Title

The effects of different doses of exercise on pancreatic β-cell function in patients with newly diagnosed type 2 diabetes (DOSE-EX): A randomized clinical trial

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Content

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Organizational structure and responsibilities

Principal investigators and research physicians

- Design and conduct of DOSE-EX
- Preparation of protocol and revisions
- Preparation and submission of registration to the Danish Data Authorities
- Clinical trials registration
- Preparation of Standard Operation Procedures brochures (SOP)
- Preparation of case report forms (CRF)
- Organizing steering committee meetings
- Publication of study reports
- Report serious adverse events (SAE) to the research ethics committee (REC)
- Members of trial management committee

Steering committee (see title page for members)

- Agreement of final protocol
- Reviewing progress of study
- Reviewing and Agreeing to changes to the protocol and/or SOP's
- Reviewing and agreeing to sub-study proposals

Trial management committee

- PI, Research Physicians, Administration
- Study planning
- Organization of steering committee meetings
- Organization recruitment and biweekly progress reports on recruitment status
- Provide annual report to the REC
- Responsible for trial master file
- Budget administration and purchases of equipment
- Advice for lead investigators
- Intervention organization
- Organization of data collection
- Assistance with international review, board/independent ethics committee applications
- Data verification
- Allocation

Introduction

Lifestyle intervention including exercise therapy is a cornerstone in clinical care of type 2 diabetes (T2D). The current exercise recommendation for T2D is 150 min/week of moderate to vigorous intensity aerobic exercise, supplemented with resistance training 2-3 days/week [1,2]. The rationale for the recommendations are primarily based on reductions in HbA1c, whereas evidence supporting an effect on central T2D pathophysiological mechanisms e.g. pancreatic β-cell function is scarce[1,2]. As exercise induces health effects that most likely are not exclusively mediated via HbA1c or other known surrogate markers, this study aims at understanding exercise dosing beyond HbA1c in order to approach minimal exercise dose to reduce the risk of micro and macro vascular complications. In fact, epidemiological studies suggest that the vigorous leisure time physical activity should be above 4 and as high as 10 hours per week to reduce the risk of vascular complications [3-6]. Thus, it may in this perspective very well be that the most efficient exercise dose is higher than the current recommendations. With the aim of developing "exercise as medicine" for patients with T2D, efficacy in relation to clinically relevant endpoints needs to be demonstrated. Moreover, dose-response efficiency of exercise on these endpoints needs to be established – i.e., establishing the minimum dose necessary to affect these outcomes. As targeting a reduction in HbA1c might not lead to a reduction in complications, knowledge about the exercise dose needed to reduce micro- and macrovascular complications in T2D is largely unknown [7-15]. In essence, as the most clinical exercise interventions in T2D base their conclusions on HbA1c, the significance of exercise in the clinical care of prevalent T2D is challenged [14,16-18].

Pancreatic β-cell function and non-pharmacological interventions in T2D.

Although T2D is in general regarded as a treatable yet chronic condition, several non-pharmacological therapies have been demonstrated to introduce remission and reversal of pancreatic β-cell function (normal/non-diabetic glycemic control without the use of glucose lowering therapy)[19-21]. These are weight reduction, dietary or surgical, and exercise. In a recent trial we observed that an exercise-based (6 sessions of exercise/week) lifestyle intervention with a hypocaloric diet component eliminated the need for glucose lowering medications in 56% of the intervention group following the 12-month intervention[22,23]. Thus, there is evidence supporting that T2D may be a reversible disease.

Glycemic control is maintained through a relationship between insulin sensitivity and insulin secretion. This relationship is inversely and proportionally related, where glucose homeostasis is tightly regulated by a feedback loop with crosstalk between pancreatic β-cells and insulin-sensitive tissue[24]. Whereas insulin resistance is the earliest detectable abnormality in T2D[25], dysfunction in the insulin secretory capacity determines the onset of hyperglycemia and concomitant treatment[26]. By the time of T2D diagnosis the insulin secretory capacity of the β-cell may be reduced by up to 50 percent[27]. This β-cell dysfunction may be caused by lipo- and glucotoxity[28,29]. The presence of high FFA availability in presence of hyperglycemia is detrimental to β-cell survival and is believed to cause β-cell apoptosis. The pancreatic β-cell in T2D is subject to oxidative stress, endoplasmic reticulum (ER) stress and amyloid deposition. Moreover, ER stress caused by an abundance of FFA, may cause a depletion of Ca^{2+} stores and prevent the release of insulin[30,31]. As previously proposed, an increased hepatic insulin resistance will increase *de novo lipogeneses*, and thereby increase delivery of lipids from the liver to other tissues[32], amongst those the pancreas where they will accumulate[33]. Excess circulating FFA may be taken up by the β-cells and stored as intracellular lipids [33]. Due to peripheral (skeletal muscle and adipose tissue) and central (liver) insulin resistance, increased levels of portal insulin develops and may stimulate the storage of lipids in β-cells[33]. Finally, local inflammation, namely glucose induced IL1-β, may cause oxidative stress by activation of the NFκB pathway and activation of infiltrated macrophages[30]. Indeed, pharmacological inhibition of IL1-β by subcutaneous injections of IL1-ra increased

the β-cell secretory function following 13 weeks of treatment in T2D patients[34]. This supports that inflammation plays an important role in the etiology of T2D and may be causally related to pancreatic β-cell dysfunction in T2D.

Bariatric surgery and extreme dietary calorie restriction along with a rapid weight loss have shown to improve β-cell function within days to weeks[35]. This coincides with a decrease in hepatic lipid deposition, followed by a decrease in pancreatic lipid deposition[33]. It has thus been proposed that secondary to the restoration of hepatic insulin sensitivity by depletion of intracellular lipid accumulation from weight loss, the delivery of TAG to other tissues are decreased and thus the function of the β-cell is restored through a depletion of TAG/FFA[33,35,36]. Indeed, to accomplish the massive weight loss needed to restore function (11-20 kg of body weight) a maintained and extreme calorie restriction or bariatric surgery is needed. Thus, it is evident that diet-induced weight loss is pivotal in reversing β-cell function in a dose-dependent manner[19,37,38], and should be considered in concert with exercise intervention in the clinical care of T2D.

The role of exercise in restoring pancreatic β-cell function

It is well established that exercise improves insulin sensitivity in the peripheral tissue[39,40] and thus may induce pancreatic β-cell rest. Only a few studies have focused on the effects of exercise on pancreatic β-cell function in T2D and discrepancies regarding the effect exists[41-45]. The discrepancies may relate to the assessment of β-cell function[46], concomitant pharmacological therapy and the pre-trial insulin secretory capacity. Moreover, exercise intensity, volume and modality may play a role[15,17,47-49].

Exercise may, independently of weight loss, relieve hepatic insulin resistance[50] and decrease *de novo lipogeneses*[51] and lower plasma TAG[52]. Accordingly, in a recent study from Heiskanen et al., it was observed that only 14 weeks of exercise decreased pancreatic ectopic lipid accumulation while improving β-cell function in both participants with and without T2D[53], lending support to the so-called twin-cycle hypothesis. The latter hypothesis postulates that chronic calorie excess leads to accumulation of liver fat with eventual spill over into the pancreas. These self-reinforcing cycles between liver and pancreas eventually cause metabolic inhibition of insulin secretion after meals and onset of hyperglycemia. However, in our recent trial, we observed that the oral disposition index (DI - a marker of β-cell function) was improved despite only a modest weight loss (unpublished data). This improvement was due to an improved insulin secretion rate as well as an improved whole body insulin sensitivity. In fact, in a post hoc analyses we found that in relation to weight change only a non-significant 3 kg difference in weight loss ($p=0.1$) and a <0.4 kg difference in loss of abdominal fat mass ($p=0.1$) were observed between participants achieving T2D remission and participants that did not, following the 12-month intervention (unpublished data). This suggests that adding exercise to weight loss may work through different mechanisms than proposed by the twin cycle hypothesis. In post hoc analyses (in preparation) we observed that IL1-ra was significantly reduced following the intervention. This reduction in IL1-ra probably reflects a reduction in IL1-β which is causatively related to β-cell function[31]. Indeed, the reduction in IL1-ra explained >60% of the improvement in DI observed in the study (in preparation). However, the methodology did not allow us to understand the mechanism behind this observation nor did it allow us to investigate the causative role of manipulating the exercise dose in the context of lifestyle intervention.

It is well recognized that the muscle is an endocrine organ and that exercise elicits an anti-inflammatory effects in a cross-talk with multiple organs[54]. Moreover, it has previously been suggested that the anti-inflammatory effects of exercise may be mechanistically linked to improved β-cell function in T2D [55], through mechanisms that are distinct different from diet induced weight loss. It is thus evident that diet induced weight loss and exercise may complement each other, and including exercise to diet-induced weight loss provide additive effects in relation the re-establish the pancreatic β-cell function. Thus, we propose that

combining a moderate diet-induced weight loss with exercise may re-establish pancreatic β-cell function through decreased metabolic stress and an anti-inflammatory effect of exercise in T2D patients with no concomitant glucose- and lipid lowering pharmacological therapy. Moreover, as the volume of exercise needed to re-establish β-cell function is unknown this needs to be established in order to gain knowledge about the role of exercise-based lifestyle intervention as a first-line therapy for T2D patients.

The patient population and concomitant care

Poor glycaemic control and poor β-cell function prior to training predict less benefit from training[56,57]. Indeed, Dela et al. showed that T2D patients with remaining insulin secretory function improved the insulin secretory capacity with exercise, whereas participants with low secretory capacity prior to the intervention did not[42]. This is in line with observations from other lifestyle interventions in T2D[58,59]. Moreover, to avoid any risk of drug-induced signs of hypoglycemia or hypotension, previous trials with an expected decrease in body weight in other populations of T2D patients have adjusted the concomitant glucose-and BP lowering medications according to symptomatology and/or standard care guidelines without any adverse effects[1959].

To use lifestyle as a first-line monotherapy it is thus sensible **a)** to focus on T2D patients with remaining β-cell function prior to the intervention and **b)** to adjust concomitant pharmacological therapy according to symptomatology and existing guidelines when investigating the effects of lifestyle intervention on pancreatic β-cell function.

The role of exercise in mechanisms leading to the development of T2D complications.

