sleep quality (Pittsburgh Sleep Quality  
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47 358 Index[67]), and self-efficacy (General Self-Efficacy Scale[68]).

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51 360 Food preferences

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4 361 Food preferences and food reward responses will be measured in fasted state and postprandially  
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6 362 with the Leeds Food Preference Questionnaire [69]. This is a computerized task where standardized  
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9 363 pictures of 20 typical Danish food items are shown in the categories: high fat savoury, low fat  
10  
11 364 savoury, high fat sweet and low fat sweet. Participants are instructed to rate each individual food  
12  
13 365 item according to liking and to systematically choose the food items they prefer the most. Speed of  
14  
15  
16 366 choices is covertly assessed as an index of implicit wanting.  
17

#### 18 367 19 20 368 Free-living assessment of physical activity and sleep

21  
22 369 An accelerometer device (GENEActiv, ActivInsights Ltd, Cambridgeshire, UK) will be worn on the  
23  
24  
25 370 wrist for seven consecutive days and nights with concomitant sleep registration (time for going to  
26  
27 371 and out of bed) to assess habitual physical activity and sleep duration.  
28

#### 29 30 372 31 32 373 *Data management*

33  
34 374 Participants will be identified by study ID. Study data is collected and managed using the REDCap  
35  
36 375 secure web-based system [70] hosted by the Capital Region of Denmark, where electronic case  
37  
38  
39 376 report forms (CRF) have been created. During the trial, data will be entered directly into REDCap  
40  
41 377 by study personnel. Extraction procedures will be performed by investigators or sponsor.  
42

43 378 Laboratory data will be transferred electronically from the laboratory performing clinical analyses  
44  
45  
46 379 and will be archived in secured hard drives with backup. All biological material (blood, adipose  
47  
48 380 tissue, faeces, urine, saliva, and semen) obtained from study participants will be kept in a research  
49  
50 381 bio-bank. Samples will be labelled with study ID. The bio-bank allows for analyses of samples to be  
51  
52  
53 382 performed simultaneously to avoid large instrumental variations. The research bio-bank will be  
54  
55 383 terminated no later than August 1<sup>st</sup> 2036. After this date, the remains of the material will be  
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57 384 transferred to a regular bio-bank in Denmark. In order to access this material, a new protocol must  
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385 be approved by an ethics committee. The Danish Data Protection Agency has been notified about  
386 the trial and the trial adheres to the Data Protection Act, which requires data to be anonymized as  
387 soon as it is practical to do so.

388

#### 389 *Data analysis plan*

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

396

#### 397 Primary endpoint

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

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4 409 medication allocation will be kept for investigators until analysis of the primary endpoint has been  
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6 410 completed.

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11 412 Secondary and exploratory endpoints

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13 413 For secondary and exploratory endpoints, general linear models will be used to test for differences.

14  
15 414 The change from V1 to V3 will be analysed using general linear models with treatment group as  
16  
17 explanatory variable and baseline weight or BMI, initial weight loss (if relevant), sex as covariates.

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20 416 Longitudinal data will also be analysed using linear mixed models as described above.

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25 418 *Auditing*

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27 419 The Good Clinical Practice (GCP) unit of the University Hospitals of Copenhagen will perform on-  
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29 site audits minimum once yearly to ensure that the trial adhere to the guidelines for good clinical  
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31 practice provided by the International Council for Harmonisation (ICH).

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36 423 **Ethics and dissemination**

