Protocol

Exercise Regulation of Human Adipose Tissue

NCT03133156

August 24th, 2020

Joslin Diabetes Center

Committee on Human Studies

Application for Review and Approval of Research and Training Projects Involving Human Research

Principal Investigator: Laurie Goodyear, PhD

Co-Investigator(s): Roeland J. Middelbeek, MD and Sarah J. Lessard, PhD

Project Title: Exercise Regulation of Human Adipose Tissue

Funding: NIH RO1 and K23 grant funding

Study Contact: [redacted]

---

1. PURPOSE OF PROTOCOL:

Exercise Regulation of Human Adipose Tissue
Background and specific aims:
The prevalence of obesity and type 2 diabetes in the United States and worldwide has risen dramatically over the last few decades, resulting in an enormous need for new therapies to treat these conditions. The discovery that beiging of subcutaneous white adipose tissue may increase energy expenditure has led to tremendous interest in beige cells as a potential treatment for diabetes and obesity. An established treatment for type 2 diabetes and obesity is endurance exercise training. Exercise training can improve systemic homeostasis, and although adaptations to skeletal muscle play a critical role in these effects, the underlying mechanisms are not fully understood. Moreover, the contributions of other tissues in these beneficial effects of exercise on metabolism have not been intensively investigated. We hypothesize that exercise training results in fundamental changes to white adipose tissue, including beiging, and these adaptations play an important role in the effects of exercise training to improve metabolic homeostasis.

This hypothesis stems from our recent animal-based studies that have led to the exciting discovery that exercise training-induced adaptations to subcutaneous white adipose tissue (scWAT) result in significant improvements in whole-body glucose tolerance and insulin sensitivity (1), and that the mechanism for this effect may be due to auto-, para-, or endocrine effects of novel exercise-induced adipokines. Importantly, we also found that exercise training in mice results in a beiging of scWAT as evidenced by an increase in beige marker genes, mitochondrial density, and multilocular droplets. Several other labs have also reported a beiging phenotype with exercise training in mice (2-5). Beiging of

adipose tissue has been postulated to contribute to non-shivering thermogenesis, increasing energy expenditure, and potentially inducing weight loss. Thus, understanding and potentially mimicking these exercise-induced changes to scWAT could lead to novel therapies for obesity and type 2 diabetes.

While we and others have clearly shown that exercise training induces beiging in rodents, a fundamental question for human physiology is to determine if exercise causes beiging of scWAT in human subjects. Of the very limited published research in this area, there has been no clear consensus. Our own preliminary data show that a 12-week endurance exercise training program in healthy young men increases *UCP1* mRNA expression by 1.8-fold in scWAT, supporting the hypothesis that beiging can occur with exercise training in humans. On the other hand, we find that several other beiging markers are minimally or not increased by this same exercise training program. We believe that the type of exercise, the pre-training fitness level, and the metabolic health of the subjects may all be important factors in determining if exercise induces a beiging of WAT in human subjects. Given the great interest in exercise mimetics as treatments for diabetes and obesity, it is essential to determine if exercise training increases the beiging and metabolic function of scWAT in human subjects, and if these responses are normal in people with obesity and type 2 diabetes. It is also important to understand the mechanisms underlying the beneficial effects of trained scWAT on glucose homeostasis, and for this goal we will investigate a number of secreted proteins that we have identified as novel, putative adipokines. The remaining fraction of the blood, which contains blood cells, will be analyzed by Fluorescence Activated Cell Sorting (FACS) to determine the number of specific cell types, including endothelial progenitor cells. This analysis will determine how exercise affects the number of specific cell types in the blood that can regulate metabolic health.

We have identified two Specific Aims:

Specific Aim 1. To determine if exercise training causes beiging of scWAT in human subjects. These studies will be done in lean, healthy-obese, and obese subjects with type 2 diabetes.
Specific Aim 2. To determine if exercise training alters the metabolic phenotype of scWAT from human subjects. This will include studies of mitochondrial function and glucose and fatty acid metabolism in scWAT.

Due to additional funding, we are now able to explore subaims to Aims 1 and 2, by enrolling additional subjects. The subaim for Specific Aim 1 will determine if exercise training causes differences in scWAT beiging in male vs. female subjects. The subaim for Specific Aim 2 will determine if exercise training alters the metabolic phenotype of scWAT in male subjects differently compared to female subjects.

2. STUDY DESIGN:

Subjects
In this section, we will provide the study design in terms of subject recruitment, exclusion criteria, visit details, exercise training program, and sample collection.

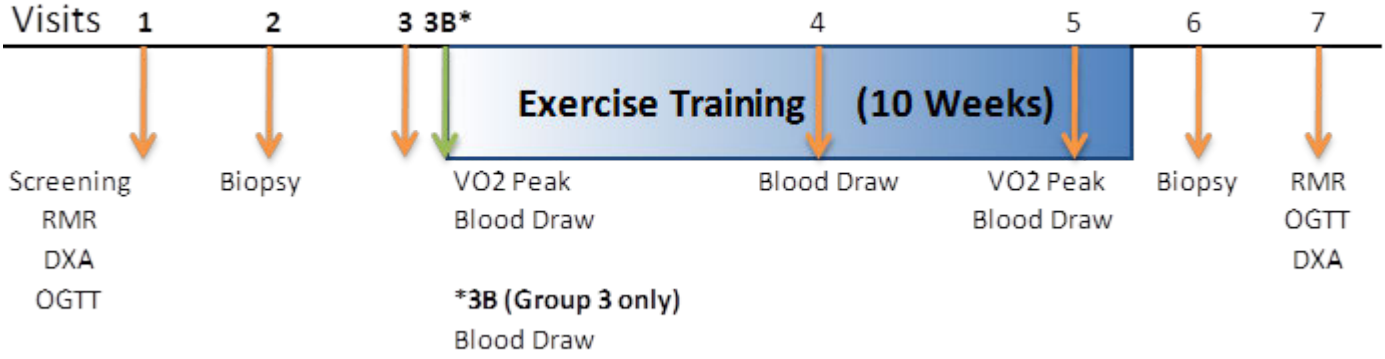
There will be two exercise training regimens. The first training regimen will determine the effects of moderate intensity endurance exercise training on scWAT in lean, healthy overweight/obese, and overweight/obese subjects with type 2 diabetes (MIT study group). The second training regimen will determine the effects of high-intensity interval training (HIT) in lean subjects (HIT study group). For both studies, the visit details, acute exercise experiments, and sample collection will be similar so they are described together.