Both a physically inactive lifestyle and abdominal adiposity create a state of chronic low-grade inflammation [60]. Together reactive oxygen species (ROS) and inflammation contribute to the development of micro- and macro-vascular complications [60,61]. The recent finding that anti-inflammatory therapy leads to a significantly lower rate of recurrent cardiovascular events in patients at high risk [62] suggests that the anti-inflammatory actions of exercise may also influence macrovascular complications to diabetes. Glycaemic variability, defined as the mean amplitude of glycaemic excursions (MAGE), is associated with coronary artery disease, vascular endothelial dysfunction, possibly through increased oxidative stress independent of HbA1c, and fasting plasma glucose [63-65]. High glycaemic variability and hyperglycemia triggers the production of ROS which may drive local and systemic low-grad inflammation[60,66,67]. It has been proposed that the accelerated formation of advanced glycation end-products (AGEs) and their interaction with the receptor for AGEs (RAGE) cause ROS and low-grade inflammation [60,68]. AGEs bind to AGE receptors that can be broadly classified into those that degrade or detoxify AGEs, and those that signal to increase oxidative stress and inflammation upon ligand binding, namely RAGE as well as other AGE receptors[60]. The activation of RAGE by AGEs results in the initiation of various signal transduction cascades and transcription factors such as nuclear factor (NF)-κB, which ultimately cause the generation of ROS and subsequently inflammation. Thus, AGE and ROS seem to represent an important, interconnected pathogenic mechanism involved in all microvascular complications [60]. As exercise represents a natural, strong anti-inflammatory strategy [55,69], exercise may reduce the risk of complications. Thus, we propose that, secondary to a reduction in glycemic variability, exercise may reduce inflammation between systemic ROS and inflammation through the AGE/RAGE axis.

Study objectives and hypotheses

Objectives

Primary objective: To investigate the dose-dependency of exercise on pancreatic β-cell function in patients with short standing T2D.

Secondary objectives: To investigate the dosing effect of exercise on markers of T2D pathogenesis beyond glucose tolerance and mechanisms that may lead to restoration of pancreatic β–cell function, vascular complications and elucidate the causality and time relation between these various causes and consequences of the disease.

Research hypotheses:

Primary: The effect of exercise on pancreatic β-cell function (disposition index) increases with increasing volumes of exercise in combination with a diet across a 4-month intervention in patients with T2D of short duration. It is expected that both moderate volume and high volumes of exercise in combination with a dietary intervention is superior to the control intervention for improving pancreatic β-cell function. It is moreover expected that high volumes of exercise are superior to moderate volumes of exercise and to dietary intervention alone in improving pancreatic β-cell function.

Secondary: Exercise reduces glycaemic variability in a dose-dependent manner followed by reductions in systemic ROS and inflammation mediated by alterations in the AGE/RAGE axis.

Methods

Design:
A parallel-group 4-arm assessor-blinded randomised clinical trial (Figure 1). Participants will be randomly allocated (1:1:1:1) stratified by sex to;

1) No intervention (CON)
2) Dietary intervention (DCON)
3) Dietary intervention + moderate volume exercise (MED)
4) Dietary intervention + high volume exercise (HED)

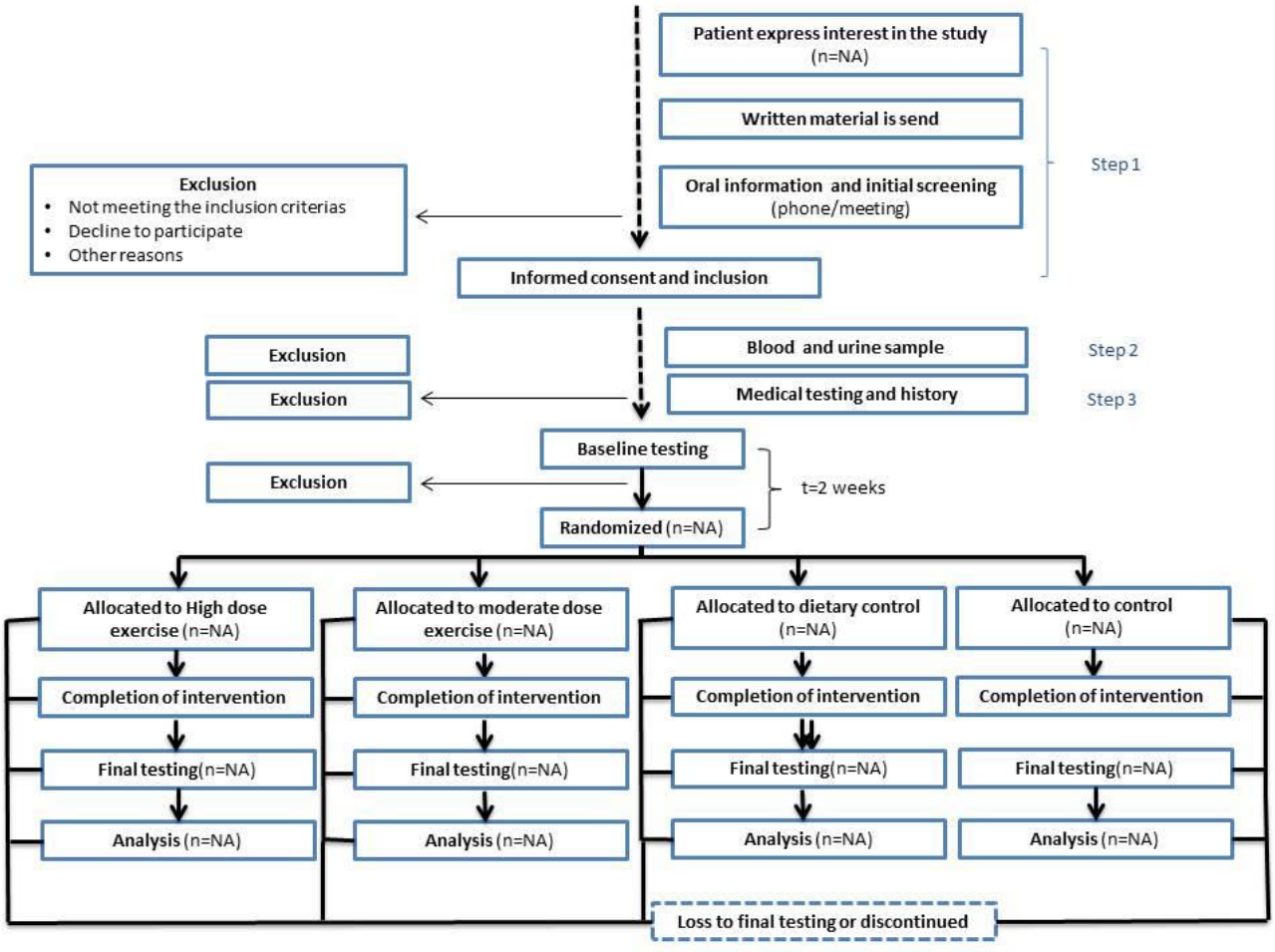

Figure 1: Flow of participants.

Primary place of study execution and data collection
Centre for Physical Activity Research (CFAS)
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Tagensvej 20, DK-2200 Copenhagen (study address)
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All data will be collected and analysed in Denmark

Eligibility criteria

Inclusion criteria

1. Men and women aged 18-80 years
2. Diagnosed with diabetes type 2 and/or HbA1c ≥ 48 mmol/mol if no treatment with anti-diabetic medication and/or use of antidiabetic medication
3. Caucasian
4. No diagnose of Type 1 diabetes, MODY-diabetes, Type 1½ diabetes or LADA-diabetes
5. T2D 0-6 years of duration
6. No treatment with insulin
7. Body Mass Index (BMI) >27 kg/m2 and <40 kg/m2
8. No known or signs of intermediate or severe microvascular complications to diabetes (retino-, neuro- or nephropathy)
9. No known cancer
10. No Known lung disease
11. No known cardiovascular disease
12. No known thyroid disease
13. No known liver disease
14. No known autoimmune disease
15. No other endocrine disorder causing obesity
16. No current treatment with anti-obesity medication
17. No current treatment with anti-inflammatory medication
18. No weight loss of > 5kg within the last 6 months
19. No diagnose of depression or treatment with anti-depressive medication, ongoing or within the last three months before enrolment
20. No diagnose of psychiatric disorder or treatment with anti-psychotic medication
21. No history of suicidal behavior or ideations within the last three months before enrolment
22. No previous surgical treatment for obesity (excluding liposuction > 1 year prior to enrolment)
23. Not pregnant/considering pregnancy
24. No functional impairments that prevents the performance of intensive exercise
25. Accept of medical regulation by the U-TURN endocrinologist
26. Inactivity, defined as < 1,5 hours of structured physical activity pr. week at moderate intensity and cycling < 30 minutes/5 km pr. day at moderate intensity (moderate intensity = out of breath but able to speak)
27. No participation in other research intervention studies

Exclusion criteria

1. HbA1c: >=75 mmol/mol with no glucose lowering medications
2. HbA1c: >=64 mmol/mol with mono glucose lowering therapy (if compliant with the prescription)
3. HbA1c: >=57 mmol/mol with >=dual glucose lowering therapy (if compliant with the prescription)
4. eGFR<60mL/min
5. Protein or glucose in the urine at pre-screening

6. No biochemical sign of other major diseases
7. Presence of circulating glutamatdecarboxylase anti body (GAD) 65
8. Objective findings that contraindicates participation in intensive exercise
9. Anamnestic findings that contraindicates participation in the study
10. Unable to allocate the needed time to fulfill the intervention
11. Language barrier, mental incapacity, unwillingness or inability to understand and be able to complete the interventions

Interventions

General description

The general intervention is based on a previous trial and adapted to the aim of this study[23,70]. The active interventions will consist of two main components; 1) increased physical activity and structured exercise and/or 2) a dietary intervention aiming at a weight loss. Whereas there will be no differences in the dietary intervention between the lifestyle groups, the volume of physical activity and structured exercise will be varied according to the frequencies of the structured exercise sessions. The study groups are prescribed:

1) **Control group (CON):** No intervention
2) **Dietary control (DCON):** Dietary intervention (see below)
3) **Moderate Exercise Dose (MED):** Two aerobic training sessions per week of 45-60 min duration and one session per week with combined aerobic (30-35 min) and resistance (30 min) training and a dietary intervention (described below)
4) **High Exercise Dose (HED):** Four aerobic training sessions per week of 45-60 min duration and two sessions per week with combined aerobic (30-35 min) and resistance (30 min) training and a dietary intervention (described below)

Increased exercise doses and dietary intervention

Detailed description of the intervention components.

