**Study Protocol**

**Project title:** Metabolic adaptations in response to high intensity interval training in obese adults (HT-Study)

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**OVERALL OBJECTIVES**

Exercise is a key component in the prevention and treatment of obesity-related metabolic complications, such as insulin resistance and type 2 diabetes. Recent evidence clearly indicates that ***high intensity interval training (HIIT)***, which involves brief high-intensity efforts interspersed with recovery periods, is a very effective and time-efficient alternative to conventional exercise training (1-3, 14, 16). Importantly, HIIT has been found to be safe and well tolerated in obese and type 2 diabetic patients (6, 9, 10). However, the ***mechanisms*** underlying HIIT-induced improvements in metabolic health remain poorly understood. A comprehensive assessment of the mechanisms responsible for the cardio-metabolic health benefits of HIIT is essential for optimizing the most effective exercise therapies. In addition, variations in key components of HIIT (# of intervals, exercise time, energy expended) may induce different metabolic outcomes, yet a systematic assessment of variations in these parameters has not been conducted. Furthermore, while available evidence clearly demonstrates HIIT can induce impressive health benefits after a few to several weeks of training, the ability/willingness of obese adults to adhere to a long-term HIIT program is not known. **Our overall objectives** are to: 1) thoroughly assess putative mechanisms underlying HIIT-induced improvements in insulin resistance at the whole-body, tissue, and cellular levels, 2) systematically compare different HIIT regimens to help identify effective “doses” of HIIT that may be optimal for improving metabolic health in obese adults, and 3) assess the ability/willingness of obese subjects to adhere to a long-term HIIT program. Findings from these studies will greatly expand our understanding about the effects of HIIT on metabolic health, and will provide valuable information for development of programs aimed at maximizing key metabolic benefits of exercise.

**SPECIFIC AIM #1**: *Determine the effects of 3 months of High Intensity Interval Training [HIIT] (10 x 1min at ~90%maximal heart rate [HRmax]; 4days/week) on insulin resistance and factors mediating insulin resistance in obese adults with impaired glucose tolerance.*

**Hypothesis 1A**: Compared with before training, 3 months of HIIT will: a) reduce peripheral insulin resistance, b) reduce hepatic insulin resistance, c) improve 24h glucose control, d) lower hepatic lipid accumulation, and e) improve blood lipid profile, f) reduce macrophage infiltration, and g) lower other key markers of inflammation within skeletal muscle, adipose tissue, and systemic circulation.

**Hypothesis 1C**: HIIT will induce superior improvements in insulin resistance compared with a “conventional” exercise training program commonly prescribed for health and fitness (45 min of steady-state exercise at 60-70%HRmax; 4days/week) - despite ~45% lower energy expenditure and time requirements for HIIT *vs.* the conventional exercise program.

**SPECIFIC AIM #2**: *Determine the impact of the number of intervals, exercise time, and energy expended during HIIT on insulin resistance after 3 months of training, and on exercise adherence throughout the next 9 months (i.e., one year after initiating the program) in obese adults with impaired glucose tolerance.*

This will be accomplished by comparing changes in insulin resistance in response to 3 different HIIT regimens:

* **HIIT#1**=10 x 1min at ~90%HRmax [25min (with warm-up and recovery); ~150kcals],
* **HIIT#2**=5 x 1min at ~90%HRmax + 10min of continuous exercise at 70%HRmax [25min; ~150kcals]
* **HIIT#3**=5 x 1min at ~90%HRmax [15min; ~90kcals].

After 3 months of supervised training, subjects will continue their prescribed exercise regimen on their own – We will track adherence to the different HIIT regimens and to “conventional” exercise over the next 9 months.

**Hypothesis 2A**: The improvement in insulin resistance after 3 months of **HIIT#1** will be greater than that found after **HIIT#2**, despite being matched for total exercise time and energy expenditure.

**Hypothesis 2B**: Improvement in insulin resistance after 3 months of **HIIT#3** will be similar to **HIIT#2**, despite ~40% less exercise time and ~40% lower energy expenditure per session in **HIIT#3** vs. **HIIT#2.**

**Hypothesis 2C**: One year after initiating the exercise programs: a) adherence to HIIT requiring only 15min of exercise per session will be superior to all others, and b) adherence to all HIIT regimens will be greater than adherence to “conventional” exercise training (45 min of steady-state exercise at 60-70%HRmax).