Subjects. For the MIT study, there will be three groups of subjects: 1) 28 lean subjects; 2) 28 overweight or obese subjects without type 2 diabetes; and 3) 10 overweight or obese subjects with type 2 diabetes. For the HIT study, there will be 10 lean subjects. Subjects will include equal numbers of males and females, aged 25-55 years, who have been cleared for regular exercise. All subjects will be sedentary according to American College of Sports Medicine's guidelines (6). Enrollment HbA1c

values will be ≤ 5.7±0.1% for lean and overweight/obese subjects and between 6.5±0.1%-9.0±0.1% for subjects with type 2 diabetes. BMI will be between ≥20±0.1 and < 27 kg/m^2 for lean subjects, ≥27 and ≤37±0.1 kg/m^2 for obese/overweight subjects, and ≥25±0.1 and ≤37±0.1kg/m^2 for subjects with type 2 diabetes.
Additional exclusion criteria will include: current smoker, type 1 diabetes, severe complications of diabetes, heart or lung disease, acute infection, current dieting or weight loss efforts, pregnancy, known history of HIV/AIDS, cancer, biochemical evidence of renal or hepatic dysfunction, neurological disease, recent blood donation, clinical history of stroke, severe hypertension (systolic >160 mmHg or diastolic >90 mmHg), history of keloid formation, and inability to exercise at 50% of predicted heart rate reserve at baseline. Participants taking beta-blockers, thiazolidinediones, a basal-bolus insulin regimen, or who screen positive for the American Heart Association's contraindications to exercise testing will be excluded (7). Subjects will be asked to maintain a constant diet and extracurricular activities during the study.

Exclusion related to COVID-19: current or recent infection of COVID-19 (previous 3 months) and any hospitalization due to COVID-19.

Subject Visits.



Pre-training visits 1, 2 and 3 will be scheduled within four weeks of each other and the order of visits may be reversed. Visit 4 will occur halfway through the exercise training program. Visit 5 will be done in lieu of the first or second training session of week 10. Visit 6 will be within 24 hours of the last training session, and Visit 7 will occur up to 3 days before or after visit 6. Visits 2 and 3, and 5, 6 and 7 may be scheduled in different order, if absolutely necessary to accommodate subject's schedules.

Testing Visit 1. Subjects will arrive between 8 and 9AM after an overnight fast of at least 8 hours. During the pre-screening, subjects will be informed about this requirement and medication instructions will be given. This information will also be mailed or emailed to the subject. At the first visit, before initiating any procedures, the study will first be explained to the participant in detail and consent forms will be signed (see below). Subjects will be asked in anticipation of this visit to hold their medication the morning of the visit (this is the same for any visit requiring fasting). The participant's medical history, blood pressure, height, weight, and BMI (weight in kilograms divided by height in meters squared) will be obtained. An estimate of body composition will be obtained by performing a DXA body composition analysis (only for lean and overweight/obese subjects in the MIT training group). Briefly, the Hologic DXA system will be used, which provides images for assessment of bone density, and body fat composition. Images will be obtained by an experienced technician. Pregnant women are excluded from the study. Participants must not have had any radiographic barium studies within the past week, as this will be seen in the digestive track and interfere with the measurement of bone densitometry. Any metallic implants or previous fractures are not a contraindication for the scan, but will be annotated

during the analysis. No radiation protection via radiographic shielding will be used as the radiation exposure is minimal. The participant will be instructed to remove all clothing or body jewelry with metal. This includes- bra, jeans and any clothing with metallic accents or embroidering. The participant will be provided a hospital gown to wear. The participant will be instructed to lay flat on the table, normal breathing, without movement for the duration of the scan. They will not be provided any analysis information by the technician. After scan completion, participants will proceed with the study visit. Next, blood will be collected for fasting glucose, insulin, C-reactive protein, TNF-α, IL-6, leptin, adiponectin, serum lipids and additional novel adipokines, kidney and liver function. We will also measure the catecholamines epinephrine, norepinephrine, and dopamine in the blood during this visit. Also, an HbA1c for screening purposes will be obtained by standard blood draw. Alternatively, an HbA1c documented in the Joslin's electronic medical record within 2 months prior to the start of the study may be used instead of a screening HbA1c. During Joslin Visit 1, participants will answer questions about their recent physical activity to ensure that they have been sedentary (see below). Also, a 24-hour standardized dietary recall will be performed. Next, a resting metabolic rate (RMR) will be measured using the metabolic cart that will also be used in Visit 3 for VO_2peak testing. The technician will briefly explain the test to the subject, who will be placed in supine position. The metabolic hood will be placed on the subjects. After 10 minutes of adjustment, the measurement period will initiate, and the data of the last 5-15 minutes of the 20 minute measurement will be used for the analyses to obtain the resting metabolic rate. After this measurement, an oral glucose tolerance test (OGTT) will be performed For the OGTT, baseline serum glucose and insulin measurements will be obtained. Participants will then be given a 75g-oral glucose solution to drink and serum glucose and insulin measurements will be obtained through an IV catheter at 15, 30, 60, 90 and 120 mins after the start of the test. Participants without a previous history of type 2 diabetes mellitus will be excluded from the study if their blood glucose rises ≥140mg/dl at 2 hours. The resting metabolic rate may be deferred to the Visit 2, and the body composition analysis may be performed instead at Visit 2 or 3 if there are time constraints. If more than 10 days have passed since the Visit 1 pregnancy test at the time of the DXA exam, the test will be repeated for female subjects. If results are found to be positive, the DXA assessment will not be performed. After the results of the baseline laboratory studies and glucose tolerance test have resulted, the subject will be notified if they are eligible to participate in the study. This usually occurs later that day.

Summary of procedures during Joslin Visit 1:
- Consent forms
- History and physical
- Vitals
- Body composition Analysis
- Laboratory studies
- Resting metabolic rate measurement
- Oral glucose tolerance test

Testing Visit 2. Subjects will report between 8 and 9AM after an overnight fast of at least eight hours. Subjects will withhold their AM diabetes medications until after the biopsy has been taken. Participants will then undergo a subcutaneous adipose tissue biopsy procedure ((8) and as described below) [redacted] Similar procedures will be followed for each participant. There has been extensive experience with this procedure at the Joslin Diabetes Center. Briefly, the peri-umbilical area will be anesthetized and the biopsy will be obtained with the Coleman Aspiration Cannula and Infiltration system, using the Miller Medical 60cc Monoject syringe with Toomey or Luer Lock tip, with a target of obtaining up to 1.5g of tissue. If there is inadequate sampling, either the contralateral side of the abdomen will be used, or the subject will be asked to voluntarily repeat the biopsy procedure within the next 7 days. Compensation for Visit 2 ($80) will then again be offered.

Summary of procedures during Joslin Visit 2:

-Laboratory studies
-Subcutaneous adipose tissue biopsy

Testing Visit 3. To determine aerobic capacity, a standard VO_2 peak test will be performed. The procedure is described in detail below. [redacted] or a covering study physician, will be present as the study physician. A ramp style protocol on the cycle ergometer will be used. Blood pressure and ECG tracing will be monitored and testing will terminate based on ACSM criteria (7). Blood will be obtained 15 min before, right after the exercise test and after 30 mins of rest, to measure novel adipokine responses to a maximal exercise bout. Lactate measurements will be obtained at baseline and at VO_2 peak by performing either a finger stick or an earlobe stick to obtain a small drop of blood for testing. Blood samples will either be sent directly to the lab for further analysis of the specified laboratory tests or will be further processed for storage. Participants will be shown the exercise facilities and may use the exercise facilities during visit 3 if desired. Joslin Visit 3 will count as the first day of the 10 week exercise program. In anticipation of the start of the exercise training program, a wearable activity tracker and a heart rate monitor will be provided to the participants. Participants will sign for receipt of the activity tracker and the heart rate monitor. They will be taught how to use this device. An online account will be set-up in order to track activity remotely. The study coordinator, co-investigators and PI, and the participant have access to the data. After completion of the study, participants will return the activity tracker.