Dietary intervention and intended weight loss (DCON, MED and HED)

The dietary intervention will be based on the recommendations from the American Diabetes Association (ADA) with increased on macronutrient quality [71]. The macro-nutrient distributions are in line with the current guidelines from the national Diabetes Association and Canadian guidelines, where individualization in macronutrient distribution should lie within the range of 45-60E% carbohydrate, 15-20E% protein and 20-35E% fat [72]. Based on recent reviews with eleven and twelve randomized controlled trials of > four weeks duration[73-75], the diet will include food items with low glycaemic index (GI) or load (GL) diets as they are associated with improved glycaemic control as compared with high GI or GL food items. Additionally, low GI diets are effective in T2D management [76]. Thus, the dietary intervention emphasis will be on low GI and low GL in shape of non-processed foods. Since T2DM is associated with co-morbidities like cardiovascular disease and due to the general consensus that saturated fat intake is related to cardiovascular disease risk [77] thus the dietary plan will aim at reducing saturated fat intake <7E% as proposed by ADA[71]. Both prevention

and a successful management of type 2 diabetes are highly related to diets rich in whole grains, fruits, vegetables, nuts and legumes and lower on refined grains, red or processed meat and sugar sweetened beverages[77], why these will be targeted throughout the study. The broad range of macro-nutrient composition allows for individual preferences.

Procedure: A dietician will prepare individual meal plans based on individual counselling (1 session/month) with proposed recipes (see appendix 1 for an example). The implementation and potential adjustments will be performed continuously based on self-report dietary records and discussed during group sessions (monthly) and at the individual sessions. The meal plans will cover three main meals and three snack meals per day. The content recipes may be adapted to individual participant preferences and recipes will be changed continuously throughout the intervention. Energy requirement will be based on the age-adjusted Oxford equations[78], aiming for a weight loss The participants' body weight is used for calculation of the energy requirement if the body mass index (BMI) is 25 kg/m2. If the BMI is >25, the body weight in the equation is adjusted to equal a BMI=25 kg/m2. On days with training sessions, 200 kcal/day will be added to the energy intake. In case of hypoglycemic events, energy intake will be reassessed. In order to reduce the risk of mild hypoglycaemia, a snack meal just before (100– 200 kcal) and after (200 kcal), and a main meal 2–3 h before a training session will be advised. In case of subjective signs of low blood glucose (hunger, sweating, increased heart rate, feeling uncomfortable, dizziness and confusion), the participants are instructed to eat either one piece of fruit, drink a glass of juice in combination with a piece of rye bread or crisp bread. To facilitate adherence, the participants are allowed to contact the clinical dietician by email once/ week in case of any issues regarding implementation of or concerns about the meal plan.

Table 1 Principles of the dietary intervention. Adapted from Ried-Larsen et al 2015[23].

Principle	Additional comment
Homemade food	Recipes will be available
Limit processed food items	
Include seasonal greens and fruits (minimum 600 g/day)	
Maximum 2 pieces of fruits per day	
Limit the amount of sodium	
Include fish (350 g/week)	200 g should be 'fat' fish e.g. salmon or mackerel
Fibre rich food items (3g/MJ)	
Hot meals should include fish once per week, one vegan meal per week	
Minced meat maximum twice per week	
Hot meals should contain minimum 200g vegetables per meal, max. ¼ of the plate should be meat, max. ¼ of the plate should be high glycaemic index/load food items.	
Ad libitum intake water and tea is allowed	
No sugar sweetened beverages (including soda pops, juice or artificial sweetened beverages	Juice is allowed in case of subjective signs of mild hypoglycaemia in relation to training
Alcohol is discouraged throughout the intervention period	

Increased physical activity and structured exercise

The training protocol will be adapted based on a previous study where the T2D participants were prescribed 6 weekly sessions of aerobic alone or combined aerobic and resistance training (averaging 360-420 min of exercise per week)[23]. Although mean adherence was high (82% of the planned session were completed), variation was high and imperfect[70]. Thus, a lower dose may be expected and thus a moderate dose exercise groups is formed based on the original protocol[23].

Procedure: As in our previous protocol, the target aerobic training intensity span in will be **60-88% of HR_{max}**, which is in line with current guidelines [79,23]. The intensity of the resistance training will also be in line with current guidelines, i.e. 3 bouts per muscle group with intensities varying between 6-15 repetitions maximum[79]. Thus, the exercise program will be adapted as previously described[23]. As previous analyses suggest that there may be an inverse dose-response relationship between reductions in HbA1c and aerobic exercise volume, this parameter will be used to adapt the training protocol[15,17]. As the effect of exercise on HbA1c is closer related to the number of training sessions[17], we will reduce the number of sessions by 50%, to three sessions/week in the moderate exercise dose group and maintain the original session frequency in the high dose exercise group (six sessions/week).

All training sessions will be completed at local fitness centres. All exercise programs are administered by the trainers and individualized on site based on preferences and risk of injuries. The target intensity of the exercise is not individualized based on preferences. An example of a weekly training program (high dose group) is found in Table 2. The initial two weeks of the intervention, a familiarization to the specific exercises will be prioritized to facilitate the training quality (i.e. to meet the prescribed training intensity) in the remaining part of the intervention. During this period the participants will be thoroughly introduced and to the Polar HR watch, training diaries and the concepts of *repetitions in reserve/rate of perceived exertion*.

To ascertain compliance to the intervention and quality of the training, all exercise sessions will be supervised by educated trainers with a medical training, physiotherapist training or exercise physiology training. The educated and experienced trainers will form individual exercise plans on a weekly basis to accommodate individual participant preferences in terms of exercise modality and to avoid potential overuse injuries. Due to the high risk of injuries with running[80], running will not be allowed as an exercise modality. All exercise sessions will be monitored and recorded (intensity: heart rate/resistance/repetitions/modality. Aerobic exercise duration: time see below).

Training will be supervised to ensure intensity and compliance. Furthermore, heart rate, training duration, numbers of repetitions and perceived exhaustion will be monitored at every bout of exercise which will enable us to precisely evaluate the amount of exercise performed by each of the participants.

Table 2 Example of a weekly training program for the high dose group. Adapted from Ried-Larsen et al 2015[23]

Week day	Aerobic training	Resistance training	Notes to the trainers
Monday	Duration: 40 min 1. 5 min warm up at 60-65 % of HR max 2. 20 min at 70-78 % of HR max 3. 15 min at 76-83 % of HR max	Duration: 30 min 1. 5 primary target exercises: Anterior chain (thigh), posterior chain (thigh), chest, back and shoulders. 2. Each target exercise is performed in 3 sets of 10-12 repetition max (RM) 3. You can use machines, free weights, barbells, body weight etc. 4. Active breaks containing core exercise are performed between each set. This means that the pause between each set is replaced with a core training exercise 5. The 5 core exercises should include 3 dynamic abdominal exercises and 2 lower back exercises	Make sure to inform the participant which muscle groups are activated and with time expand their "box" of different exercises. This will help them in the long run and create variety in relation to motivation but also to minimize injuries.
Tuesday	Duration: 60 min 1. 5 min warm up at 60-65 % of HR max 2. 5 min at 70-75 % of HR max 3. 20 min at 74-79 % of HR max 4. 10 min at 80-88 % of HR max 5. 5 min at 70-75 % of HR max 6. 15 min consisting of 2 HR min at 76-80 % of HR max, 2 min at 83-90 % of HR max and 1 min active recovery. Repeat 3 times.		
Wednesday	Duration: 60 min. 1. 5 min warm up at 60-65 % of HR max 2. 10 min at 68-73 % of HR max. 3. 15 min at 75-80 % of HR max 20 min at 77-84 % of HR max. 4. 10 min consisting of 30 sec max effort and 30 sec active recovery.		
Thursday	Duration: 30 min 1. 5 min warm up at 60-65 % of HR max 2. 25 min at 73-83 % of HR max	Duration: 30 min 1. 5 primary target exercises: Anterior chain (thigh), posterior chain (thigh), chest, back and shoulders. 2. Each target exercise is performed in 3 sets of 10-12 RM 3. You can use machines, free weights, barbells, body weight etc. 4. Active breaks containing core exercise are performed between each set. This means that the pause between each set is replaced with a core training exercise 5. The 5 core exercises should include 3 dynamic abdominal exercises and 2 lower back exercises	
Friday	Duration: 60 min 1. 5 min warm up at 60-65 % of HR max		

		2. 15 min at 73-83 % of HR max 40 min consisting of 5 min at 76-82 % of HR max, 3 min towards max and 2 min active recovery. Repeat 4 times.	
Saturday	Rest day		
Sunday		Duration: 60 min 1. 45 min walking 2. 15 min walking/jogging in hills or on stairs.	Duration: 15 min 1. Core training. Free of choice.

Training of Intervention personnel: 4-6 trainers with minimum a bachelor degree in sports science or educated physiotherapists will be recruited for the training intervention and a dietician will be recruited for the dietary intervention. The primary responsibility and working tasks for the intervention staff is to assure compliance with the protocol and prevent loss-to-follow-up. As they will be responsible for motivating participants and making individual adjustments to the participants' training programs in order to reduce the risk of excessive load or injuries previous experience is required. All intervention personnel will attend a adapted (to T2D) certification course based on the ambassador program at provided to health care professional by the sponsor (see http://aktivsundhed.dk/da/ambassadorer). The course is led by the lead investigators, clinicians and psychologists and will contain the following:

- The research protocol
- Disease pathology (type 2 diabetes)
- The intervention: Organizations, exercise, physical activity, diet, sleep and coaching
- Motivation
- Medical issues during intervention (including a course in cardiopulmonary resuscitation)

Mandatory biweekly meetings for all persons (management (clinical and research) and intervention personal) will be enforced to discuss all issues related to the intervention (medical issues, challenges in relation to recruitment, motivation and drop-outs etc.)

Modifications and strategy to maintain and improve compliance

Exercise intervention: For participants in the exercise intervention group, compliance is monitored continuously through the study by the trainers. If any participants complete less than 80% of the training volume prescribed over a 1-week period, procedures to prevent drop-out are initiated. A 1-week vacation will be allowed, where the participants will receive exercise programs that will be feasible to complete at the vacation location. The programs will closely mimic the assigned intervention. In case that the exercise volume is unfeasible during vacation, the volume will be reduced. A specific plan to reach the overall exercise volume (across 16 weeks) will be drafted in collaboration with the participant (e.g. to increase the existing exercise sessions by 10 min over a period until the target volume has been reached).

The drop-out prevention procedures for the exercise session include;

1. The participant is offered an interview with an exercise trainer to help handling the worries and to help manage the time. If the lacking compliance relates to injuries, pain or resistance to exercise modality, the exercise modality may be altered, whereas the exercise intensity will be maintained.
2. If compliance is not corrected/maintain based on action 1 within a week, a temporary adjusted plan is made in collaboration between the trainers and participant with the aim of maintaining the weekly

training volume by reducing the sessions of exercise per week but increase duration (unchanged intensity).