OVERALL RESEARCH STRATEGY

* Obese adults with impaired glucose tolerance will be randomized into one of four different exercise training groups for a 3 month exercise intervention (4 days/wk)

1. **High intensity interval training (HIIT#1)** [10 x 1min at ~90%HRmax - with low intensity warm-up, recovery, and cool-down - total of 25 min/session]
2. **Moderate-intensity continuous training (MICT)** = steady-state exercise at 60-70%HRmax for 45min] - representing a commonly prescribed exercise program]
3. **HIIT#2**=5 x 1min at 90%HRmax + 10min steady-state exercise at 70%HRmax [25min; ~150kcals; 4d/wk]
4. **HIIT#3** = 5 x 1min at 90%HRmax [15min; ~90kcals; 4d/wk]

* ***HIIT#1*** *and* ***MICT*** *training regimens will be used to address Specific Aim #1 and all four training regimens will be used to address Specific Aim #2*
* Before and after the interventions, subjects will participate in a “clinical study” in which we will perform a battery of metabolic tests (e.g., hyperinsulinemic-euglycemic clamps, tracer infusions to measure substrate kinetics) and we will also collect skeletal muscle and adipose tissue biopsy samples.
* Subjects will participate in one overnight “clinical study” before the 3-month training program, and two similar clinical studies after training. The first post-training clinical study will include a supervised exercise session upon admittance – the second post-training clinical study will be performed exactly 3 days later, to "washout" the effects of the most recent session of exercise. Performing these two clinical studies after training will allow us to make the very important distinction between the ***acute*** effects of the most recent session of exercise and the ***chronic adaptations*** in response to the exercise training.



**Figure 1 – Overall timeline of the training intervention**

* In addition to measuring insulin sensitivity using a hyperinsulinemic-euglycemic clamp, we will also assess “free-living” 24h glucose control using a continuous glucose monitor (CGM), as well as several other relevant clinical markers (e.g., subcutaneous and visceral adiposity, hepatic lipid accumulation accumulation and degree of hepatic fibrosis [MRI analysis], blood lipid profile, resting metabolic rate, HbA1c).
* Exercise training will be supervised during the first 3 months of the program. Then, participants in all groups (**HIIT#1, MICT, HIIT#2, and HIIT#3**) will be asked to continue their prescribed training program on their own. Adherence to their assigned training program will be tracked for 9 months after completion of the initial 3 months supervised training program (1 year total).
* One year after initiating their training program, subjects will return to the clinic for a quick battery of standard clinical assessments (e.g., body weight, body composition, HbA1c, blood lipid profile, blood pressure).

**RESEARCH DESIGN AND METHODS**

**Subjects**

A total of 150 obese, pre-diabetic women [n=75] and men [n=75] will be recruited for this study - see inclusion and exclusion criteria, below. All subjects must be weight stable (±3kg for ≥ 6 months) and they will be screened with a detailed history and physical examination, routine blood tests, an exercise stress-test (VO2max test), and body composition assessment (DEXA). Subjects will not be taking medications known to affect lipid or glucose metabolism. Anyone with evidence and/or history of cardiovascular disease and type 2 diabetes will be excluded. All women will be pre-menopausal and will have regularly occurring menses. To avoid potential confounding hormonal effects, all women will be studied in the early follicular phase of their menstrual cycle.

Inclusion criteria

* Age: 18-40
* Body Mass Index: 30-40 kg/m2
* Waist circumference: 88-98cm for women and 100-110cm for men
* No regularly planned exercise/physical activity
* Women must have regularly occurring menses and must be premenopausal

Exclusion criteria

* EKG abnormalities as assessed by the cardiologist on the research team (Dr. Hummel)
* Evidence/history of cardiovascular or metabolic disease
* Medications known to affect lipid or glucose metabolism
* Pregnant or lactating
* Tobacco or e-cigarette use

**Preliminary testing**

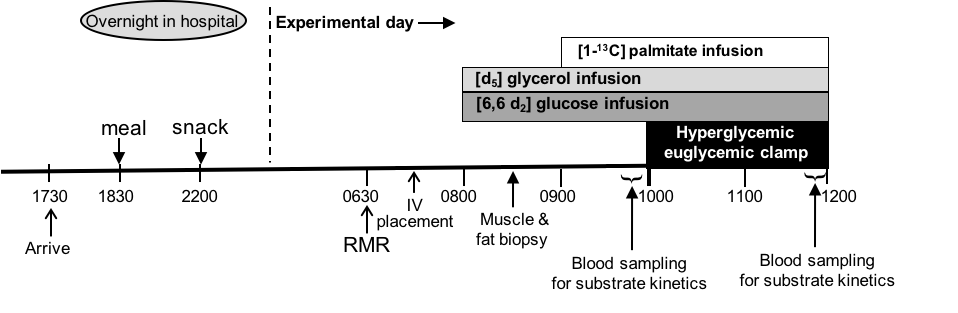
Before participating in the experiment, eligible participants will undergo the following preliminary procedures:

Subjects will complete a food frequency and physical activity questionnaire.