Summary of procedures during Joslin Visit 3:
-VO_2 peak testing
-Laboratory studies
-Exercise program instruction
-Wearable activity tracker and heart rate monitor instructions

Testing Visit 3b:
For the enrolled subjects with type 2 diabetes, we are interested in the effects of an exercise bout on reductions in blood glucose. For this group of subjects, we ask subjects to perform a 45min aerobic exercise session, prior to starting the exercise training protocol.
For testing visit 3b, subjects will be given a standardized drink to be taken at home at 7AM, and report to the CRC around 10AM. Subjects can start the visit with a finger stick blood glucose between 100-200mg/dl. An IV catheter will be placed and baseline plasma and serum samples will be collected. Subjects will exercise on the treadmill for 45 mins at approximately 75% of VO_2peak as determined during Visit 3. For the determination of blood glucose, the YSI machine will be used. Blood samples will be collected through an IV catheter at baseline, 15 min into exercise, at the end of exercise, and 15, 30, 45, 60 and 75 min after exercise completion, since the vast majority of the decrease in blood glucose is seen within the first 2hr after exercise [3]. The study visits will be concluded after the last blood sample is taken. Blood glucose will be measured at the indicated time points. Hypoglycemia will be treated per established protocols if blood glucose decreases <70mg/dl. If a subject reports hypoglycemic symptoms, blood glucose will directly be measured.

Testing Visit 4. During week 5, subjects in groups 1-3 will undergo a submaximal exercise bout for 45 min at the heart rate target corresponding to approximately 75% of VO_2peak. Blood draws will be obtained before, immediately after, and after 30 minutes of rest post the exercise session. Participants do not need to fast for this visit, but will be given a standardizeddrink. Subjects in group 4 will undergo a regular HIT exercise session. Two lactate measurements will be obtained at the beginning and at the end of this visit with the same procedure as outlined under Visit 3.

Testing Visit 5. This visit will be identical to Visit 3, except it will occur during week 10 of training.

During the last week of the exercise intervention, participants will undergo VO_2 peak testing during a visit in week 10 to measure a training effect compared to baseline. Submaximal exercise capacity will be recorded, as well as time to achieve target heart rate [see below for details of VO_2 testing]. To assess the effects of acute exercise, laboratory studies will be obtained 15 minutes before, immediately after and after 30mins after the end of the exercise test with a standardized blood draw as described above. Lactate measurements will be obtained at baseline and at VO_2 peak as outlined under Visit 3. Blood samples will either be sent directly to the lab for further analysis of the specified laboratory tests or will be further processed for storage.

Summary of Procedures during Joslin Visit 5:
-VO_2 peak testing
-Laboratory studies

Testing Visit 6. This visit will be identical to Visit 2, with the exception of providing instructions about the activity tracker, and will take place 24 hours after the last training session. Similar to Visit 2, a repeat biopsy during visit 6 may be offered if limited sample is obtained.

Summary of procedures during Joslin Visit 6:
-Subcutaneous adipose tissue biopsy

Testing Visit 7. Subjects will arrive after an overnight fast of at least 8 hours. Next, a RMR will be measured using the metabolic cart that will also be used in Visit 2 for VO_2peak testing. The technician will briefly explain the test to the subject, who will be placed in supine position. The metabolic hood will be placed on the subjects. After 10 minutes of adjustment, the measurement period will initiate, and the data of the last 5-15 minutes of the 20 minute measurement will be used for the analyses to obtain the resting metabolic rate. Body composition with DXA will again be performed as described above; the study team may opt to instead perform this assessment at Visit 5 or 6 to facilitate scheduling. A urine pregnancy test will be repeated prior to the DXA Exam for female subjects; if a positive result is found, this assessment will not be performed. After this measurement, an IV catheter will again be placed and a standard 2h oral glucose tolerance test (OGTT) will be performed with serum glucose and insulin measurements as described above. At the baseline blood draw of the OGTT, blood samples will be collected for assessment of kidney function, liver function, HbA1c, lipids and cathecholamines. After completion of the OGTT, the study has completed and subjects will return the activity tracker.

Summary of procedures during Joslin Visit 7:
- Laboratory studies
- Resting metabolic rate (RMR) measurement
- Body composition analysis
- Oral glucose tolerance test

MIT Exercise Training Program. Subjects will participate in a supervised aerobic Moderate Intensity Training (MIT) program for 10 weeks. Based on the true VO_2 peak obtained during Joslin visit 3, a target heart rate range [heart rate with range of about 10 beats per minute] will be calculated, that corresponds with 70-75% of VO_2 peak using the heart rate reserve formula. This will be used as a target heart rate for the aerobic portion of participants' exercise regimen during the subsequent training period of 10 weeks. The exercise program will be designed for each individual by our Joslin Exercise Physiologist. Subjects will exercise on a treadmill or other aerobic equipment. Initially, subjects will exercise 20-30min per day (as tolerated) at approximately 40-50% VO_2peak 3 days per week. The duration, intensity and frequency of exercise will gradually increase with the goal to reach 45-60min of exercise per day, 4 times per week by week 4, at an intensity of approximately 70-75% of VO_2peak based on target heart rate determined by the VO_2peak testing. Participants will be wearing the wearable activity trackers and heart rate monitors and will be reminded how to use them. For this MIT Exercise Training Program, from the start of the study, subjects will be encouraged to complete up to

two exercise training sessions per week at home, outdoors or at a local gym if available. Exercise will be logged by the activity tracker with heart rate monitor and reviewed remotely to ensure compliance, as well as during the visits at Joslin, which occur at least twice a week. This model is chosen given the high cost of parking and use of the exercise facility, to improve adherence to the study and to provide subjects with long-term exercise goals after the completion of the study. Participants will be provided with a target heart rate, as obtained during the VO_2 peak test, to target 70-75% of VO_2 peak during the aerobic part of the exercise training. If illness or travel prevents attending any of the required exercise sessions, the missed time will be made up at the latter half of the 10-week period.

Subjects with type 2 diabetes will monitor blood glucose 3-4 times per day and asked to report hypoglycemia in order to adjust anti-hyperglycemic medication. Subjects with type 2 diabetes will have a medical visit with the study physician at 3, 6 and at 10 weeks where dose adjustments to the subject's diabetes regimen can be made as needed. Participants will be reimbursed for parking if they decide to exercise at Joslin, up to four times per week. Participants will be instructed to log their physical activity on their wearable activity trackers and exercise logs will be monitored remotely. Participants may be contacted by phone or email for encouragement if a decrease in activity is noted. The staff at Joslin Diabetes Center is highly trained to work with type 2 diabetes patients who are sedentary. Our exercise physiologists have overseen successful exercise and weight loss programs such as Why WAIT, YOU-Turn, Do-It and LookAHEAD.