3. If this is not sufficient to correct/maintain compliance, the training volume will be reduced for a short period of time by retracting 1/3 of the exercise sessions per week for 2 weeks. During this procedure a plan to restore the training volume is formed in collaboration between the trainer and the participant.

Dietary intervention: If participants exceed +/- 30% of the prescribed energy intake as assessed by their dietary records the procedures below are initiated. Moreover, if the participant contacts any intervention staff and expresses concerns about satiety, food preferences or food preparation techniques the procedures, described below are initiated. A 1-week vacation will be allowed, where the participants will receive dietary guidance that will be feasible to complete at the vacation location. Moreover, the participant will be asked to complete at dietary record during the vacation, in order to adjust the following dietary program to reach the pre-specified energy intake.

The drop-out prevention procedures for the dietary intervention include;

1) An interview regarding compliance to the meal plan is performed, and the participant is provided with specific guidelines to practical changes in the plan by the clinical dieticians. E.g. to increase adherence to food items increasing satiety or exchange some food items match preferences
2) If action 1 is in sufficient then the energy intake is increased in steps of 100 Kcal/day until the level of satiety is acceptable by the participant.

Adherence assessment

Posture allocation and physical activity behaviours are measured using three axial accelerometer-based physical activity monitors (Axivity AX3, Newcastle, UK). See procedures below. All heart rate profiles are recorded during in the intervention group (not control) (Polar V800, Polar, Holte, Dk). As multiple modalities are allowed to target the muscle groups described in the training plan (Table 2), all participants in the active group receive a sheet with pictograms of possible exercises available in the training center (see appendix 2 for an example). The final sheets are formed when final training locations are identified. The participant will note the resistance, rate of perceived exertion and number for repetitions for each exercise and general notes about the quality and resistance modifications. Moreover, if the participant does not complete or partially complete the session as prescribed, the reasons for that is noted on the sheet. The trainers will randomly check the validity of the self-report during the sessions. At the end of the sessions the sheets are collected by the trainers and stored at CFAS for later data management. The participants are moreover asked to complete weighed dietary records during the intervention. See procedures below.

Control intervention:

The control group will be instructed to maintain regular physical activity and diet behaviors throughout the study. No additional intervention is introduced.

Concomitant care (all participants)

Pharmacological treatment procedures and algorithm:

In order to avoid any interferences of drugs or exercise on the various measurements all glucose lowering drugs and exercise will be discontinued (48 hours) to visit 1, 2, 6 and 7. In relation to visits 0, -1, 4 and 12 HbA1c, cholesterol, and home blood pressure monitoring will be reviewed. Glucose-lowering,

antihypertensive, may be changed or discontinued to e.g. avoid hypotensive or hypoglycemic events (see procedures below). With regard to lipid lowering treatment patient will continue any given dosage unless LDL provided LDL cholesterol is >2,5 mmol/L at inclusion, in which case dosage of statin will be increased, e.g. atorvastatin 10 mg to 20 mg or 20 mg to 40 mg, and then the patient will continue on this dosage throughout the study. The clinical responsibility of the pharmacological T2D treatment will be transferred from the patient general practitioner (GP) to the trial endocrinologist (Consultant diabetologist Thomas Peter Almdal) throughout the study period. The participant GP will be informed about study participation and procedure and encouraged to contact the study nurse in case of questions (see appendix 3 for letter to GP). During the intervention (at 0, 4 and 12 weeks follow-up) the study nurse presents anonymized data (HbA1c, triglyceride, low density lipoprotein and total cholesterol, home-based diastolic and systolic blood pressure [18 home-based resting measurements across three days] to the trial endocrinologist, who will adjust the medical treatment according to the scheme shown below. If the patients report symptoms, between these three scheduled visits, the trial endocrinologist will be allowed to adjust the medical treatment based on clinical parameters and the symptoms (see procedures below). The trial endocrinologist will be blinded to group allocation, but all necessary information in relation to medical treatment, medical history and adverse events will be provided through the study nurse. If considered necessary, the blinding will be repealed, and the participant will be contacted directly by the trial endocrinologist. If the participant receives GLP-1 receptor agonists, Pioglitazones or Sulphonyl urea class drugs at baseline, the participant is transferred to the pharmacological treatment described in the algorithm below. To avoid this transfer to mask the intervention effects, the inclusion and exclusion criteria is re-assessed 6 weeks later before attending the baseline measurements.

In case of premature termination (due to any reason), the participant will be offered a consultation aiming at adjusting the pharmacological therapy if needed and the responsibility for the pharmacological therapy will be transferred back to the GP. The participant will be advised to contact their GP. Participants completing the trial, will have readjusted their pharmacological therapy based on the current guidelines following completion and the responsibility for the pharmacological therapy will be transferred back to the GP[81]. Whenever the participants are transferred back to the GP a letter will be send and the participants will be strongly encouraged to contact their GP upon completion of the trial.

Safety criteria and rescue medication:

An experienced endocrinologist will be in charge of the regulation of medication in accordance with the pre-defined algorithms mentioned below. Participants will in details be informed about side effects as well as subjective signs of increased hypo- or hyperglycaemia (thirst, fatigue, polyuria, confusion) or hypotension (dizziness, especially at raising, confusion and fatigue) and urged to contact the study nurse in case of any adverse symptoms. Safety criteria include adverse events, health related outcomes (for instance episodes of angina pectoris or signs of cardiac arrhythmias) and participant-reported hypoglycemic episodes (plasma glucose < 4mmol/l). Minor hypoglycemic episodes are defined as those that can be self-treated; major episodes are defined as plasma glucose <3mmol/l or episodes requiring third-party assistance or medical intervention. In case of unacceptable adverse effects, medication is changed according to titration described below.

In case of patient-reported (see above) symptoms of hypo-, or hyperglycemia or hypotension, the patients are asked to complete glucose (3 consecutive days: morning (fasting), before evening meal and 2 hours after evening meal (postprandial)) measurements or BP (18 home-based resting measurements across three days) profiles. The profiles will be reviewed by the nurse and presented to the study endocrinologist in a blinded and stepwise manner (see algorithm below). The endocrinologist will adjust the medications based on the algorithm below. All events are registered in the database.

Rescue pharmacological algorithm and titration:

According to Danish national guidelines (https://vejledninger.dsam.dk/media/files/4/guidelines-2018-final.pdf) the goal in relation to glucose, blood pressure, and LDL cholesterol are as follows:

	Glucose	Blood pressure	Lipids – LDL Cholesterol
Goal	HbAc < 48 mmol/mol	130 / 80 mmHg	2,5 mmol/l

These goals will also apply to patient included in the DOSE-EX Trial, as it did in the U-Turn trial[22].

Based on the results reported from the U-TURN and Direct trial[58], is expected that a HbA1c1 in the HED and possibly in the MED group on average will be decreased by 5 – 7 mmol/mol in the period from week 0 – 12, and in the same period a modest reduction in blood pressure is expected. In the DIRECT trial the average number of prescribed antihypertensive medications decreased from 1.1 to 0.5 in the intervention group. At weeks -2, 4 and 12 it is pre-planned to measure HbA1c and blood pressure and medical treatment will adjusted in order to reach HbA1c and BP goals as given below.

Medication	Treatment goals*	Intensification of treatment at inclusion and follow-ups	Reduction in medication at inclusion and follow-ups
Glucose lowering /Antidiabetics	HbA1c = 48 mmol/mol	HbAc = 53 mmol/mol	HbA1c < 48 mmol/mol
	Fasting glucose < 7 mmol/l *Postprandial glucose < 7,8 mmol/l*	*Fasting BG > 7,0 mmol/l* *Postprandial PG > 11 mmol/l*	*Fasting BG < 7 mmol/l and* *Postprandial BG < 7,8 mmol/l*
Antihypertensive	BP ≤130/80 mmHg	BP >140/85 mmHg	BP < 125 / 75 mmHg
	BP ≤130/80 mmHg	*BP >140/85 mmHg*	*BP < 125 / 75 mmHg*

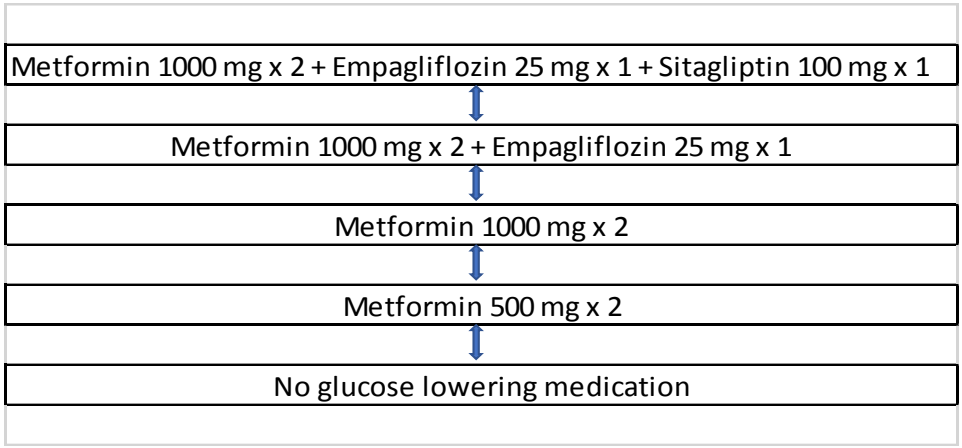
*) Goals given normal letters at used in relation to scheduled visits at weeks -2, 4 and 12, whereas goals given in italic are used in case the patient reports symptoms, and hypo or hyper-glycemia or hypo- or hypertension are documented

Algorithm for reduction in glucose lowering treatment:

It is anticipated that the patient at when seen the first time is treated with none or 1 to 3 glucose lowering drugs given as tablets, one of them being metformin. The two will be changed so that these will include Empagliflozin and Sitagliptin. Thus, the patients at inclusion and during the trial can be treated on any of the 5 below mentioned steps, and at weeks 4 and 12 they can change from one step to another.