* + Subjects will be screened with a detailed medical history survey
  + Subjects will complete a resting EKG
  + Subjects will complete a brief graded exercise test (bike or treadmill) to determine maximal aerobic capacity (VO2max). This exercise test is routinely used in clinical research projects to assess aerobic fitness in lean, obese and other clinical populations.
  + Subjects will be asked to complete an AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire, which will be used as a guideline to identify the subjects’ history of cardiovascular disease. We will not exclude subjects who identify as being inactive or overweight as these are important inclusion criteria for our study. In addition, we will not exclude subjects who report not knowing their blood cholesterol levels, and blood cholesterol will be measured as part of the study. We will also not exclude all subjects taking prescription medications, because exercise is not contraindicated with many medications - and there are many of these medications that would not interfere with the measurements we are making in this study.
  + Eligibility to participate in the study will ultimately be determined on a case by case basis. Dr. Charles Burant, MD will be reviewing the detailed medical history survey and Dr. Scott Hummel, MD will be reviewing the resting EKG for eligibility to participate.

These preliminary tests could be completed in one visit or multiple visits, depending on the schedule and convenience of the participants.

Clinical study procedures for Specific Aim #1:

Before the exercise intervention and twice after the intervention, subjects will participate in an overnight clinical study performed at the Michigan Clinical Research Unit (MCRU) in the University of Michigan Medical Center. The timeline of events during these clinical studies is outlined in Figure 2. Upon arrival for each overnight clinical study, female subjects will undergo a urine pregnancy test to confirm continued eligibility. Briefly, after staying overnight in the hospital, subjects will undergo stable isotope infusions of [13C]palmitate, [d5]glycerol, and [d2]glucose to assess the rates of fatty acid mobilization, lipolytic rate, and hepatic glucose production, respectively. Skeletal muscle (vastus lateralis) and subcutaneous abdominal adipose tissue biopsy samples will be collected in the overnight fasted state. We will then begin a hyperinsulinemic-euglycemic clamp (insulin infusion rate = 40 mU/m2/min) to assess insulin resistance, as described previously by Defronzo, et al.(4). Detailed descriptions of these procedures can be found in our previous publications (7, 8, 11, 15). During overnight clinical study #1 and #3 we will also obtain a blood sample to assess the subjects HbA1c and liver function. Subjects’ diets will be tightly controlled during these in-patient studies, and their body composition will be measured by DEXA before and after the 3-month exercise interventions. Female subjects will undergo a urine pregnancy test to confirm continued eligibility before the completion of each DEXA scan. We also plan to measure hepatic fibrosis, the amount of fat stored in the liver and visceral fat (or intra-abdominal fat) using magnetic resonance imager (MRI) with elastography. This test will be performed twice, one before exercise training and again after 3 months of training. If this test is not able to be scheduled due to appointment availability, etc., in close proximity to subject’s pre- and post- training clinical studies, then these MRI tests will not be completed.

**Figure 2 – Timeline of events during the “clinical study”** (times are approximate)

To evaluate the responses to a single session of exercise, we may ask subjects to return to the laboratory on a separate occasion after they acclimate to their training program (i.e., after a few weeks of training). During this visit, we will obtain abdominal subcutaneous adipose tissue biopsies and blood samples before and after a single exercise training session. These visits will be performed in the Substrate Metabolism Laboratory in the Central Campus Recreation Building (CCRB). Data collected during this experiment will be analyzed separately from data collected as part of the main training intervention in order to specifically assess the ACUTE responses to exercise. Therefore, this separate visit is not required for interpretation of the data collected for the main project, which focuses on the adaptive responses to exercise training. As such, if this separate visit for assessing the acute responses to exercise is not able to be scheduled due to subject willingness, appointment availability, or required enrollment numbers are met for power calculations/analysis of the Mid-Training samples collected, etc., then this separate visit will not be completed. We are currently conducting a very similar project involving adipose tissue biopsies and blood draws in our lab in the CCRB to assess the acute effects of conventional exercise on adipose tissue metabolism in overweight adults (HUM00102522). This project will expand on this current project by comparing the effects of an acute session of high intensity interval training vs conventional exercise on adipose tissue metabolism in obese adults.

As noted above, subjects will undergo two separate overnight clinical studies after the 3-month training periods in order to distinguish between the acute effects of exercise after training from the more chronic adaptations to training (these studies will be conducted 3 days apart). Diet will be controlled and no exercise will be allowed between these visits.

