Exercise Dose and Metformin for Vascular Health in Adults With Metabolic Syndrome

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INTERVENTIONAL RESEARCH PROTOCOL
(HRP-503a)

STUDY INFORMATION

- **Title of Project:**
  Exercise dose and metformin for vascular health in adults with metabolic syndrome

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1.0 Research Design

1.1 Purpose/Specific Aims

The overall purpose of the study is to test the effect of metformin on exercise intensity based training adaptations in adults with metabolic syndrome.

A. Objectives

Understand effect of metformin on training intensity mediated vascular versus metabolic insulin sensitivity in adults with metabolic syndrome for clinical health.

B. Hypotheses / Research Question(s)

The overarching hypothesis is that metformin may blunt adaptation following high intensity exercise by lowering/attenuating oxidative stress. Thus, compared with high intensity exercise plus metformin, low intensity exercise plus metformin will produce greater vascular insulin sensitivity and metabolic insulin sensitivity changes following 16 weeks of treatment. In addition, we anticipate that high intensity exercise based training alone will produce greater effects than low intensity exercise. Lastly, we hypothesize that these changes in metabolic and vascular insulin sensitivity will correlate with glycemic control and blood pressure changes.

1.2 Research Significance

Metabolic Syndrome (MetS) is associated with a 3-fold cardiovascular disease (CVD) mortality risk compared with healthy controls, and it is estimated that 40% of the U.S. will have MetS by 2020 (1). MetS is characterized by elevated blood glucose, hypertension, carotid artery thickness, dyslipidemia, and obesity (2). Although skeletal muscle insulin resistance is an important underlying cause of MetS, vascular insulin resistance, particularly at the microvasculature level, may actually contribute to the pathogenesis of metabolic insulin resistance and drive the CV complications in type 2 diabetes (T2D) (3). Here we propose that vascular insulin resistance is central to CVD risk reduction in MetS and interventions are needed that target enhancing endothelial function and decreasing arterial stiffness. High intensity exercise (HiEx) is an established treatment to reduce CVD risk in MetS patients (4-7) by, in part, improving basal endothelial function and arterial stiffness (8-10), although this has not tested under insulin-stimulated conditions. Metformin is a 1st line agent to treat hyperglycemia due to in part to its ability to reduce hepatic glucose output, and protect against CV morbidity and mortality as evidenced by the UKPDS (11). Surprisingly, our preliminary work in MetS/prediabetic individuals shows that metformin may ameliorate CVD risk, but when combined with HiEx there tends to be a blunting effect on skeletal muscle metabolic insulin resistance (12). One attractive alternative to promote vascular and metabolic adaptation, and test the additive effects of metformin on exercise, is to prescribe exercise that does not primarily rely on mitochondrial oxidative stress to induce adaptation, i.e. low intensity exercise. Herein, we propose that metformin may interact with exercise in an intensity based manner for adaptations related to skeletal muscle metabolic and/or vascular insulin resistance. These findings will have direct impact on public health for glycemic control and blood pressure regulation as well as inform how metformin interacts with physical activity behavior.

1.3 Research Design and Methods

This is a randomized by sex, double-blind, placebo controlled trial. People with metabolic syndrome will undergo exercise at low and high intensity with placebo, and this will serve as the control to the same exercise programs but with metformin. During this trial, subjects in the metformin group will receive only regular metformin, not metformin ER.

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A. Research Procedures

Note: All assessments outlined in this consent are being done for research purposes only. In light of COVID-19, we will follow routine safety checks per University guidelines until otherwise deemed unnecessary. These checks will be implemented at time of in person visits and consist of: temperature check and questions related to exposure yourself or to others with COVID-19. Face masks should also be worn unless otherwise indicated.

PHASE 1: SCREENING AND TESTING PROCEDURE
*Note: Portions of screening visits 1 and 2 can be combined to ease your burden and number of visits.