1. Metformin 1000 mg x 2 + 2 two more glucose lowering drugs (Empagliflozin 25 mg x 1, and Sitagliptin 100 mg. Patients treated with sulfonylureas at inclusion will be switched to one (or both) of the two above mentioned drugs)

2. Metformin 1000 mg x 2 + 1 or two more glucose lowering drugs (First line drug will be Empagliflozin 25 mg x 1, 2nd line drug will be Sitagliptin 100 mg. Patients treated with sulfonylureas at inclusion will be switched to one of the two above mentioned drugs)
3. Metformin 1000 mg x 2
4. Metformin 500 mg x 2
5. Stop glucose lowering medication



Algorithm for reduction/intensification of antihypertensive treatment:

It is anticipated that the patient at inclusion will be treated with 1–3 agents from the following classes of antihypertensives; AT-2 or ACE inhibitors, thiazide diuretics, and Calcium channel blockers. If this is not the case the medication will be adjusted to comprise the 3 agents. If only 1 antihypertensive drug is given this must be AT-2 or ACE inhibitors. Based on BP measured at 18 home-based measurements done immediately before weeks - 2, 4 and 12, antihypertensive medication may be continued unchanged, may be reduced or may be intensified in a stepwise manner corresponding to the steps showed for glucose lowering drugs. The steps are:

1. Losartan / Enalapril 100 mg / 20 mg or equivalent dosages of any other AT-2 antagonist or ACE inhibitor + 2 other antihypertensives (a Calcium channel blocker and a thiazide diuretic in recommended dosages)

2. Losartan / Enalapril 100 mg / 20 mg or equivalent dosages of any other AT-2 antagonist or ACE inhibitor + 1 other antihypertensive drug (can be either a Calcium channel blocker or a thiazide diuretic)

3. Losartan / Enalapril 100 mg / 20 mg or equivalent dosages of any other AT-2 antagonist or ACE inhibitor

4. Losartan / Enalapril 50 mg / 10 mg or equivalent dosages of any other AT-2 antagonist or ACE inhibitor

Prohibited medications

Biological medicine, beta blockers, glitazones, anti-psychotic medicine, lithium or metabolism disorder treatment. Anti-cancer treatment, daily use of systemic- or local glucocorticoids. Furthermore, no regular or daily use of non-steroid anti-inflammatory drugs. Also, no daily use of proton pump inhibitors. The day prior to visits 1 and 6 no intake of paracetamol is allowed.

Outcomes

Primary outcome

- The change in the late-phase disposition index (DI) from baseline (0 weeks) to follow-up (16 weeks) during the final 30 minutes of hyperglycemic phase of the hyperglycemic clamp. The late-phase DI is defined as the insulin secretion rate (ISR) divided by blood glucose concentration multiplied by the insulin sensitivity index (S_I) ((ISR/glucose)×S_I). The DI will form basis for hierarchical co-primary outcomes assessment based on per protocol as the differences in change of the DI between study groups;
    1. CON vs. HED
    2. CON vs. MED
    3. DCON vs. CON
    4. DCON vs. HED
    5. DCON vs. MED
    6. MED vs. HED
- The data collection in the *per-protocol population* is independent of group allocation. The per protocol population is defined as participants (all criteria present):
    1. CON
        - The primary outcome is assessed at both baseline and after 16 weeks follow-up should be available for analysis for both groups (i.e. complete case).
    2. DCON
        - The primary outcome is assessed at both baseline and after 16 weeks follow-up should be available for analysis for both groups (i.e. complete case).
        - Do not exceed +/- 30% of the prescribed energy intake as assessed by their dietary records (assessed as the mean energy intake mean across that latter 12 weeks, excluding 1-week vacation administered following week 2 of the intervention)
    3. MED and HED
        - The primary outcome is assessed at both baseline and after 16 weeks follow-up should be available for analysis for both groups (i.e. complete case).
        - >=70% of the prescribed exercise volume across the intervention period (excluding weeks 1, 2 + 1-week vacation administered following week 2 of the intervention).
        - Do not exceed +/- 30% of the prescribed energy intake as assessed by their dietary records (assessed as the mean energy intake across that latter 12 weeks, excluding 1-week vacation administered following week 2 of the intervention)

Key secondary outcome

- Intention-to-treat analyses of the primary outcome.

Secondary outcomes (difference in changes between groups from baseline to 16 weeks follow-up)

- Secondary measures of pancreatic α- and β-cell function
    - GLP-1 stimulated insulin, C-peptide and glucagon secretion
    - Arginine stimulated insulin, C-peptide and glucagon secretion
    - 1st phase C-peptide and insulin secretion defined as the peak concentration during the initial 10 minutes of the hyperglycaemic clamp
    - Late-phase S_I (mean Glucose infusion rate over last 30 min of clamp phase/(mean insulin×glucose))

  - Early phase DI (DI from 0-30 minutes)
  - Rate of glucose appearance (R_a)
  - Rate of glucose disappearance (R_d)
- Glycaemic control parameters derived from 4-7 days free-living continuous glucose monitoring
  - Mean amplitude of glycemic excursions will be calculated based on min 3 days sensor glucose profiles. MAGE is calculated by taking the arithmetic mean of the blood glucose increases or decreases (from blood glucose nadirs to peaks or vice versa) when both ascending and descending segments exceed the value of 1 SD of the blood glucose during a 24-hour measurement period[82,83]
  - Coefficient of variation defined as (mean glucose/the standard deviation (SD)) of min 3 days sensor glucose profiles
  - The SD of the glucose from min 3 days sensor glucose profiles
  - The mean glucose from min 3 days sensor glucose profiles
  - Time in hyperglycaemia defined
  - Time in hypoglycaemia defined
- Body composition and lipid deposition
  - Pancreatic fat deposition (MRS)
  - Hepatic fat deposition (MRS)
  - Visceral abdominal fat mass
  - Body composition (body weight, body mass index, lean body mass, total fat mass)
- Clinical, functional, metabolic and urinary metabolic markers of mechanisms
  - Urinary systemic markers of oxidative stress (8-oxo-Guo and 8-oxo-dG)
  - Circulating advanced glycation end-products (AGE),
  - Circulating receptor for AGE (sRAGE)
  - Circulating inflammatory markers (high sensitive C-reactive protein, interferon-ϒ, IL-10, IL-8, IL-6, IL1, TNFα).
  - Haemoglobin 1Ac (HbA1c)
  - Lipoedema (total cholesterol, Triglyceride, low and high density lipoprotein)
  - Blood pressure (Resting systolic and diastolic blood pressure)
  - Glycemic control during mixed meal tolerance test (iAUC, tAUC of glucose, insulin, glucagon and C-peptide)
  - Circulating markers of appetite regulation
  - Rate of gastric emptying
  - Orthostatic tolerance
  - Blood volume
- Physical fitness
  - Maximal aerobic capacity (VO_2 peak)
  - One repetition maximum (RM) strength
- Patient reported outcomes
  - Physical and mental well-being
  - Quality of life
  - Satiety

Exploratory outcomes

- Outcomes from muscle and fat biopsies

  - Mitochondrial density
  - Mitochondrial function
  - mRNA
  - Protein expression
  - Inflammation
  - Metabolic signalling
- Metabolomics and proteomics from urine and blood samples
- Circulating biomarkers of organ and/or arterial function

Sample size considerations

Based on a previous study with an aerobic exercise volume similar to the guidelines (moderate dose group) in a T2D with short T2D duration, it is expected that an exercise intervention will increase late-phase disposition index derived from a hyperglycemic clamp by 1.5 (au.) more than the control group, with a standard deviation of 1.5 (au.) of the change in the exercise and 1.0 (au.) in the control group[41]. For a contrast in a one-way ANOVA with four means (1.5, 1, 0.5, 0) and contrast coefficients (1, 0, 0, -1) using a two-sided significance level of 0.05, assuming an error standard deviation of 1.5 and a balanced design, a total sample size of 80 yield a power of 0.877. Thus, to obtain a sufficient statistical power up to 20 participants are recruited per group (N=80 in total).

Recruitment

Recruitment is initiated upon approval from the Regional Scientific Committee and is open until N=80 T2D patients are randomly allocated or until 01-12-2021, whichever is reached first. To prevent screen failures, participants are recruited and screened for eligibility in three separate steps (described below).

Step 1 Individuals will be recruited in collaboration with Center for Diabetes, Municipality of Copenhagen, Steno Diabetes Center Sealand (contact person Director Lise Tarnow), The Danish Diabetes Association (Diabetesforeningen) and through advertisements in media (e.g. newspaper, television, radio), at social media (e.g. facebook), and by the internet, posters, and flyers in local areas. Interested persons will contact the project investigators by either the CFAS/CIM website, email, mail or by phone. Persons will then receive "information for participants" by mail with an encouraging note to read the material within two days. The project investigators will, by phone, give oral information about the study, ask whether the subject has any question to the written or oral information, and perform a screening of the eligibility criteria. All possible participants will be informed about the possibility of inviting a private counsellor to an information meeting and all possible participants have the possibility to reconsider participation for at least 24 hours before signing the informed consent. If the interested person is eligible for inclusion and oral and written informed consent is provided, the person is included and a blood and urine screening is booked at the laboratory (step 2) and the baseline screening visits (visit 1-3) are planned. The person will be informed about the possibility to contact the responsible study personnel in case of questions and information from the phone screening will not be used for later evaluation in the project.
If inclusion criteria are not met, the person is informed hereof and the process is discontinued.
If an information meeting is inquired it will take place in a closed room with only the project investigator, the potential participant and potentially the private counsellor will be present. Once again, the interested persons will be given the chance to consider engagement in the study before signing the informed consent. Following this procedure, the person is included in the study.

Step 2 Included participants are invited to Rigshospitalet (department of clinical biochemistry, 5001) for an initial blood test and urine screening. The blood- and urine test are performed to identify potential exclusion criteria not identified in step 1. In theory, this screening can also identify unknown disease (e.g. kidney, liver or thyroid disease). In such case the participant will be excluded and referred to his/her GP.

Step 3 Medical history and medical examination will be conducted at the beginning of the first baseline screening visit at CFAS (visit 1). Medical history will be obtained based on a relevant screening program and physical examination including electrocardiogram (ECG), blood pressure measurement, cardio-pulmonary stethoscopy and foot examination will be performed by a MD. If the medical history or examination reveals contraindications for further participation the participant will be inform hereof and excluded. Following this step, the participant will undergo baseline screenings and receive allocation.

Allocation

Participants are randomly allocated following successful completion of the baseline measurements. An independent statistician generates a computer-generated randomization schedule in a ratio of 1:1:1:1 stratified by sex. In order to ascertain concealment, the block sizes will not be disclosed. The schedule is forwarded to a secretary not involved in any study procedures and stored on a password protected computer. Following the baseline measurements, the participants are given consecutive numbers which is forwarded to the secretary who subsequently returns the corresponding allocation to a study nurse not involved with any testing procedures. The study nurse will then assign the allocation to the participant in person.

Blinding

Participants are blinded for treatment allocation until group assignment. However, following the baseline assessment blinding of the participants is no longer possible. All study personal involved with data collection will be blinded throughout the study. Participants are not allowed to discuss group allocation while participating in the assessments. The endocrinologist assessing the need for medication and safety will be blinded to allocation. The clinical values will be presented to the endocrinologist by a study nurse without disclosing treatment allocation.