***SOP for Skeletal Muscle and Subcutaneous Adipose Biopsies***

**Screening and pre-visit procedures**

* Anti-coagulant medication (e.g., Coumadin, Rivaroxaban) and Lidocaine allergy/sensitivity are exclusion criteria for the biopsy procedure.
* Aspirin (ASA) and/or other non-steroidal anti-inflammatory medications (NSAIDS; e.g., Ibuprofen, Naproxen) must be avoided for 3 days before the biopsy procedure (Tylenol can be used).

Before performing the tissue biopsies, Dr. Horowitz (or his qualified co-investigator who is performing the biopsies) will:

1. confirm the participant’s identification
2. thoroughly explain the biopsy procedures to the participant, describing what they may experience during the procedures.
3. confirm the location of the procedures (e.g., left or right thigh for muscle biopsy - and left or right of the umbilicus for the adipose tissue biopsy/aspiration

**Skeletal Muscle Biopsy Procedure**

* Confirm that the participant does not have any known allergies/sensitivity to local anesthetic (e.g., Lidocaine/Xylocaine) – this should also have been addressed before/during informed consent process.
* If necessary, shave ~5” x ~5” square above the vastus lateralis.
* Put on surgical gown, mask and bonnet (assistants in procedure room also must wear mask and bonnet).
* Disinfect skin above the vastus lateralis with betadine.
* Put on sterile gloves and all the following procedures must be performed using aseptic technique.
* Create sterile field on tray top and around the betadine covered area of the participant’s thigh.
* Infiltrate skin and underlying tissue with 2% lidocaine using a 25g x 1.5” needle (~6-10 ml of lidocaine: total dose of lidocaine not to exceed 4.5mg/kg).
* Make an incision above the vastus lateralis with a #10 scalpel (cut skin and try to cut fascia if possible).
* Apply pressure to incision site with sterile gauze until bleeding stops (or is very light).
* While pressure is being applied to the thigh, the biopsy assistant attaches a 3-way stopcock to a 60cc syringe.
* One end of sterile pressure tubing is attached to the sterilized biopsy needle (4-5g Bergstrom or UCH biopsy needle), and the other end of the tubing is handed to the assistant to attach to their 3-way stopcock/60cc syringe set-up for suction.
* The biopsy needle is inserted into the incision with firm pressure. The biopsy needle is inserted past the fascia, in the location/direction of the applied anesthetic.
* Once the biopsy needle is fully inserted, the biopsy assistant pulls back firmly on the plunger of the 60cc syringe to apply steady suction. The sample is collected by closure of the biopsy needle – To increase sample yield, the biopsy needle can be quickly rotated then opened and closed 3-5 times to obtain a few “snips” of sample before removing the biopsy needle from the thigh.
* The sample is placed on a sterile absorbent pad, and pressure is applied to the biopsy site with sterile gauze.
* The biopsy assistant quickly rinses/cleans the sample with saline and gently dabs the sample on the absorbent pad to remove excess liquid. The sample is placed in collection vial - and placed in liquid N2.
* While assistant is cleaning and storing the sample, manual pressure is applied to the incision site.
* The biopsy procedure can be repeated in the same incision site (or separate pre-prepared site) if more tissue is needed.
* When the entire biopsy procedure is finished, firm direct pressure is applied to the incision site until bleeding stops (~10 minutes).
* Incision site is cleaned with 70% ethanol and dried with sterile gauze.
* The incision is closed with steri-strips and an overlaying Tegaderm bandage (with absorbent pad).
* A pressure dressing is applied to the thigh until bedtime the night of the biopsy procedure.
* Participant is instructed to avoid water submersion for 2 days and to keep the bandages on for 5 days.
* Upon discharge, provide participant with pre-printed post-procedure care instructions with emergency contact information.