During sessions at the Joslin Diabetes Center, blood glucose measures will be taken to prevent hypoglycemia. Prior to each exercise session, participants with type 2 diabetes will be asked to check their blood sugar with a finger stick to ensure that it is at a safe level for exercise. For patients on insulin or insulin combined with oral medications, blood glucose must be at least 120 mg/dL before beginning exercise. For patients on oral medications alone, blood glucose must be at least 90 mg/dL before beginning exercise. Patients will be given juice and a snack to help raise blood glucose if it is below the required value, and study staff will re-check patients' blood sugar every 15 minutes until it is above the required value. The exercise physiologist will also explain 1) ways to gauge appropriate exercise intensity, 2) the importance of hydration, and 3) the need to report symptoms of hypotension to their clinician or study physician. During exercise, participants will rate exercise intensity using Borg Ratings of Perceived Exertion.

HIT Exercise Training Program. Ten lean subjects will be randomized to a high-intensity interval (HIT) training program, which will be outlined to the subjects. The total number of HI efforts each session will increase over the 10-week training period. For the initial two interval sessions, HIT training will consist of 4 x 30s all-out cycling efforts (HI) with 4min of recovery (LI; 15-25% PPO) between HI efforts. For the 3rd and 4th training sessions, 5 x 30s efforts will be performed. For the remainder of the sessions, the subject will perform 6 x 30s HI efforts. Each exercise bout will start with 5 sec of acceleration with light resistance to the maximal cadence followed by a sudden increase in resistance (starting at 100% of peak power output from VO2 assessment and adjusted as necessary) and continued with maximal effort cycling for 30s. The HIT Exercise Training Program will be performed at Joslin three times/week without an 'at home' component. If illness or travel prevents attending any of the required exercise sessions, the missed time will be made up at the latter half of the 10-week period.

For both the MIT and HIT exercise training program, subjects may perform the exercise training session at Joslin both during normal business hours (8am-5pm) and outside normal business hours in coordination with the exercise physiologist. Outside regular business hours, emergency medical assistance at Joslin may not be readily available and subjects may need to proceed to the ER in case medical assistance is needed.

Activity Assessments: To confirm that participants are sedentary pre-intervention, we will assess their physical activity by physical activity recall for the last three months during Joslin Visit 1. Participants will be considered sedentary according to the most current guidelines of the American College of Sports

Medicine if they meet the following criteria: sedentary lifestyle is defined as not participating in at least 30 minutes of moderate intensity physical activity (estimated at 40-60% VO_2 reserve) on at least three days of the week for at least 3 months (9). Self-reported data regarding their level of activity from the pre-screening will be used to determine eligibility.

VO_2 Peak Testing: At Joslin Visit 3 and Visit 5, a true VO_2 peak fitness assessment will be performed with a physician and exercise physiologist present. By performing this assessment prior to the start of the exercise training program, baseline fitness will be assessed. The VO_2 peak test does not evaluate the causes or mechanisms of exercise limitations. At the end of the study, participants will be reassessed using the VO_2 peak testing protocol during the VO_2 visit. This will be done to assess overall improvement in fitness after the 10-week exercise training program.

VO_2 peak is a measure of oxygen consumption. The VO_2 peak protocol allows participants to stop the test upon discomfort, as defined by volitional fatigue, and considers oxygen consumption at this point to be peak oxygen consumption. Prior to beginning the fitness assessment, the participant will have a resting electrocardiogram (ECG) performed by the study physician and will not be able to participate if any abnormalities are noted, unless this has already been obtained during Visit 2, in which case a repeat resting ECG would not be needed. During the VO_2 peak test, rating of perceived exertion, and heart rate and blood pressure will be monitored. The American Heart Association's Contraindications for the VO_2 testing (10) are also exclusion criteria for the study (see Appendix).

We will use a ParvoMedics Metabolic Measuring system (http://www.parvo.com/trueone-2400/), which also monitors heart rate. Participants will be also be monitored with an electrocardiogram and blood pressure cuff every 2 minutes. Participants will rate exercise intensity using Borg Ratings of Perceived Exertion every 2 minutes during the assessment (see Appendix for Borg scale).

Prior to VO_2 peak testing, participants who have type 2 diabetes will be asked to check their blood glucose with a finger stick to ensure that it is at a safe level for exercise. For type 2 diabetes participants on basal insulin or insulin combined with oral medications, blood glucose must be at least 130 mg/dL before beginning exercise. For participants on oral medications alone, blood glucose must be at least 100 mg/dL before beginning exercise. These criteria have been used in Why WAIT and are more conservative than those recommended by the American College of Sports Medicine (9). Participants will be given juice and a snack to help raise blood glucose if it is below the required value, and study staff will re-check participants' blood sugar every 15 minutes until it is above the required value.

We will utilize a cycle ramp style protocol, which has been used extensively in at-risk populations. The procedure involves monitoring inhaled and exhaled oxygen concentration using a face mask while steadily increasing speed and grade on a treadmill every 2 minutes (see Appendix for modified Bruce protocol). The modified Bruce protocol is identical to the Bruce protocol but it starts at a lower intensity and increases intensity more gradually, so it is better suited for sedentary individuals. VO_2 testing will terminate upon volitional exhaustion or if any of the criteria described in the American College of Sports Medicine's (9) indications for stopping an exercise test are met, including the following:

1) drop in blood pressure of $\geq$10 mmHg from baseline blood pressure despite an increase in workload, when accompanied by other evidence of ischemia;
2) moderate to severe angina;
3) increasing nervous system symptoms (e.g. ataxia, dizziness, or near syncope);
4) signs of poor perfusion (cyanosis or pallor);
5) technical difficulties monitoring the ECG or systolic blood pressure;
6) subject's desire to stop;
7) sustained ventricular tachycardia;
8) ST elevation (≥1.0 mm) in leads without diagnostic Q-waves (other than V_1 or aVR).

Upon completion of the test, participants will be seated and ECG, heart rate, and blood pressure measurements will continue every 1 to 2 minutes every 5 minutes or until exercise-induced changes return to baseline.

If during the test any abnormalities on the electrocardiogram are observed, the test will be stopped. The participant will be assessed by the study physician; a history and physical examination will be performed and a 12-lead ECG will be obtained. Depending on the clinical situation, the participant will be either referred to the emergency room, and/or the participant's primary care physician will be notified. The study physician will be present during the VO_2 peak testing.

At the fifth week, adherence to the exercise training regimen will be measured by assessing the heart rate reserve. Also, data from the wearable activity trackers will be monitored throughout the study period through an online account, which will be set up at the time the wearable activity tracker will be provided (Joslin Visit 2). Participants will be contacted by phone or email for encouragement if there is a decrease in the logged exercise activity.