To obtain a blinded outcome assessment the following procedure is enforced. Upon completion of the study and prior to breaking the allocation code, a data-collection form is generated by a statistician (RC) and the PI (MR-L). The data-analyst breaks the allocation code and labels the participants according to the assigned treatment and analyzes the outcomes. Following the analyses, group allocation will be concealed in all data outputs and the N per group and present the data to the writing committee in a blinded fashion. Then the writing committee will provide their blinded interpretations.

Emergency un-blinding

As all necessary information about intervention, medical history and adverse events can be provided to the endocrinologist by the study nurse, the blinding of the endocrinologist can be repealed if considered necessary.

Data collection, management and analysis

The participant timeline for outcomes assessment are described in Table 3 and the procedures are described below.

Table 3 Participant timeline										
	Week	-3	-2	-1	-1	4	12	17	18	18
	Appropriate staff	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Primary outcome										
Hyperglycemic clamp	Study MD			X					X	
Secondary and exploratory outcomes										
Clinical examination and history	Study MD		X					X		
Clinical blood sampling	Study nurse	X	X			X	X	X		
Urine sampling	All team members	X	X			X	X	X		
Mixed meal tolerance test	Study nurse		X					X		
Continous glucose monitoring	All team members		X					X		
Muscle and fat biopsies	Study MD			X					X	
Cardio-vascular procedures										
Home blood pressure	All team members		X			X	X	X		
Tilt-table	All team members		X					X		
CO-rebreathing	All team members		X					X		
Body composition										
Magnetic resonsance imaging	Radiologist				X					X
Magnetic resonsance spectroscopy	Radiologist				X					X
Dual-energy x-ray absorptiometry	All team members		X					X		
Physical function										
Cardio-respiratory fitness	All team members		X	X				X		
Muscular strengt	All team members		X					X		
Physical activity behavior	All team members		X			X	X	X		
Patient reported outcomes										
Mental and physical well-being	All team members			X					X	
Quality of life	All team members			X					X	
Satiety	Study nurse		X					X		
Dietary records	All team members		X			X	X	X		

MD: Medical doctor, CO: Carbon mono-oxide

Data collection methods

Training and certification plans: All test personal will receive extensive training in all relevant standard operating procedures. Moreover, relevant staff will receive training and certification in DXA scanning. The training will be organized and executed by expert staff members.

Primary outcome

The hyperglycemic clamp

For the 3-stage hyperglycemic clamp an antecubital venous catheter is placed for infusion. For blood sampling a retrograde venous catheter is placed in the contralateral hand and kept warm with a heating blanket. After baseline blood sampling (t=-120 min.) a primed, continuous [6,6-^{2}H2]glucose infusion and a primed, continuous palmitate and glycerol infusion is started and maintained throughout the duration of the clamp in order to assess rate of appearance and disappearance of glucose and FFA during the procedure.

At t=0 the hyperglycaemic stage is initiated. We aim to increase baseline glucose concentration by 5.4mM above fasting level by a square-ware glucose infusion lasting 15 min. After this, glucose concentration will be kept constant and glucose infusion rates (GIR) will be adjusted based on blood glucose measurements (ABL 8 series, Radiometer, Denmark) obtained every 5th min according to an automated algorithm based on[41]. At t=120 min, the hyperglycemic+GLP1 stage is commenced by infusing a primed (0.2 pmol/kg), continuous GLP-1 infusion mimicking postprandial levels in healthy individuals. At t=240 the arginine stage is commenced with injection of arginine hydrochloride (5g given over 30 s) to assess maximal insulin secretory capacity. At t=280 min, the clamp is terminated. Following the termination, participants will be monitored with blood glucose measurements for 30-60 min into to safe recovery.

Secondary and explorative outcomes

Clinical examination, clinical blood, and urine sampling

A medical history and examination (vital parameters (HR, BP), weight, height, waist and hip circumference, ECG) will be measured by standard procedures. Moreover, socio-demographic information; education, age, ethnicity, civil status, occupation, smoking status and alcohol consumption will be collected. Blood sampling will be conducted at all visits by standard procedures. The blood samples (25 ml) will be analysed immediately for serum concentration of cholesterols, triglycerides, glucose, C-peptide, insulin and HbA1c, haematology, electrolytes, metabolites, liver- and renal status, endocrinology (including HCG if relevant) at Clinical Biochemical department 3011 (Rigshospitalet). Participants are asked to collect urine samples throughout the study. They will be provided a spot urine-sampling kit to use at home prior to visit 1, 4, 6 and 7. The samples should be obtained no more than 2 days before the visit and frozen immediately after collection. Urine will be used to measure 8-oxo-Guo and 8-oxo-dG using a validated method of ultra-performance liquid chromatography and tandem mass spectrometry[84].

Mixed meal tolerance test

To evaluate the postprandial glucose metabolism and gastric emptying time a standard 3-hour mixed meal tolerance test (MMTT) with the addition of 1.5 g paracetamol will be performed at baseline and after the training intervention. Serial blood samples will be drawn at baseline, at 0, 15, 30, 60, 90, 120, 150, 180 min after intake of 360 ml of a liquid meal (E%: 55/30/15, respectively carbohydrate/fat/protein). Blood samples will be collected in relevant tubes and analyzed for GLP-1 (active and total), glucagon, GIP, Leptin, Ghrelin, PYY, insulin, pro-insulin, C-peptide, glucose, and rate of gastric emptying. The rate of gastric emptying will be calculated as previously reported[85].

Continuous glucose monitoring (CGM)
A blinded CGM sensor (iPro2, Medtronic, Copenhagen, Denmark) will be inserted in the subcutaneous adipose tissue on the lower part of the abdomen (under the umbilicus). Blinded CGM devices are considered minimally invasive. Enzyme-coated electrodes are used to measure interstitial glucose concentrations. Using an applicator device, a thin plastic sensor is inserted just under the skin of the abdomen. The information stored in the receiver is converted into estimated profiles of glucose. The measured glucose values will be standardized to capillary blood glucose levels measured during calibration, with calibrations performed by the subject 4 times a day. Thus, the participants are asked report home glucose levels from measurements prior to breakfast, before lunch, before main evening meal and before bedtime using a glucometer (Contour XT, Ascensia Diabetes Care Denmark ApS, Copenhagen, Denmark). The receiver can store information for up to 7 days. The values are downloaded in the laboratory and stored for later analyses and calculation of outcome measures.

Biopsies
The muscle biopsy (approx. 2-300 mg, will be obtained with Bergstrøm needle from m. vastus lateralis. 5 ml of lidocaine (20 mg/ml) will be administrated as local anaesthetic before the biopsy. The wound will be closed with patches and is expected to heal within days. Immediately after the biopsy, we will isolate muscle progenitor cells. After isolation we will use immunomagnetic sorting (MACS) of CD56+ cells to ensure that the cell population is a pure fraction of myoblasts. Single cell capture, specific reverse transcription of miRNAs and cDNA pre-amplification will be performed using the Fluidigm® C1™ System.

The abdominal subcutaneous fat biopsies (approx. 1-200 mg), will also be obtained by Begstrøms needle from the subcutaneous adipose tissue on the abdomen after administrating 2 ml lidocain (20 mg/ml) as local anaesthetic. The wounds will be closed with patches and is expected to heal within days. Qualified health professionals will take the biopsies under sterile conditions.
Subjects are only allowed to receive acetylsalicylic acid (e.g. Magnyl) or dipyridamol as antiplatelet therapy and do not have to pause their treatment prior to biopsy.
Thus, subjects taking anticoagulants or other blood thinners will not be offered muscle – or fat biopsies due to risk of bleeding. On the day that the biopsies are taken, the subjects will be handed a physical – and easy-read pamphlet where a plan of action is described should there be signs of infection or bleeding.

Cardiovascular procedures

Blood volume (BV) status
Hemoglobin mass (Hbmass) will be determined using a carbon monoxide (CO) rebreathing technique with a typical error of 1.0 %, as previously described[86]. In brief, all individuals will rest for 20 min in the supine position before each measurement. During this time, a catheter will be inserted in an antecubital vein. Subsequently, individuals will breath 100 % O2 for 4 min to flush nitrogen from the airways. At 3 min of O_2 breathing, 2 ml of blood will be sampled and analyzed immediately in triplicates for percentage carboxyhaemoglobin (% HbCO) and hemoglobin concentration [Hb] (ABL800, Radiometer, Denmark) at CFAS. Then, a bolus of 1.2 ml kg^{-1} body weight of 99.997 % chemically pure CO (CO N47, Air Liquide, France) will be administrated into the breathing circuit. Individuals will re-breath this gas mixture for 10 min in a closed circuit where O_2 is added on a demand basis. An additional 2 ml blood sample will be obtained and analyzed in triplicates at CFAS. The change in % HbCO will be used to calculate Hbmass. Total RBCV, PV and BV will be derived from measures of Hbmass and hematocrit[86,87].

Orthostatic tolerance test

Following 30 min rest in the supine position, individuals will be moderately tilted to -20, -10, 0, +10, +20, +30 and +40 degrees each step holding for 10 min[88]. A bicycle saddle and a harness will be installed to gently hold their body weight throughout the tilting protocol. Mean arterial pressure (MAP) will be obtained during the last three minutes of each stage using Finometer PRO (Finapres, Medical Systems, Netherlands) with data exported into acquision software (Labchart 7, AD Instruments, UK).

Body composition

Abdominal Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS)

MRI and MRS were performed using a Siemens Magnetom Prisma 3 Tesla matrix magnetic resonance scanner (Erlangen, Germany) at 3 mm intervals. All adipose tissue located from the diaphragm to pelvic floor inside the peritoneum is traced manually as the visceral fat region of interest. MRS to assess liver and pancreatic fat is performed based on the MRI and analyzed as described elsewhere[89]. The MRI will be performed at Rigshospitalet (dept. 3024) by a trained radiologist. Relevant software will be used for post-processing. All MR scans will be analysed in a blinded manner.

Body composition

Dual x-ray absorptiometry will be used to assess body fat and lean mass before, during and after the intervention. To distinguish between subcutaneous adipose tissue and visceral adipose tissue in the android region, additional software (enCORE software version 13.6.) will be used.

Physical function

Maximal aerobic capacity

The participants will undergo a maximal graded exercise test on a bicycle ergometer for evaluation of cardiovascular function and determination of the peak oxygen uptake (VO2peak). The test starts with a 5 min warm up at 70 watts (the watts may have to be adjusted to the individual fitness level). Warm up is immediately followed by a 15 watt increase every 1 min until exhaustion. Ventilation and expired gases will be measured during the test via an indirect calorimetric system, and heart rate will be assessed simultaneously. From watt-max the maximal aerobic work capacity will be calculated using ACSM standard metabolic calculation. The test will be continued until the following criteria were met: plateauing of heart rate and VO_2 with incremental workloads, respiratory exchange ratio > 1.1 [90]. Oxygen consumption will be assessed using continuous indirect calorimetric measurements (CPET, Cosmed, Italy) and heart rate monitoring (Polar Electro, Holte, DK).