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38 424 *Harms*

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40 425 All adverse events (AE) will be collected from the first drug administration and in all following  
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42 contacts with participants throughout the trial. A serious adverse event (SAE) is defined as any  
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44 untoward medical occurrence that results in death, is life-threatening, requires hospitalization or  
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46 prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is  
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48 428 a congenital anomaly or birth defect. Once yearly, sponsor will send a report regarding SARs  
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50 429 occurring in the trial and safety of the study participants in regards to continuation of the trial to the  
51  
52 430 Danish Medicines Agency and the Ethics Committee. In case of any deadly or life-threatening  
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54 431 suspected unexpected serious adverse reaction (SUSAR), sponsor will immediately (and no later  
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433 than 7 days after becoming aware) notify the Danish Medicines Agency and the Ethics Committee.  
434 No later than 8 days after reporting of a SUSAR, sponsor will notify the Danish Medicines Agency  
435 and the Ethics Committee of all relevant information about sponsor's and investigator's follow-up  
436 of the SUSAR. All other SUSARs will be reported to the Danish Medicines Agency and the Ethics  
437 Committee no later than 15 days after sponsor becoming aware of this. All AEs and SAEs will be  
438 noted in the CRF and recorded in the End-of-Trial Form to the Danish Medicines Agency and in the  
439 report to the Ethics Committee (if requested) no later than 90 days after trial completion.

440

#### 441 *Ethical considerations*

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

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4 457 minimal discomfort. DXA scans use radiation with a radiation dose of approximately 0.02 mSv per  
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7 458 examination. This dose is very low compared to the background radiation in Denmark  
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9 459 (approximately 3 mSv/year). The FMD and carotid intima-media thickness ultrasound scans do not  
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11 460 use radiation. Applying nitroglycerine may cause transient headache, dizziness, decreased blood  
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14 461 pressure, and increased heart rate. Half-life of nitroglycerine is 1-3 min. Nitroglycerine will not be  
15  
16 462 used if systolic blood pressure is under 100 mmHg. The risks that are associated with this study are  
17  
18 463 assessed as minimal. By participating in this project, the participants will contribute with new  
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20  
21 464 important knowledge about the interaction between GLP-1 and exercise and their importance for  
22  
23 465 weight loss maintenance and metabolic health. Overall, we consider that any potential risks and side  
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25 466 effects are outweighed by the advantages of participation. The trial is approved by the ethical  
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27 467 committee of the Capital Region of Denmark (H-16027082) and the Danish Medicines Agency  
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29  
30 468 EudraCT Nr.: 2015-005585-32. The trial will be carried out in accordance with the Declaration of  
31  
32 469 Helsinki, and adhere to the GCP-ICH guidelines. After careful written and oral information about  
33  
34 470 the trial and associated risks have been given, a written informed consent form will be obtained by  
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37 471 investigator or sub-investigators before any study-related activities are performed. An English  
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39 472 translation of the informed consent form is provided in supplementary file 1.  
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#### 41 473 42 43 474 *Dissemination plan*

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46 475 All study results (positive, negative, and inconclusive) will be presented at international scientific  
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48 476 conferences as oral presentations or poster presentations. Furthermore, results will be published in  
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50 477 international peer-reviewed scientific journals.  
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#### 52 478 53 54 55 479 *Protocol version*

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

## 483 **Discussion**

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

## 494 **Contributors**

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

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1  
2  
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5  
6 505 by Novo Nordisk. Cambridge weight plan diet products for the initial 8 weeks weight loss program  
7  
8  
9 506 are provided by Cambridge weight plan. JL is funded by a PhD grant from Faculty of Health and  
10  
11 507 Medical Sciences, University of Copenhagen. CJ is funded by a PhD grant from Danish Diabetes  
12  
13 508 Academy. The planning and conduct of the study, interpretation of data, and writing of manuscripts  
14  
15  
16 509 are completely independent of the funders.  
17

### 18 510

### 19

### 20 511 **Competing interests**

22 512 SST has received research grant from Novo Nordisk. SM has received research grants from Novo  
23  
24 513 Nordisk and Boehringer Ingelheim, lecture fees from AstraZeneca, Boehringer Ingelheim, Bristol-  
25  
26 514 Meyers Squibb, Eli Lilly, Merck Sharp & Dohme, Novartis, Novo Nordisk, and Sanofi Aventis, and  
27  
28 515 is a member of advisory boards of AstraZeneca, Boehringer Ingelheim, Bristol-Meyers Squibb, Eli  
29  
30 516 Lilly, Intarcia Therapeutics, Johnson & Johnson, Merck Sharp & Dohme, Novartis; Novo Nordisk,  
31  
32 517 and Sanofi Aventis. JJH has served on advisory panels for GlaxoSmithKline, Novo Nordisk,  
33  
34 518 Zealand Pharma, AstraZeneca, MSD, Intarcia and Hanmi and as a consultant for Novo Nordisk, and  
35  
36 519 has received research support from Merck, Sharp & Dohme.  
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50 716 **Figure 1 legend**