Adipose tissue biopsies

Participants will undergo a subcutaneous adipose tissue biopsy procedure during Joslin Visit 2 and Visit 6. For this procedure, participants will be properly positioned on an examination table. Prior to the biopsy a resting ECG (only during Visit 2, not Visit 6) will be obtained to ensure that there are no rhythm abnormalities or ST-T segment changes. The peri-umbilical area will be disinfected with betadine. Subsequently, the area will be anesthetized with a 1% lidocaine injection without epinephrine. As needed, EMLA crème may be applied prior to the start of the procedure, but this will be cleaned at the time of disinfection. Up to 5-10ml of 1% lidocaine may be used to ensure proper analgesia. After the peri-umbilical area has been disinfected and anesthetized, subsequently, a subcutaneous adipose tissue biopsy will be obtained with the Coleman Aspiration Cannula and Infiltration system and the Miller Medical 60cc Monoject Toomey and B-D Luer lock syringe. The needle will slowly be inserted into the subcutaneous adipose tissue at a 45 degree angle. If needed, an incision of a few millimeters may be made with a #11 blade. The needle will then be inserted and kept at a <45 degree angle. Up to 10ml of normal saline at any time can be infused subcutaneously, to facilitate dislodging of the adipose tissue. Negative pressure will be applied and the needle will be moved up and down the subcutaneous adipose tissue, while avoiding other tissues and the saline will be aspirated. When an adequate sample has been obtained, the area will be cleaned, a sterile gauze and a band-aid will be applied and manual pressure will be applied for up to 5 minutes. If there is inadequate sampling, the contralateral side may be used for a similar procedure. Aftercare instructions will be provided.

After the sample has been removed, blood clots will be removed from the sample. The largest piece of the adipose tissue will be prepared for histological analysis by formalin fixation. This will then be sent to the histology core at Beth Israel Deaconess Medical Center (BIDMC) for analysis. Samples will be prepared for H&E staining and up to 10 slides per sample will be prepared for immunohistochemistry. Immunohistochemistry will be performed to evaluate for markers of white adipose tissue browning. The remainder of the obtained adipose tissue will be processed by collagenase digestion and will then be quickly frozen in liquid nitrogen. The sample will then be prepared for mRNA and protein analysis.

Gene expression changes: In order better understand the effects of exercise training on subjects, we propose future investigation of changes in individual genes at the mRNA level by using microarray technology. The Joslin Diabetes Center's Genetics Core has been successful in performing these analyses on human subcutaneous adipose tissue. We would ask subjects consent to store the remainder of the subcutaneous adipose tissue and blood for further genetics testing, once consent has been obtained. Subjects may also opt out for this part of the study. No extra procedure will be involved.

3. INCLUSION / EXCLUSION CRITERIA

Inclusion Criteria: For the MIT study, there will be three groups of subjects: 1) 28 lean subjects; 2) 28 overweight or obese subjects without type 2 diabetes; and 3) 10 overweight or obese subjects with type 2 diabetes. For the HIT study, there will be 10 lean subjects. Subjects will include equal numbers of males and females, aged 25-55 years, who have been cleared for regular exercise. All subjects will be sedentary according to American College of Sports Medicine's guidelines (6). Enrollment HbA1c values will be ≤ 5.7±0.1% for lean and overweight/obese subjects and between 6.5%±0.1-9.0±0.1% for subjects with type 2 diabetes. BMI will be ≥20±0.1 and < 27kg/m^2 for lean subjects, ≥27 and ≤37±0.1kg/m^2 for obese/overweight subjects, and ≥25±0.1 and ≤37±0.1kg/m^2 for subjects with type 2 diabetes. The study physician will review the participants' medical records for medical and treatment history to ensure the presence of type 2 diabetes. Subjects may be enrolled in the type 2 diabetes group if they meet diagnostic criteria during the screening visit (HbA1c, fasting plasma glucose or 2-hour OGGT). Participants must be following a sedentary lifestyle defined as less than 30 minutes of moderate to intense activity 3 days per week in the last 3 months at time of study enrollment (11). Participants must be cleared for exercise, including a VO_2 peak test, by their clinician or the study physician. Lean subjects who enroll in the study will be randomized to either MIT or HIT training.

Exclusion Criteria: Potential participants will be excluded for the following criteria: Ages <25 and >55; HbA1c ≤ 6.5±0.1% and ≥9±0.1% for participants with type 2 diabetes (to maximize safety) and >5.7±0.1% for overweight or obese participants without diabetes; current smoking status, type 1 diabetes, heart or lung disease; acute systemic infection accompanied by fever, body aches, or swollen lymph glands; current dieting or weight loss efforts; current pregnancy; known history of HIV/AIDS; cancer; biochemical evidence of renal or hepatic dysfunction; End-Stage Renal Disease and any renal disease, defined as a creatinine above the normal limit; liver disease; demyelinating diseases such as multiple sclerosis or amyotrophic lateral sclerosis; recent blood donation; clinical history of stroke; severe hypertension (systolic > 160 mmHg or diastolic > 90 mmHg); type 1 diabetes; history of keloid formation, and inability to exercise at 50% of predicted heart rate (HR) reserve at baseline. Participants taking beta-blockers, thiazolidinedione, and a basal-bolus insulin regimen will be excluded as well. Participants will also be excluded if during Joslin Visit 1, participants without established type 2 diabetes reach a glucose value of 140mg/dl or higher at the 2 hour point during the oral glucose tolerance test.

Either a current pregnancy or breastfeeding are exclusion criteria for this study, in particular for scientific reasons, since pregnancy and breastfeeding may affect myokine and adipokine secretion and interfere with the ability to exercise. To confirm that a female subject is not pregnant prior to participating in the study, a urine pregnancy test will be performed at Joslin Visit 1. Pregnancy tests will be repeated prior to performing any DXA body composition analysis for female participants if greater than 10 days have passed since prior testing. If a positive result is found, this assessment will not be performed. Participants will also be asked to use contraception during the remainder of the study.

Participants who screen positive for The American Heart Association's contraindications to exercise testing (10) (see Appendix) during pre-enrollment screening by the study physician or their clinician are ineligible for this study.

Potential participants will also be excluded if they have severe complications from diabetes, including the following: severe peripheral diabetic neuropathy and/or severe peripheral vascular disease, symptomatic severe autonomic neuropathy, nephropathy (microalbumin/creatinine ratio > 300 mg/g), and untreated proliferative diabetic retinopathy based on dilated eye examination within one year of study entry.