Muscle strength

Maximum muscle strength will be assessed in four functional exercises performed in resistance training machines (leg press, chest press, leg extension, and seated row) by 1-repetition maximum (1-RM). Participants will be positioned correctly in machine and will perform a light warm-up (8 repetitions on a light load). Following a short rest, a progressive loading procedure is performed (i.e. a higher load is selected and 1 repetition is performed, after another short rest, an even higher load is tested for 1 repetition) until the single repetition cannot be completed in good form. The load of the last successful repetition will be recorded as the 1-RM.

Physical activity

All participants will be equipped with two accelerometers (AX3, Axivity, Newcastle, UK) for 4-6 consecutive days. One accelerometer placed on the right thigh, and one accelerometer placed on the right side of the lower

back. Both accelerometers will be attached on the participant with a patch (Fixomull stretch, BSN medical, Germany). Posture allocation will be determined by orientation of the thigh-mounted accelerometer, while intensity of physical activity will be determined by the accelerometer counts of the hip mounted accelerometer. For a given measurement sequence, it will be possible to determine time stamps on posture allocation, duration of sedentary periods, breaks in sedentary time, and the intensity of physical activity during breaks in sedentary time. Using the AX3 to monitor posture allocation and basic physical activity behaviour will take place four times (before, during (week 4 and 12) and after the intervention).

Patient reported outcomes

Mental and physical well-being from the Short Form 36 (SF-36)

The SF-36 is a short-form health survey with 36 questions. It yields an 8-scale profile of functional health and well-being scores as well as psychometrically-based physical and mental health summary measures and a preference-based health utility index [91].

Diet record

A self-reported three-day record of their total dietary intake will be obtained at baseline, during (at week 3, 6, 9 and 12) in the intervention period, and after the intervention. On the basis of these records the energy intake of each participant will be estimated and used to adjust the dietary intervention.

Satiety

Satiety is recorded during the MMTT (immediately before, 60 min into the test and immediately after the test) using a 5 item visual analogue scale (VAS). The participants are asked to rate immediate sensation of hunger, sensation of thirst, desire to eat, nausea and fullness.

Biological specimens and research biobank

Separate blood samples (20 mL) from the clinical examinations will be aliquoted, frozen at and stored at -80°C a project specific biobank at (CFAS) 7641 (Rigshospitalet) for future analyses of measure AGE, sRAGE, inflammatory markers, hormones and biomarkers of disease progression and organ function.

During the hyperglycemic clamp separate blood samples (232 mL mL) are aliquoted, frozen at and stored at -80°C a project specific biobank at (CFAS) 7641 (Rigshospitalet) for future analyses of for future analyses of inflammatory markers, C-peptide, insulin, glucose, paracetamol, cholesterols, triglycerides, free fatty acids, glucagon, incretins, metabolomics and proteomics. Total blood volume loss during the hyperglycemic clamp from blood samples and blood waste is estimated to be approximately 280 mL

During the MMTT separate blood samples (140 mL) are aliquoted, frozen at and stored at -80°C a project specific biobank at (CFAS) 7641 (Rigshospitalet) for future analyses of inflammatory markers, C-peptide, insulin, glucose, paracetamol, cholesterols, triglycerides, free fatty acids, glucagon, incretins, metabolomics and proteomics.

Urine samples (15 mL) are frozen at -80°C until analysis. Remaining muscle tissue (~250 mg) is immediately frozen in liquid nitrogen and stored for batch isolation of mRNA and protein. Gene expression analyses will be conducted for regulation of protein degeneration and regeneration signaling, as well as muscle specific pathways. Protein expression analyses will be performed to elucidate related pathways in muscle degeneration, regeneration and oxidative stress. Adipose tissue biopsies (100-200 mg) are immediately frozen in liquid nitrogen and stored for batch isolation of mRNA and protein. Gene expression analyses will be conducted for markers of immune cell infiltration, inflammatory cytokines, adipose tissue browning, and

regulation of cellular metabolism. Similarly, protein expression analyses will be performed to elucidate browning, inflammatory and metabolic signaling.

Finally, we will ask participants to give separate consent a blood sample (10 ml) which will be frozen and stored for non-specified future research until termination of the study (2028). Before future analyses are performed of these samples, we will apply local ethical committee for approval. Any excess biological material will be stored for 20 years (01.11.2038) and then be transferred to CFAS' biobank registered with Datatilsynet (RH-2017-60 I-suite nr: 05329). Biological material will not be exchanged with researchers in other countries unless reported to and approved by the ethical committee.

Retention

All participants will receive up to DKr. 4.000 Dkr (€530) to cover lost earnings, transport and discomfort. The transaction is completed upon completion of the study (all four full laboratory days (v1, v2, v6 and v7) or upon withdrawal). For every completed full day of laboratory testing, participants will receive 1.000 DKr. Moreover, 500 Dkr. in compensation will be added per biopsy (up to 4 in total). To prevent loss-to-follow-up amongst participants in the CON, they are offered a standard care lifestyle intervention program, provided by the Municipality of Copenhagen, Center For Diabetes. All participants are allowed to contact the study nurse by phone in case of project related questions (e.g. pharmacological treatment, sports injuries etc.).

Data management

Data forms, entry, transmission, entry and editing

The web-based Clinical Trial Management System EasyTrial is used for data entry and management (EasyTrial ApS). EasyTrial has been approved by the Danish Data Protection Board. Electronic case reports forms (eCRF) and questionnaires have been generated by the sponsor in EasyTrial. Fields have been programmed with acceptable ranges for data entry. EasyTrial is also used to send reminders to the participants prior to visits and to remind participants in the exercise intervention group to upload heart rate data for supervision. During the study, data are entered directly into the system by the investigators, and after study completion data will be extracted directly from the system by the sponsor/investigators and stored behind a firewall on a secure served with logging of activity retrospectively for 6 months. The servers are backed up continuously.

All paper material (CRF, blood screen results, questionnaires and dietary records) is collected and store in a locked cabinet on CFAS, Rigshospitalet Denmark. All information from the paper material is entered (twice by in non-consecutive order) into the electronic back-end system. Consistency is checked using appropriate software. In case of discrepancies between the entries, the original paper record is consulted. Upon completion of the study all paper material is scanned and store on the secured hospital server in an electronic form. All paper material, except for the consent form, is the destroyed. Data management is performed using appropriate statistical software.

Statistical methods

Analysis of the primary outcome

The primary analysis will be based on the set of participants that is as close as possible to the intended intervention protocol (i.e. per protocol – see definition above).

The analyses of the primary outcome will be performed using mixed linear modeling with the mean change score of DI as dependent variable and group (2 levels), sex (2 levels) and the baseline value of DI as independent variables. The assumptions for using the linear models will be checked to confirm normal

distribution of the residual and the homogeneity of the variance (standardized residuals vs. the predicted values).

The primary analysis is based on a hierarchical analytic approach in order to maintain the type 1 error rate. Between group comparisons for effect size estimation ((difference in change from 0-16 weeks, based on a superiority assumption) is completed in the following order;

1) CON vs. HED. If a difference is present ($p<0.05$, 2-sidet) then the next between group comparison is performed. If not – then sequence is terminated
2) CON vs. MED. If a difference is present ($p<0.05$, 2-sidet) then the next between group comparison is performed. If not – then sequence is terminated
3) CON vs. DCON. If a difference is present ($p<0.05$, 2-sidet) then the next between group comparison is performed. If not – then sequence is terminated
4) DCON vs. HED. If a difference is present ($p<0.05$, 2-sidet) then the next between group comparison is performed. If not – then sequence is terminated
5) DCON vs. MED. If a difference is present ($p<0.05$, 2-sidet) then the next between group comparison is performed. If not – then sequence is terminated
6) MED vs. HED.

The assumptions for using the linear models will be checked to confirm normal distribution of the residual and the homogeneity of the variance (standardized residuals vs. the predicted values).

Analyses of the secondary outcomes

The ITT analysis of DI (key secondary outcome) will be based on the *as-observed population* (missing data will not be imputed in the primary analysis)[92]. Thus, the participants are analyzed according to the planned treatment regimen rather than the actual treatment, i.e. irrespective of their compliance with the planned course of treatment. The 'Full Analysis Set' will thus be derived from the set of all randomized participants by minimal and justified elimination of participants. Therefore, all participants allocated to a treatment group (CON, MED or HED) will be followed up, assessed and analyzed as members of that group irrespective of their compliance to the planned course of treatment. Sensitivity analyses will be performed using *baseline observation carried forward* and multiple imputation procedures[92]. Patterns of missing data will be investigated. *A priori*, the less restrictive missing at random (MAR) assumption is considered more reasonable than the missing data be missing completely at random (MCAR). Assuming that the data on potential drop-outs are missing at random multiple imputation procedures would be applicable to handle missing data for all participants with baseline measurements.

Other continuous secondary outcomes assessed pre- and post-intervention will be analyzed analysis of covariance with the mean change score of the variable as dependent variable and group (3 levels), sex (2 levels) and the baseline value of the variable as independent variables. Continuous variables, additionally assessed during the intervention period, are analyzed within the framework of repeated-measures linear mixed models, an analysis of covariance model will be used to analyze mean changes in continuous end points. The model includes treatment (4 levels), time (3 levels), sex (2 levels), and the possible interaction between treatment (group) and time (weeks) as fixed effects, with the baseline value of the relevant variable as a covariate and participant ID as random effect. The assumptions will be investigated as described above. Variables not meeting the model assumptions will be transformed using appropriate transformations. If no suitable transformation is identified, the median change with interquartile ranges will reported and testing is

performed using suitable non-parametric statistical tests (e.g. wilcoxons signed rank tests). Binary outcomes are reported as numbers and proportions and tested using a X^2 test or exact statistics.

Harms, risks and discomforts

Adverse events (AE) and safety evaluation
In this study, we have adopted the ICH definition of adverse event (AE) (E2A).

An AE is thus defined as; "*An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product"*[93].

Serious AE (SAE) is defined as;*"[…] any untoward medical occurrence that at any dose: * results in death, * is life-threatening, NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. * requires inpatient hospitalisation or prolongation of existing hospitalisation, * results in persistent or significant disability/incapacity, or * is a congenital anomaly/birth defect"*[93].