**Subcutaneous Adipose Biopsy Procedure**

* Confirm that the participant does not have any known allergies/sensitivity to local anesthetic (e.g., Lidocaine/Xylocaine) – this should also have been addressed before/during informed consent process.
* Clean the anterior abdominal wall in the peri-umbilical area with betadine.
* Put on sterile gloves and all the following procedures must be performed using aseptic technique.
* Create sterile field on tray top and around the betadine covered area of the participant’s abdomen.
* The location of the incision will be 5-10cm lateral to the umbilicus – infiltrate the skin and underlying tissue in this location with ~1-2ml of 2% lidocaine using a 25g x 1.5” needle. Then, with the lidocaine needle inserted at the intended incision site direct the needle at ~20 degree angle with the abdomen and anesthetize a quadrant of the abdomen with a 1.5” radius between the intended incision site and the umbilicus (this will anesthetize the sampling region). Total lidocaine dose for abdomen = 5-8ml of 2% lidocaine; total dose of lidocaine must not exceed 4.5mg/kg – this includes any lidocaine administered for any other procedures during the test day).
* Make an incision in this anesthetized site (5-10cm lateral to the umbilicus) with #10 or #11 scalpel.
* Apply pressure to incision site with sterile gauze until bleeding stops (or very light).
* While pressure is being applied to the abdomen, the biopsy assistant attaches a 3- way stopcock to a 60cc syringe.
* One end of sterile pressure tubing is attached to the sterilized biopsy needle (4-5g Bergstrom or UCH biopsy needle), and the other end of the tubing is handed to the assistant to attach to their 3-way stopcock/60cc syringe set-up for suction.
* For collection of the core tissue sample, insert the biopsy needle (4-5g Bergstrom or UCH biopsy needle) into the incision, directed at ~20 degree angle with abdomen. The biopsy needle is inserted ~2cm in the direction of the anesthetized area (toward the umbilicus).
* Once the biopsy needle is inserted ~2cm, suction is applied to the needle (by an assistant) via an attached extension tubing and 60cc syringe. The sample is collected by closure of the biopsy needle – and extracted.
* The sample is placed on a sterile absorbent pad. The biopsy assistant rinses and cleans the sample with saline and gently dabs the sample on a on absorbent pad to remove excess liquid.
* The sample is placed in collection vial (typically containing formalin).
* The biopsy procedure can be repeated in the same incision site if a larger core sample is needed.
* After completing the core sample collection, more tissue can extracted using aspiration.
* For the subcutaneous adipose tissue aspiration method, attach a sterilized aspiration needle (e.g., Spirotri cannula) to sterile pressure tubing, attached to sterile 60cc syringe with stopcock attached.
* Insert aspiration needle into incision site (~3cm), retract plunger, and apply constant negative pressure with the syringe during the aspiration process.
* With needle inserted into this incision site, one hand lightly pinches the surface of the biopsy site, while the other hand gentle maneuvers the biopsy needle under the skin for 30-60 seconds to extract tissue (tissue will accumulate in the syringe).
* Remove aspiration needle from incision site – The extracted tissue can be removed from the syringe either by ejecting through the tip of the syringe – or by removing the syringe plunger and pouring the tissue out. In both cases the tissue should be placed on a sterile absorbent pad for the biopsy assistant to clean and process the sample.
* Aspiration procedure can then be repeated in the same incision, but try to direct the needle in a slightly different “track” within the anesthetized area in order to avoid tissue collection within exactly the same area.
* After completion of all tissue extraction, pressure should be applied to the biopsy site for 5-10 min. • Clean biopsy site with alcohol, place steri-strip on site to close the small incision, and tegaderm bandage is then placed over the steri-strips.
* Apply ice to biopsy site for 10-20 min.
* Participant is instructed to keep the bandages on for 5 days.
* Upon discharge, provide participant with pre-printed post-procedure care instructions with emergency contact information.

As an alternative approach, a 1.5in x 16g needle or a “Coleman needle” may be used for tissue aspiration instead of the Spirotri cannula, under the same conditions as described above. However, when using these alternative needles, more aspiration “passes” may be required to extract the necessary amount of adipose tissue.

**Continuous glucose monitoring (CGM)**

In addition to measuring insulin resistance in the clinical studies via the hyperinsulinemic-euglycemic clamp, we will also assess “free-living” 24h glucose control using CGM (IPRO2 Professional; Medtronic MiniMed, Inc.) We will perform CGM over a 48h period (from 0900h on one day until 0900h two days later - on an out-patient basis). Before beginning the exercise treatments, subjects will come in for CGM placement and then they will be discharged to resume their normal sedentary lifestyles during this 48h collection period. At the end of the 3-month exercise interventions, after placement of the CGM device on the first morning of the measurement, subjects will be required to perform their prescribed exercise regimen at 1000h, and they will not exercise the next day. This approach will allow us to capture 24h glucose control on both an exercise- and non-exercise day. Importantly, standardized meals will be provided and will be consumed at specific times each day of the CGM measurement period. Subjects will log the exact time of each meal for confirmation. These meals will be identical on day 1 and day 2 of measurement and they will also be the same before and at the end of the intervention. These CGM measurements will be performed 1-2 weeks before the in-patient clinical studies. Dr. Rodica Pop-Busui (Internal Medicine) has extensive experience using these CGM devices for her IRB-approved protocols, and she will be assisting with this portion of the study.

**Exercise training interventions**

After completing the baseline overnight clinical study, subjects will be randomized into one of four exercise training groups (details below). There will be a total of 30 subjects in each training group and the distribution of men and women will be divided equally among the groups.