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52 717 Study design

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57 719 **Tables**  
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Table 1. Eligibility criteria for participants in the S-LiTE trial

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**Inclusion criteria**

- BMI: 32-43 kg/m<sup>2</sup>
- 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
<b>Time point (week)</b>	-	-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	

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3				
4	ECG	X	X	X
5				
6	Physical fitness testing	X	X	X
7				
8	Questionnaires	X	X	X X
9				
10	Food preference test	X	X	X X
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12				

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

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For peer review only

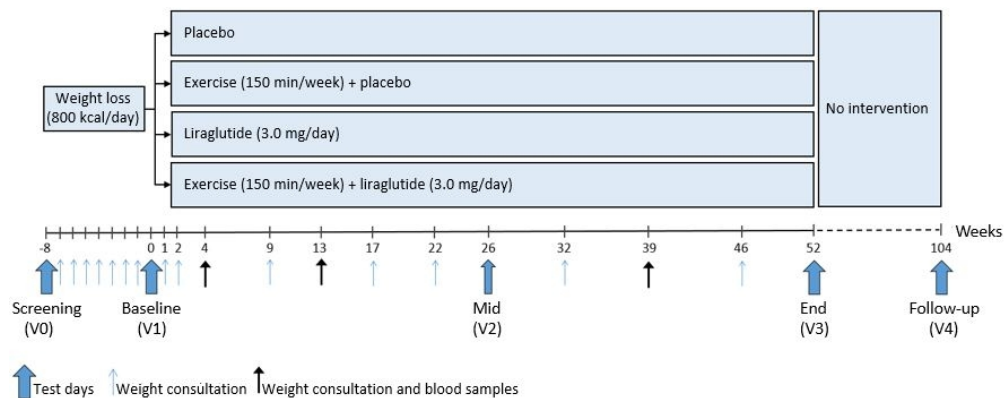


Figure 1. Study design

187x73mm (120 x 120 DPI)

**English translation of informed consent form**

Project title: 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
<b>Administrative information</b>			
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

1	<b>Introduction</b>			
2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	4, 5
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	5
7				
8	Objectives	7	Specific objectives or hypotheses	5, 6
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	6
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
12				
13				
14	<b>Methods: Participants, interventions, and outcomes</b>			
15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	6
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	6, 7, Table 1
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	7, 8, 9
23			administered	
24				
25		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	9
26			change in response to harms, participant request, or improving/worsening disease)	
27				
28		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	8
29			(eg, drug tablet return, laboratory tests)	
30				
31		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
32				
33	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	9, 10
34			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
35			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
36			efficacy and harm outcomes is strongly recommended	
37				
38	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	12, Table 2
39			participants. A schematic diagram is highly recommended (see Figure)	
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1	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
2				
3				
4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6, 7
5				

### 6 **Methods: Assignment of interventions (for controlled trials)**

#### 7 Allocation:

8				
9				
10	Sequence	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
11	generation			
12				
13				
14				
15				
16	Allocation	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
17	concealment			
18	mechanism			
19				
20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
21				
22				
23				
24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	6
25				
26				
27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11, 12
28				
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### 31 **Methods: Data collection, management, and analysis**

32				
33	Data collection	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
34	methods			
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39		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
40				
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42				

1	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
2				
3				
4				
5	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
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17, 18
9				
10		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
11				
12				
13				
14	<b>Methods: Monitoring</b>			
15				
16	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
17				
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22		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
23				
24				
25	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
26				
27				
28	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
29				
30				
31				
32	<b>Ethics and dissemination</b>			
33				
34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21
35				
36				
37	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
38				
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	21
2				
3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
5				
6				
7	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
8				
9				
10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	22, 23
11				
12				
13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	22
14				
15				
16	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
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18				
19				
20	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
21				
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	22
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	n/a
27				
28				
29	<b>Appendices</b>			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Available on request
32				
33				
34	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
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