AEs/SAEs (anticipated and unanticipated) will be recorded on adverse event forms. These forms will include a description and classification of the event, date of onset, date resolved, whether the event was serious or not (ICH criteria), relationship of the event to the study (1=none, 2=unlikely, 3=possible, 4=probable, 5=definitely), action taken, and whether the study was suspended or not. All SAEs, will be reported to the Regional Ethical Committee by the sponsor. AEs observed by any investigator and/or reported by the participant must be reported in the source data and case report form from the first (= signature of informed consent) to the last protocol-specific procedure allocation[94].

VO_2-max test and 1RM: Physical fitness and strength tests, where subjects must put in maximal effort. The tests can cause some degree of breathlessness and exhaustion, but is standard methods used for scientific purpose at the CFAS laboratory.

DXA scan: Is not expected to cause any discomfort and involves very little radiation (0.0004mSv) corresponding to approximately 1/10 of the radiation acquired for a thoracic x-ray. The dose is smaller than received when flying in a commercial jet from (11-12 hours) (SST.dk - Strålingsguiden). The DXA scan is considered safe and is part of standard scientific testing at CFAS.

Hyperglycemic clamp: The hyperglycemic clamp combined with GLP-1 infusion and arginine bolus may cause hypoglycaemic symptoms (dizziness, headache and fatigue) following the trial. However, blood glucose will be monitored for up to 1 hour after the test and a meal will be provided when testing has finished. Furthermore, there is a minor risk of infection or hematoma due to blood lines being placed. All researchers are experts in these procedures so the risks are minimal. The hyperglycaemic clamp with GLP-1 and arginine has previously been used for scientific purpose in our laboratory. The introduction of arginine may give the participants a transient metallic taste which is short and fully reversible.

Stable isotope tracers will be used to obtain knowledge about glucose and lipid distribution. The isotopes are not radioactive and are considered safe to use. They have previously been used in the CFAS laboratory and we do not expect any additional discomfort than the ones already described according to the clamp.

Blood sampling: A small peripheral venous catheter (PVS) will be placed and can cause slight discomfort and a small risk of local infection and edema. The blood volume collected (maximum 965 ml/4 months) is considered too small to cause any symptoms.

Biopsies: Prior to the muscle biopsy local anaesthesia will be administered, which is associated with short-lasting discomfort. After a few minutes, the area is numbed and a small skin incision is made. The Bergstrom cannula is inserted in M. vastus lateralis and the biopsy is taken. This part of the procedure might be associated with slight pain. The procedure takes a few minutes and after the biopsy is taken, the wound will be closed with Steri-Strips, which must be kept on for five days. There will be an additional patch, which can be removed after two days. Subjects might experience some degree of muscle pain after the biopsy. Paracetamol (1000 mg) max. four times a day will be recommended as pain killers. The fat biopsy (subcutaneous adipose tissue from the abdomen) is taken following the same procedure; these biopsies in general cause much less discomfort. Complications are rare and usually mild. The procedures can leave small bruises, but generally heals nicely. Temporary decreased sensation at the incision area or where the local anaesthetic has been injected can be seen. Usually, nerve lesions and altered sensation at the incision area heals within months. In theory, infections can appear when piercing the skin. This occurs in 1 out of 25,000 times and can in some cases require treatment with antibiotics. In order to avoid infection all participants will be informed to abstain from swimming in seawater or swimming pools within the first five days. Furthermore, they will be informed to contact a project doctor in case of any signs of infection (heat, redness, swelling or fever).

MRI/MRS: The measurement is pain free. It is based on radio waves and thus the participant is not exposed to x-rays or other sources of radiation. The scan is performed in a tight cylinder which may cause claustrophobia with some participants. The scans are not performed with a specific clinical purpose but rather for quantification of site-specific ectopic adipose tissue. It cannot and will not be used for diagnostic purposes. However, all scans will be screened by a trained radiologist and therefore unexpected abnormalities may be detected. If deemed necessary, the department of radiology (Rigshospitalet, dept. 3024) will help perform further diagnostics if the participant so wishes (according to the consent form).

Continuous glucose monitoring (CGM): The method is safe and routinely used by diabetic patients to continuously monitor the blood glucose level. A tiny electrode (glucose sensor) will be inserted to the subcutaneous tissue at the upper arm and 4 daily peripheral blood samples will be performed for calibration purposes. To this end, there is a small risk of infection and the study participants will be informed and instructed to take action in case of symptoms of infection.

Tilt-table: The inhalation of CO and the attendant increase in % HbCO is the equivalent to the habitation for 12 – 48 hours in a big city. Accordingly, this procedure is not of great risk. The highest HbCO concentrations, which was ever measured with this method by us was 12 % and the lowest HbCO concentration, where they could diagnose small side effect of CO intoxication was over 15 %. Therefore, there are no risks associated with this method. The only notable side-effect will be a mild reduction in exercise capacity. Circulating carboxyhemoglobin and exercise capacity will return to normal levels within ~ 12 hours. As we never perform a subsequent measurement within this time, there is no additive effect.

CO-rebreathing: The orthostatic test can cause pre-syncopal symptoms which include nausea, dizziness and sweating. However, the test is supervised at all times and if any abnormalities appear, the table can be tilted back to the supine position whereupon normal orthostatic function is regained quickly.

Ethics and dissemination

The study is expected to result in minimal discomfort and risk and the participants can discontinue their engagement at any time and without any reason. The study examines the effects of various volumes of physical activity and exercise on glucose levels and pancreatic β-cell function in participants with shorter term T2D.
Both a rapid weight loss through diet and diet/physical activity may induce subjective signs of hypo-glycemia. Thus, procedures to adjust medications in both the Look AHEAD and direct DIRECT studies are described[58,95]. It is thus reasonable to adjust glucose and BP lowering agents with a safety mechanism in a blinded standardized algorithm in all groups. Based on a previous study a large proportion of the exercise/diet groups are expected to discontinue their glucose-lowering and BP lowering medications within the study period without any adverse events[22]. Immediately following the study the clinical parameters are reviewed by the research physicians and the participants are asked to contact their GP with the purpose of continuing their treatment based on the clinical guidelines[81]. The participants receiving DCON/MED/HED groups will benefit from the study in terms of thorough medical examination and increased physical capacity and increased T2D management. Based on previous research from our group, it is expected that a large proportion will maintain or even improve T2D control with this intervention[70]. Moreover, in contrast to the previous study, all exercise session are fully supervised, thus it is expected that compliance to the lifestyle intervention is even higher than previously reported (82%)[70]. The control group will also benefit from an extensive health checkup and achieve insight to basic anti-diabetic lifestyle alterations. After the project has finished all participants will be re-referred to the various activities for patients with type 2 diabetes in their local municipalities. If participants in the control group do not wish or is unable to attend the rehabilitation program, then a dietary plan and an extensive individualized training program (based on the intervention provided in the (DCON/MED/HED groups) will be provided.

The study is important, valuable, sound, and will contribute to the essential knowledge of possible T2D remission induced by non-surgical and non-pharmacological lifestyle intervention. Specifically, this study will improve knowledge about if and how exercise intervention, when administered in concert with diet-induced weight loss may revert of pancreatic β-cell function in patients with T2D and possibly provide a sound alternative to conventional high-risk procedures. Moreover, the study will elucidate the time dependency and causality between the pathophysiological processes of cardiovascular damage and add to the knowledge about how and if exercise intervention will decrease the risk of the micro- and macro vascular complications induced by T2D. In the end the project will be a crucial stepping stone in the process of developing efficient lifestyle interventions with both a curative and secondary prevention purposes in the clinical care of T2D

Participants may withdraw with no obligation to provide a reason. Participants may be withdrawn from the study prior to the expected completion of the study if steering committee decides to discontinue the study (possible at any time for any reason) or the clinical condition of the participant is such that the investigator recommends withdrawal.

Ethical approval will be applied at the Scientific Ethical Committee at the Capital Region of Denmark and the study will be conducted in accordance with the Declaration of Helsinki (1964) with its subsequent revisions. No study procedures (including recruitment procedures) will be initiated until ethical approval has been obtained.

Protocol amendments

Changes to the protocol can be induced by scientific rationale or evidence of potential harms/risks of the intervention or data collection methods. Potential amendments have to be accepted by the DOSE-EX scientific committee before submission to The Scientific Ethical Committee of the Capitol Region of Copenhagen. No amendments to the protocol will be implemented until approval by the ethical committee.

Consent or assent

Trained research personal will provide oral and written information about the project to all possible participants. On basis of an informed discussion the research personal will obtain written and oral informed consent from the participants willing to be part of the trial.

Confidentiality

To anonymize data all participants will be ascribed a unique participant identification (ID) number. The identification key (ID number to personal information) on a password protected computer separate from the unique ID number and the database. All local databases will be secured with passwords and logged. Printed data will be kept in a separate locked area with limited access. All patient-related information obtained during the study will be handled in accordance with the Danish law for protection of personal data ("lov om behandling af personoplysninger") and the Danish health law ("sundhedsloven"). Information will be obtained from patient journals in relation to blood samples that are drawn as part of this study and analyzed by the biochemical department at Rigshospitalet. Moreover, information about medications prescriptions and redemption from inclusion and until completion or discontinuation of the study will be obtained from the patient journals. The blood samples include screening for side effects during the study and a broad medical blood screening in the beginning and end of the study. The blood samples will be registered from the hospital blood sample portal (Labka) and para-clinical observations will be obtained through "Sundhedsportalen". Upon ethical approval, the study will be submitted to the Danish Data Protection Agency ("Region H's paraplyanmeldelse") for approval.

Declaration of interests

The authors declare no conflicts of interest. The funding agency (Trygfonden) has not taken part in protocol drafting and will not take part in completion of the study, data collection nor interpretation or publishing of the data from this trial.

Access to data

All data is the property of CFAS and access to data is overseen by the DOSE-EX steering committee. All members of the DOSE-EX study group have access to the anonymized cleaned dataset upon completion of the final post-intervention testing upon approval from the steering committee (based on an approved proposal or with specific reason provided). Following publication of the primary outcome, other research may request access to data following an approved (by the steering committee) research proposal.

Ancillary and post-trial care

Participants enrolled in the study will be covered by Patienterstatningsordningen.

Dissemination policy

The data from the DOSE-EX study will be published in international peer-reviewed journals. All results will be reported according to the CONSORT guidelines [96]. Positive, negative and inconclusive data will all be disseminated and published.

Principal investigators and other senior members of the steering committee are considered lead authors of the material derived from the project. All authors must comply with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" [97].

Informed consent materials:

Prior to inclusion in the study written and oral informed consent. Please see ***Appendix 5 for the consent form.***

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