All subjects will first undergo a familiarization program for 1 week (4d/wk) during which they all will perform “conventional” exercise (i.e., steady-state) at 65%HRmax for 25 min/session. After this 1 week familiarization period, subjects will begin their specific training program based on their training group randomization.

1. **HIIT#1** = *10 x 1min at ~90%HRmax [25min; ~150kcals]***:** The first session of the “ramp-up” period for HIIT will entail 19min of conventional exercise at 65%HRmax, followed immediately by 2 x 1min intervals at 90%HRmax with a 1 min active recovery period between intervals at 50%HRmax, and a 3min cool down at 60%HRmax after the second interval. After that first ramp up training session, 2 additional high intensity intervals will be added to each training session, so by the 2nd week of HIIT training, subjects will reach the full HIIT training protocol of total of 10 x 1min intervals at 90%HRmax (Figure 3), and they will continue this training protocol (4d/wk) throughout the 3 month intervention. Subjects who may feel challenged by the rate of increase in the number of intervals during this ramp up period will be allowed to increase the number of intervals at a slower pace, but all subjects must reach the full HIIT protocol during the 4th week of HIIT training. We do not believe that completing a few less intervals each session during the first few weeks of the 3 month training program will influence the outcomes.

3min

warm-up

60%HRmax

3min

cool-down

60%HRmax

10 x 1min 90%HRmax intervals with

1min recovery periods at 50%HRmax

Total exercise time = 25min

**Figure 3 – HIIT Protocol**

1. **MICT** =steady-state exercise at 60-70%HRmax for 45min**:** Subjects randomized to **MICT** will undergo a ramp-up period to build up to a total exercise time of 45min. During the first week of ramp-up, subjects will warm up for 3 min at 60%HRmax, followed by 25min at 70%HRmax and a 3 min cool-down. Subjects will increase exercise time at 70%HRmax by 10 min each week, until they reach a total exercise time of 45min (Figure 4), which should be achieved by week 3 of training. Subjects who feel challenged by the rate of increase during this ramp up period will be allowed to ramp up at a slower pace, but all subjects must reach a total exercise time of 45min by the 5th week of training.

Total exercise time = 45min

Steady-state exercise

@ 70%HRmax

3min

warm-up

60%HRmax

3min

cool-down

60%HRmax

**Figure 4 – MICT Protocol**

1. **HIIT#2** = *5 x 1min at ~90%HRmax + 10min of steady-state exercise at ~70%HRmax [25min; ~150kcals].* During the first session of ramp-up for **HIIT#2**, subjects will warm up for 3 min at 60%HRmax, then the exercise intensity will increase slightly to 70%HRmax for the next 10min, followed immediately by 2 x 1min intervals of exercise at 90%HRmax with a 1 min active recovery period between intervals at 50%HRmax, and a 3min cool down at 60%HRmax after the second interval. After this first ramp up training session, 2 additional high intensity intervals will be added to each training session, so by the 1st week of **HIIT#2**, subjects will reach the full **HIIT#2** training protocol (Figure 5), and they will continue with this training protocol (4d/wk) throughout the intervention. Similar to all other training protocols if subjects feel challenged by the rate of increase during the ramp up period, they will be allowed to increase the number of intervals at a slower pace, but all subjects must reach the full HIIT#2 protocol during the 3rd week of **HIIT#2** training.

3min

warm-up

60%HRmax

3min

cool-down

60%HRmax

5 x 1min 90%HRmax intervals with

1min recovery periods at 50%HRmax

Total exercise time = 25min

10min

@ 70%HRmax

**Figure 5**

1. **HIIT#3** =*5 x 1min at ~90%HRmax [15min; ~90kcals]*: The first ramp-up session for **HIIT#3** will entail 9 min of conventional exercise at 65%HRmax, followed immediately by 2 x 1min intervals of exercise at 90%HRmax with a 1 min active recovery period between intervals at 50%HRmax, and a 3min cool down at 60%HRmax after the second interval. Thereafter, 2 additional intervals will be added to each training session, so by the 1st week of the **HIIT#3** training, subjects will reach the full **HIIT#3** training protocol (Figure 6), and they will continue with this training protocol (4d/wk) throughout the intervention. Subjects who feel challenged by the rate of increase in the number of intervals during this ramp up period will be allowed to increase the number of intervals at a slower pace, but all subjects must reach the full HIIT#3 protocol during the 3rd week of training.