4. DATA ANALYSIS / SUBJECT SELECTION

Sample Size

The initial sample size calculation of this study was done in consultation with [redacted] through Harvard Catalyst's biostatistical consulting service. The initial study design consisted of four study groups with a sample size of N = 10 in each of the four groups. These analyses will provide an estimate of effect size. The data collected in our study are unique and sample size estimates cannot be based on previous human studies as ours is the first study to investigate how short-term exercise training impacts these novel adipokines in obesity and type 2 diabetes and few studies have evaluated scWAT beiging. However, sample size calculation in collaboration with the Harvard Catalyst biostatistician team revealed that a sample size of 9 in each group will have power of 0.85 to detect a change of 2.8 ml/kg/min in VO_2peak, assuming a standard deviation of that change of 2.4 based on a linear mixed models (repeated measures) analysis framework at an alpha of 0.05. Since we are interested in the effects of exercise training on changes in human adipose tissue as a primary outcome, we also calculated the sample size needed to detect changes in TGF-beta 2 mRNA expression. A sample size of 8 in each group will have similar power to detect a difference in fold change of between 1.53-1.91 (SD 0.5) over basal in adipose tissue TGF-beta 2 mRNA expression levels. The fold increase in mouse serum TGF-beta 2 after exercise was 1.56 (SD 0.5), in line with and supporting our sample size calculations based on mRNA expression. Analysis of preliminary data examining the change in UCP1 mRNA levels suggests similar magnitude of effect (i.e. fold-change on the order of 2.0 with exercise training) with a more variable response (SD of change equal to size of change), and consequently 12 subjects would be required to achieve a power of 0.88 to detect this effect at an alpha of 0.05. Conversely, with the planned sample size of 10 per group, we estimate a power of 0.80 to detect this effect size at an alpha of 0.05 in the planned analyses. We anticipate that 90% of volunteers will comply with the study protocol and, therefore, plan to include a total of 11 participants in each group.

These original power calculations did not include an adequate number of subjects to discern differences between males and females. We have secured additional funding to determine sex-specific effects of moderate intensity exercise training on adipose tissue metabolism in lean and overweight subjects. Thus, we will increase the enrollment numbers for these two specific groups of subjects, to allow for adequate power to identify sex-specific changes in adipose tissue metabolism. The expanded groups will include: 14 lean males, 14 lean females, 14 overweight/obese males, 14 overweight/obese females, all undergoing moderate intensity exercise training.

We will evaluate changes in adipose tissue and serum as defined in the Specific Aims. Univariate analysis will be used to examine potential outliers and missing data. Categorical variables will be summarized and presented using frequencies and proportions while continuous variables will be summarized and presented using means, medians and standard deviations.

Analysis of Primary Outcomes

Change in VO_2peak in lean subjects, obese subjects, and type 2 diabetes subjects groups and high-intensity group
Change in VO_2peak will be calculated by subtracting baseline VO_2peak from final VO_2peak for individuals within each group. Pre- and post-test values for all four groups will be compared via repeated measures ANOVA (linear mixed model). If significant differences are identified, post-hoc t-tests will be performed to determine where these differences lie.

Mean fold changes in beiging proteins, mRNA expression, and novel adipokines in tissue and serum
Mean fold change values will be calculated to determine relative changes in relevant proteins, mRNA expression and adipokines in tissue and serum for each group. Log transformation may be used to compare up and down changes in some markers. Pre- and post-test values for all four groups will be compared via repeated measures ANOVA. If significant differences are identified, post-hoc t-tests will be performed to determine where these differences lie.

Conclusions will be drawn with caution due to small sample size and multiple comparisons. Regression analysis would be optimal to determine the amount of change attributable to the intervention, accounting for other factors.

Local Interim Data Analysis/Data Monitoring Plan

For this study of the effect of exercise on adipose tissue in type 2 diabetes, data safety monitoring will be performed on an ongoing basis by the Principal Investigator, Laurie J. Goodyear, PhD. In particular, the well-being and safety of subjects will be monitored closely by both the exercise physiologist and [redacted], who will be delegated as responsible for subject safety monitoring. Any adverse events, together with notification of appropriate action will be recorded and reported to the Principal Investigator as soon as possible. For subject safety, a screening history, physical examination, and safety check on laboratory equipment will be performed prior to the initiation of study protocol. Inclusion and exclusion criteria will be printed in each chart and will be reviewed for each subject after screening procedures are complete, prior to initiation of study procedures. Reasons for screen failure will be documented.

The Joslin Institutional Review Board reviews overall risk annually. Charts will be kept in locked offices to protect subject privacy but will be available for inspection by the safety officer upon request.

All major adverse events will be reported immediately to members of the Joslin Committee for Human Studies (CHS-IRB) whether related or unrelated to the study procedures. Minor and expected adverse events, such as those listed in the study consent form, will be reported annually to the Joslin CHS-IRB.

The Principal Investigator (Laurie J. Goodyear, PhD) is responsible for adverse event reporting and will report to the Joslin CHS (IRB) as follows:

a. All fatal, life-threatening, or serious adverse events will be reported verbally and faxed to the IRB and to the Safety Officer as necessary within 24 hours.
b. Unexpected adverse events will be reported verbally in real time to the CHS.
c. Unexpected moderate or severe adverse events will be reported in writing to the CHS within 10 days of occurrence or recognition.

Study participation is voluntary, and participants may discontinue participation at any time without penalty. In the setting of expected and minor adverse events, a subject may be advised to discontinue participation upon medical grounds at the discretion of the study physician. The study will be terminated immediately should there be one major adverse event occurring in a study subject and related to study procedures.

The Principal Investigator's name and contact information will be provided to all research participants along with instructions to report any potential adverse reaction:

[redacted]

5. POSSIBLE BENEFITS:

The overall risks to subjects for this study are minimal, and the scientific information to be gathered may be of considerable importance. There is currently very little information regarding the effects of exercise training on adipose tissue browning in subjects with obesity and type 2 diabetes. Moreover, the effects of exercise training on novel adipokines are also unknown. Understanding novel mechanisms that improve glucose homeostasis in obesity and type 2 diabetes is essential given the increasing prevalence of obesity and type 2 diabetes and these adipokines may lead to novel therapies for type 2 diabetes. Therefore, the benefits of this study appear to outweigh the risks by a considerable degree.

6. POSSIBLE RISKS:

Risks Associated with Exercise: There are risks associated with the exercise intervention. Although all participants will be carefully screened and cleared for exercise, there is still a remote possibility of adverse events such as abnormal blood pressure, fainting, dizziness, arrhythmia, and in very rare instances heart attack or stroke. There also exists the risk of bodily injury including injuries to muscles, ligaments, tendons, and joints. To minimize the risk of an adverse event, all participants will be pre-screened by a physician prior to participation. All participants must be cleared for exercise before they begin the intervention. Further, an assessment of physical fitness will be performed upon study enrollment, and the exercise intervention will be tailored to each participant's fitness level. Participants will exercise to percentages of heart rate reserve that are proven to be safe. In case of an emergency, arrangements for further emergent care will be made. Another risk associated with exercise is the risk of hypoglycemia. During sessions at the Joslin Diabetes Center, blood glucose measurements will be taken to prevent hypoglycemia. Prior to each exercise session, participants will be asked to check their blood sugar with a finger stick to ensure that it is at a safe level for exercise. For patients on insulin or insulin combined with oral medications, blood glucose must be at least 110 mg/dL before beginning exercise. For patients on oral medications alone, blood glucose must be at least 90 mg/dL before beginning exercise. For the VO_2peak testing, higher blood glucose targets of 130 mg/dl and 110mg/dl respectively will be used. Patients will be given juice and a snack to help raise blood glucose if it is below the required value, and study staff will re-check patients' blood sugar every 15 minutes until it is above the required value.