3min

warm-up

60%HRmax

3min

cool-down

60%HRmax

5 x1min 90%HRmax intervals with

1min recovery periods at 50%HRmax

Total exercise time = 15min

**Figure 6**

*Participant safety and well-being during the exercise intervention*s

Caution is always warranted when initiating an exercise training study in sedentary obese adults, but there is certainly additional concern about risk when participants are required to exercise at high intensity. We take this issue very seriously and extreme caution will be taken throughout the project to greatly reduce risk associated with the exercise training programs. Subjects will be closely monitored throughout the training period. All training sessions during the first 2 weeks of the training period will be supervised. These supervised sessions will occur either in the Physical Medicine and Rehabilitation’s “Transitions Studio” facility in the Burlington Building, the Physical Activity Laboratory (PAL) of the Michigan Nutrition and Obesity Research Center (Burlington Building), or in Dr. Horowitz’s Laboratory (1210 CCRB). No high intensity exercise will be performed during the first week of training for any group – and subjects in the HIIT groups will be slowly ramped up to the prescribed training program at a rate that is comfortable for them (as described above). It is important to note that several studies have now been published demonstrating that these HIIT protocols identical to that proposed in this application are safe and well-tolerated in clinical populations, including obesity, cardiovascular disease, and type 2 diabetes (for review see: Gibala, et al., J. Phyisiol. vol. 590, 2012. ((5))). For the next 2.5 months we will supervise at least 2 training sessions per week and non-supervised training sessions will be monitors by activity monitoring devices (e.g., Polar A300 Fitness and Activity Tracker). During this 2.5 month period of the training program the supervised exercise sessions may occur in the University of Michigan Recreational Sports facilities (e.g., CCRB, IMSB, NCRB) in addition to the exercise training locations listed above. After the first 3 months of the intervention, we will be testing participants willingness to adhere to the different exercise program over the longer-term in a more “real-world”/unstructured paradigm (see details below). Therefore, after 3 months of training, adherence to the exercise programs will be monitored using the activity monitoring devices, with no direct supervision. Throughout the entire training program the exercise modality will be self-selected (e.g., walk/run (outdoors or treadmill), elliptical machine, stair climbing, cycling), and subjects will be encouraged to regularly vary the exercise modality used during the 3-month training period to reduce risk of overuse injury and to enhance the variety of the exercise program. Any injuries that occur during training will be managed on a case-by-case basis. For relatively minor injuries (e.g., strained muscle, shin splints, minor joint discomfort) the Exercise Physiologists on the research team will closely monitor the participants’ activity and revise (or suspend) the training program to aid in recovery. For more severe musculoskeletal injuries (e.g., sprained ankle/knee, etc…) Research Physicians (e.g., Dr. Charles Burant) will be consulted and the subjects’ continued participation in their prescribed exercise protocol will be evaluated on a case by case basis. All injuries will be monitored closely, and we will assure that all subjects receive appropriate medical attention.

***Exercise adherence***

After completing the first 3 months of supervised exercise training, subjects will be asked to continue their prescribed training program on their own for an additional 9 months. Subjects will be provided with a wearable (wrist) physical activity monitor (Polar A300 Fitness and Activity Tracker) with a visible heart rate display. Importantly, the data from these devices are downloaded wirelessly to a computer and/or mobile device, which then automatically updates their physical activity profile on their personal web-page. Our research study coordinator (Suzette Howton) will receive permission to access each subject’s physical activity profile, enabling us to assess their adherence remotely. The data reported on the website includes 24h profile of heart rate and calories expended allowing us to clearly distinguish adherence to the HIIT and MICT regimens. We will assess each subjects’ physical activity profile on a weekly basis – and record how many prescribed exercise sessions were successfully completed. During the final month of training, subjects will be contacted to make arrangements for a brief follow-up visit for a quick set of standard clinical assessments (e.g., body weight, body composition, HbA1c, blood lipid profile, blood pressure).

The physical activity monitors will be used from the beginning of the study, so the use of these devices will be routine well before the participants are asked to continue exercising on their own. Based on our past research participant database, nearly all subjects (>95%) provide a mobile phone number and/or e-mail address as their primary contact. Therefore, it is likely most study participants will have appropriate technology necessary for our planned method for tracking their adherence. However, in instances where participants do not have access to the appropriate technology, we will make alternative arragements. Some alternative options include: providing them with a relatively inexpensive mobile device with the necessary technology (e.g., older model ipod touch), provide recordable heart rate monitors to wear during each exercise session, collect training logs.

*Dietary control during the exercise interventions*

Subjects will adhere to a weight-maintaining diet throughout the first 3 months of the training period. They will meet with our research dietitian before starting the training program and periodically throughout the training protocol. We will also track their dietary intake via the “My Fitness Pal” program. Subjects will record their dietary intake in *My Fitness Pal* (available for mobile devices and desktop computers), and our research dietitian (Suzette Howton) will gain permission to access their personal *My Fitness Pal* accounts. For participants who do not have easy access to the necessary technology for this approach, alternative accommodations will be made to capture their dietary information (e.g., diet journals, diet recalls). Body weight will be recorded before every supervised exercise training session and if body weight changes ±2kg from their initial weight, subjects will consult with our research dietitian for strategies to maintain weight during this 3-month training period. Diets will not be controlled during the 9-month outpatient adherence portion of the study.

**Statistical power/sample size calculation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 1. Powers/sample size calculations for Specific Aim #1** | | | | | |
|  | **Power** | **** | **Expected**  ** (%)** | **Estimated**  **SD (%)** | **# of subjects** |
| **Insulin resistance** | 0.8 | 0.05 | 15% | 12% | 13 |
| **24h glucose control** | 0.8 | 0.05 | 10% | 10% | 17 |
| **Plasma [LDL]** | 0.8 | 0.05 | 10% | 10% | 8 |
| **Hepatic lipid accumulation** | 0.8 | 0.05 | 20% | 25% | 24 |
| **Visceral Adiposity** | 0.8 | 0.05 | 12% | 14% | 22 |
| **Muscle lipid accumulation** | 0.8 | 0.05 | 20% | 20% | 17 |
| **Macrophage infiltration** | 0.8 | 0.05 | 20% | 20% | 17 |
| **Cytokine abundance** | 0.8 | 0.05 | 30% | 30% | 17 |

The number of subjects necessary to find statistical differences in insulin action between **HIIT** and **MICT** (Specific Aim #1)was calculated based on recent work by Karstoft and colleagues (12, 13). These authors reported insulin sensitivity (measured using clamp methods) was ~25% greater after 4 months of HIIT compared with MICT (13). Using an even more conservative estimate for the difference in insulin resistance between our HIIT vs. MICT compared with that reported by Karstoft, et al., our sample size calculation indicates that in order for a 15% difference in insulin resistance between the groups to be statistically significant, we will need 13 subjects in each group (Table 1). In a separate study, Karstoft et al., (12) found mean 24h glucose concentration after HIIT to be ~10% lower than after MICT. From these data, we calculate we would need 17 subjects in each group in our study for this difference to be statistically significant. Because a primary objective of Specific Aim #1 is to determine if HIIT improves other key clinical and sub-clinical cardio-metabolic health outcomes, our sample size calculations for the other major endpoints of this Aim were performed based on the estimated/anticipated changes in these endpoints in response to HIIT (from our preliminary findings and data from previous studies (12, 13)) rather than on expected differences between HIIT and MICT. Our sample size calculations for these endpoints indicate that we may need up to 24 subjects for the anticipated changes in response to HIIT to be statistically significant (Table 1). We anticipate ~20% attrition in this training study, so we plan to recruit **30 subjects in each training group.**

For Specific Aim #2, we anticipate that the differences in insulin resistance will be a bit more subtle between the different HIIT programs compared with the difference between HIIT and MICT (as in Specific Aim #1). Based on the calculations presented in Table 1, we predict we will need a total of 23 subjects in each group in order for a 10% difference in insulin resistance to be statistically significant. We also anticipate the difference in adherence between HIIT and MICT after 1 year of training will be smaller than the 25% difference we observed after 1 month in our preliminary study, while the variability may be greater. Therefore, we may need up to 24 subjects in each group (Table 2). Similar to Specific Aims #1 and #2, because a primary objective of this Aim is to determine if 3 months of the different HIIT programs reduce markers of inflammation, as well as lipid accumulation in muscle and liver, our sample size calculations for the other major endpoints of this Aim were performed based on the estimated/anticipated changes in these endpoints in response to HIIT (rather than on comparisons among groups). Our sample size calculations for these endpoints indicate that we may need up to 24 subjects for the anticipated changes in response to HIIT to be statistically significant (Table 2). We anticipate ~20% attrition in this training study, so we plan to recruit **30 subjects in each training group.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Table 2. Powers/sample size calculations for Specific Aim #2** | | | | | |
|  | **Power** | **** | **Expected**  ** (%)** | **Estimated**  **SD (%)** | **# of subjects** |
| **Insulin resistance** | 0.8 | 0.05 | 10% | 12% | 23 |
| **Exercise Adherence** | 0.8 | 0.05 | 20% | 25% | 24 |
| **Macrophage infiltration** | 0.8 | 0.05 | 20% | 20% | 17 |
| **Cytokine abundance** | 0.8 | 0.05 | 30% | 30% | 17 |
| **Muscle lipid accumulation** | 0.8 | 0.05 | 20% | 20% | 17 |
| **Hepatic lipid accumulation** | 0.8 | 0.05 | 20% | 25% | 24 |

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