Risks Associated with VO_2 peak testing: It is possible that this test could cause heart abnormalities (such as irregularities of the heartbeat, lack of oxygen to the heart), dizziness or weakness. Every effort will be made to minimize these risks. Participants will be pre-screened for evidence of heart disease prior to study enrollment. They will also undergo a complete history and examination by study physician or their clinician, as well as an ECG before the treadmill test. If any abnormalities are detected that would put participants at risk during the treadmill test, they will not be allowed to perform the test or participate in the study. During this test, if participants experience 1) any uncomfortable symptoms, 2) abnormalities on the ECG monitor suggesting that a heart abnormality is occurring, or 3) significant changes in blood pressure, the test will be stopped immediately and the information will be sent to the participant's physician (see addendum and above for a list of contraindications to VO_2 testing). A physician will be present in the fitness room during this procedure. Essential equipment and medical supplies are immediately available if there are any unexpected problems. The treadmill test will be performed with standardized methods and will be monitored by certified individuals. This exercise test may cause muscle soreness for 2-3 days. The risks of carefully monitored exercise tests are very low, but do rarely include non-fatal complications requiring immediate medical treatment (in less than 1 out of 10,000 tests), abnormal heartbeats requiring immediate treatment (rare, less than 1 out of 30,000 tests), or death (in less than 1 out of 150,000 tests).

Risks of Use of Glucose: The major risk of infusing glucose is hyperglycemia. The participant may feel thirsty. The participant should be able to rapidly clear the glucose they are given in this study during the oral glucose tolerance test.

Risks Associated with Blood Draws and Intravenous Catheterization: The total amount of blood drawn during the study is approximately 450 ml (or 30 tbs.). This is about less than the amount that is taken when blood is donated and should not pose any risk. Participants will be asked not to donate blood for at least 3 weeks before and after the study.

One small polyethylene catheter is placed in a peripheral vein for the withdrawal of blood samples. The participant will feel a sharp pain or sting when the needle is inserted. The potential risks of this procedure include superficial thrombophlebitis, small local hematomas, and mild local pain. In our experience, these have occurred in about 1-2% of the participants studied and resolve with local

application of heat. If the symptoms have not resolved within 2 days after the application of heat, the participant will be instructed to return to the clinic for evaluation and further treatment as indicated.

Risks Associated with Adipose Tissue Biopsies: During the study, adipose tissue biopsies will be obtained. Participants may experience mild pain, swelling, soreness or bruising after the procedure. Local anesthesia with 1% lidocaine will be used. Participants may take acetaminophen after the procedure if required. There is a potential risk for infection. In order to avoid this, sterile procedures will be used. The area will be cleaned with betadine and sterile gloves and needles will be used. A small hematoma can collect under the skin where the needle was inserted. In order to minimize this, the periumbilical area will be selected and any areas with visible veins will be avoided. Also, pressure will be applied after the procedure.

Risks of DXA Exam: The DXA exam uses a small amount of X-ray radiation, so there is a very slight risk of radiation exposure. The radiation exposure is comparable to what a participant would get if he/she were in the sunshine for 3 hours or if he/she took a flight from California to New York.

7. CONSENT PROCEDURES:

Joslin patients will be contacted by letter and then followed up by a telephone call from a study research assistant. If they are interested, they will be asked to come in to the Joslin for a more complete explanation of this study at which time, written informed consent will be obtained. The study risks and benefits will be explained as will each of the study procedures. Patients answering an ad will be screened by telephone using the same procedures.

A detailed description of the consent procedures appear below:

Written informed consent will be obtained from each subject at entry into the study. Informed consent is obtained by the following process:

- Subject reviews the study consent form.
- PI or co-investigators meet with the subject to review the consent form, confirm subject's understanding, and answer questions
- Once the investigator or co-investigator is convinced that the subject verbally demonstrates understanding and agrees to the process, the consent form is signed in the presence of a witness. Individuals authorized to obtain written consent are the principal investigator, co-investigators, and assigned research staff specifically designated by the principal investigator to work on this project.

8. RECRUITMENT / SOURCE OF SUBJECTS:

Current Joslin patients who meet study criteria may be contacted through letter, and flyers will also be placed around the Longwood Medical Area neighborhood and inside the Joslin Diabetes Center. To recruit non-Joslin patients and participants without diabetes, the use of online social media and job recruitment websites (i.e. Facebook, Craigslist, university job websites, etc.) will help identify potential participants. Additionally, we will be using the company Trialfacts to help recruit participants for the study, which uses recruitment websites including Facebook, Instagram and targeted ads on websites. The website for Boston University's online job application can be found here:

http://www.bu.edu/link/bin/uiscgi_student_employment.pl/uismpl?ModuleName=se_job.pl

Flyers with contact information that potential participants can contact regarding the study will also be placed at appropriate and approved buildings and academic institutions around the Longwood Medical Area. Examples of these buildings would include: Karp Research Building at Boston Children's Hospital, Simmons University, and the Longwood Galleria. If these methods do not identify enough potential participants, an ad will be placed in a local newspaper. Some eligible participants have already been identified by Joslin colleagues and these individuals will also be contacted.

We will enroll 38 lean participants, 28 overweight/obese participants and 10 participants with type 2 diabetes. Current studies with exercise components, including the Why Wait and LookAhead programs have 85% and 95% retention rates, respectively. Since the intervention is relatively short, the retention should be quite high. To further encourage retention, we will compensate participants for their time spent attending assessment appointments and exercising, and they will receive the exercise program free of charge. We will also provide participants with parking in the Harvard/Longwood Medical area.

Patients will be recruited through referrals from the Joslin Clinic and other Harvard-affiliated clinical practices in the Boston Metropolitan area, advertisements in the Joslin Newsletter and newsletters of the Diabetes Associations of Massachusetts, Rhode Island, and New Hampshire, extensive mailings to Joslin mailing lists, and advertisements in local newspapers. No demographic group will be intentionally excluded and minorities will be encouraged to apply. Given the gender breakdown of the Joslin Diabetes Center, we anticipate that about 50% of the sample will be women. Approximately 60% of the 22,000+ patients treated at the Joslin Clinic have type 2 diabetes.

9. RIGHTS AND PRIVACY:

In addition to the procedures for protecting and minimizing potential risk, we will maintain the confidentiality of all the participants by keeping all records locked, stored in locked facilities at the Joslin Diabetes Center's Integrative Physiology and Metabolism laboratory. Data will be maintained using an ID number only. Subject names and ID numbers will be kept separately. The key to subject names and ID numbers will also be kept in a different locked area of the laboratory to limit access to information about specific participants. Deidentified participant data may be shared with other researchers, after permission of local institutional review boards, and without disclosure of protected health information.

Please answer the following questions:

- Will medical history/clinical information be obtained from the subjects' medical records for the purpose of this study? If yes, please list what information will be recorded.
  X YES o NO

  - Most recent HbA1c and past recorded HbA1cs
  - Medical history, including history of neuropathy, retinopathy, nephropathy
  - List of current medications

- Will information resulting from this study (i.e. results of clinical/research lab tests, etc…) become part of the subjects' medical record or provided to the subject and/or others for clinical purposes? If, no, please list what information will not be given to the subject or recorded in their medical record and why.

  X YES o NO

  No information will become part of the subject's medical record. The results of adipose tissue analysis and testing for gene changes will not be provided to the subjects because the tests will be

done for research purposes only. We will share the results of other laboratory tests and fitness measures with the subject.

- Will subjects' identifiable health information* be shared with others outside of Joslin Diabetes Center? If yes, list whom this information will be shared with (please be specific, include names of collaborators, study sponsor contacts)?

  o YES X NO

10. OMIT PROCEDURES / LEAVE STUDY:

At any time, subjects will be free to leave the study. If during the study course they decide to omit particular procedures, they will be free to leave the study without bias to their clinical care or any information about their own study results.

11. INCENTIVES / REMUNERATION:

Joslin Visits 1-7 will take about 4-5 hours each to complete (including history and physical, oral glucose tolerance test, blood work and VO_2 peak testing). The exercise training visits will take about 60 minutes to complete. Participants will be compensated as follows:
Parking will be provided on the days the participants visit the Joslin for the study (exercise visits included).
If participants complete Joslin Visit 1, they will be compensated $100.
If participants complete Joslin Visit 2, they will be compensated $80.
Starting at Joslin Visit 3, participants will be compensated by $20 for each week of the exercise intervention they complete by either exercising at Joslin or logging their activity in the online activity tracker account.
Subjects with type 2 diabetes who complete Joslin Visit 3b will be compensated $50.
Subjects who undergo an additional adipose tissue biopsy will be compensated $80 per visit.
If participants complete the Joslin Visit 7, they will be compensated $80.
Additionally, if they complete the study with at least 90% participation in the described exercise training sessions, participants will be compensated an additional $120.
The total compensation for this study will be a maximum of $580 (or $630 for subjects with type 2 diabetes and up to $740 if extra adipose tissue biopsies take place). Participants will receive a check in the mail 4-6 weeks after study competition.

Besides compensation with money, participants will also receive the opportunity to exercise for free at the Joslin facilities. They will also receive individualized exercise training by an exercise physiologist for free, which can serve as an incentive to complete the study.

Since this study involves complex procedures and equipment, there may be unforeseen findings, which could make participants ineligible to continue with the study, in which case they will be compensated as follows: If it is determined that they are not eligible for the study after completing Joslin Visit 1, they will be compensated $20. Subjects will not be separately compensated for the time spent completing the exercise training visits.

Please answer the following questions:

12. Where and how will your project utilize the Joslin Diabetes Center?

- ❑ X General Clinical Research Center (GCRC)
- ❑ Clinical Trials Unit (CTU)
- ❑ Joslin Clinic
- ❑ Eye Unit
- ❑ X Other (please specify) __Clinical Exercise Physiology Research Laboratory and Facility

13. Will your project involve research on living human fetuses?

- ❑ Yes
- X No

14. Does your project involve the use of any new drug or device?

- ❑ Yes IND# or IDE# _____
- X No

15. Is review required by risk management foundation?

- ❑ Yes
- X No

7/8/2020

Signature of Principal Investigator ***Date***

I have read and reviewed this application for approval by the Committee on Human Studies

7/8/2020

Signature of PI's Section Head ***Date***

Please bring the original and twenty-four (24) copies of
this form and the informed consent form for the above research project to
Leigh Read in the Office of Sponsored Research
by the appropriate CHS meeting deadline.

Reference List

1. Stanford,KI, Middelbeek,RJ, Townsend,KL, Lee,MY, Takahashi,H, So,K, Hitchcox,KM, Markan,KR, Hellbach,K, Hirshman,MF, Tseng,YH, Goodyear,LJ: A Novel Role for Subcutaneous Adipose Tissue in Exercise-Induced Improvements in Glucose Homeostasis. *Diabetes*db140704, 2015
2. Trevellin,E, Scorzeto,M, Olivieri,M, Granzotto,M, Valerio,A, Tedesco,L, Fabris,R, Serra,R, Quarta,M, Reggiani,C, Nisoli,E, Vettor,R: Exercise training induces mitochondrial biogenesis and glucose uptake in subcutaneous adipose tissue through eNOS-dependent mechanisms. *Diabetes* 63:2800-2811, 2014
3. Cao,L, Choi,EY, Liu,X, Martin,A, Wang,C, Xu,X, During,MJ: White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab* 14:324-338, 2011
4. Sutherland,LN, Bomhof,MR, Capozzi,LC, Basaraba,SA, Wright,DC: Exercise and adrenaline increase PGC-1{alpha} mRNA expression in rat adipose tissue. *J Physiol* 587:1607-1617, 2009
5. Bostrom,P, Wu,J, Jedrychowski,MP, Korde,A, Ye,L, Lo,JC, Rasbach,KA, Bostrom,EA, Choi,JH, Long,JZ, Kajimura,S, Zingaretti,MC, Vind,BF, Tu,H, Cinti,S, Hojlund,K, Gygi,SP, Spiegelman,BM: A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481:463-468, 2012
6. American College of Sports Medicine: *ACSM's Guidelines for Exercise Testing and Prescription*. Wolters Kluwer/Lippincott Williams & Wilkins Health, 2013,
7. Fletcher,GF, Ades,PA, Kligfield,P, Arena,R, Balady,GJ, Bittner,VA, Coke,LA, Fleg,JL, Forman,DE, Gerber,TC, Gulati,M, Madan,K, Rhodes,J, Thompson,PD, Williams,MA: Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation* 128:873-934, 2013
8. Goldfine,AB, Crunkhorn,S, Costello,M, Gami,H, Landaker,EJ, Niinobe,M, Yoshikawa,K, Lo,D, Warren,A, Jimenez-Chillaron,J, Patti,ME: Necdin and E2F4 are modulated by rosiglitazone therapy in diabetic human adipose and muscle tissue. *Diabetes* 55:640-650, 2006
9. American College of Sports Medicine: *ACSM's Guidelines for Exercise Testing and Prescription*. Wolters Kluwer/Lippincott Williams & Wilkins Health, 2013,
10. Fletcher,GF, Ades,PA, Kligfield,P, Arena,R, Balady,GJ, Bittner,VA, Coke,LA, Fleg,JL, Forman,DE, Gerber,TC, Gulati,M, Madan,K, Rhodes,J, Thompson,PD, Williams,MA: Exercise standards for testing and training: a scientific statement from the American Heart Association. *Circulation* 128:873-934, 2013
11. Baker,LD, Frank,LL, Foster-Schubert,K, Green,PS, Wilkinson,CW, McTiernan,A, Cholerton,BA, Plymate,SR, Fishel,MA, Watson,GS, Duncan,GE, Mehta,PD, Craft,S: Aerobic exercise improves cognition for older adults with glucose intolerance, a risk factor for Alzheimer's disease. *J Alzheimers Dis* 22:569-579, 2010