Screening Visit 1 at the Clinical Research Center (CRC) or Institute for Food, Nutrition and Health (IFNH) (Day 1) (will last about 1- 2 hours)

Prior to signing this consent, subjects were contacted by a member of the study following your interest in this study. If subjects agree to participate, subjects will sign this consent form before any study related procedures take place. Before subjects can start the study, there will be a screening period. Subjects will have tests and procedures during this time to make sure they are eligible, and it is safe for participation. Subjects are to fast (nothing to eat or drink after midnight) for blood work. Water is ok. These include the following:

- Health history and Physical Activity Readiness Questionnaire (PAR-Q) that will take about 30 minutes to complete
- Vital signs (blood pressure, heart rate)
- Subjects height, weight, and waist circumference will be measured.
- Blood draw will also be performed for laboratory testing.
- Subjects body mass index (BMI) will then be calculated. It must be between 25-47 kg/m^2 for you to be able to continue in this study.

PLEASE NOTE: Subjects continued participation in the study after this point will depend on the results of the vital signs, waist measure as well as blood sugar and lipid results. Further, if subjects are a woman who is able to bear a child, subjects will have a pregnancy test that must be negative in order to participate – this will be determined via urine collected at the screening.

Screening Visit 2 at the Clinical Research Center (CRC) or IFNH or Foran Hall
(will last about 1-2 hours)

IMPORTANT: Subjects must fast (not eat, water ok) for 4-10 hours before this visit. During the second screening visit the following assessments will take place at the CRC or IFNH or Foran Hall:

- Review of your medical history
- Physical exam and vital signs (blood pressure, heart rate) will be conducted.
- Resting electrocardiogram (ECG) to see the electrical activity of your heart will also be done.

If subjects continue to qualify for the study the remainder of the visit will involve the study procedures described below:

A. Body Composition and Resting Metabolism:

- Subjects must be fasting for at least 4-10 hours before this procedure.
- Subjects weight will be measured without shoes and while wearing minimal clothing.
- Subjects waist and hip circumference ratio will be obtained with a tape measure to determine how much fat is located in the central part of your body.

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- The total amount of fat and muscle in the subjects body will be measured via DEXA in Foran Hall.
- Subjects will be required to wear clothes without metal on them (including zippers and bras with an underwire). If their clothes have metal, we will ask that them to change into a pair of shorts and a t-shirt or wear a hospital gown provided by the lab.
- If there are technical issues with the DEXA machine, we will measure the total amount of fat and muscle in the subjects body with the BodPod® in the IFNH.
    - We will provide a swimsuit and swim cap for subjects to wear during this procedure to help standardize your results. The swimsuit and swim cap are provided to get accurate results. Subjects will not get wet at any time during this measurement. Subjects will enter the device and sit down. A door will close while they sit in the BodPod®. Subjects are asked to breath normal and be still. The BodPod® looks like a large white egg and has a window on the door.
- We will also measure subjects resting metabolic rate, a measure of the amount of energy it takes to burn calories. While laying down subjects will wear a canopy (cover) that is connected to a special device (metabolic cart) that measures exhaled carbon dioxide (CO_2) and oxygen through indirect calorimetry. These measurements are then used to calculate the number of calories burned to determine food needs.

B. Treadmill exercise testing for cardiovascular fitness (i.e. VO2max):

- Subjects will be asked to perform a maximal treadmill exercise test in the CRC or the IFNH. The test will begin with low speed on the treadmill, and the resistance will increase gradually every 2 minutes. Subjects will be asked to go as long as you can, that is, until you feel exhausted.
- During the test, Subjects may have continuous electrocardiogram (ECG) heart monitoring and blood pressure monitoring if you are considered at risk.
- During exercise testing we will also measure subjects metabolic rate, a measure of the amount of energy it takes to burn calories. During exercise they will wear a facemask that is connected to a special device (metabolic cart) that measures your exhaled carbon dioxide (CO_2) and oxygen through indirect calorimetry. These measurements are then used to calculate the amount of calories you burn.
- During the exercise test, subjects will wear a mask over your nose and mouth. This allows us to measure how much oxygen they are using and will tell us what their fitness level is.
- The visit ends after the subject completes the exercise test. Subjects will receive the results of their test after the completion of the study.
- Subjects will also be provided with an **accelerometer** and instructions on how to use the accelerometer. The accelerometer is a small device, similar to a pedometer, which is worn on their belt and records the amount of activity performed. Data will be downloaded when the device is returned.

C. Physical Activity questionnaire

- *Minnesota Leisure Time Physical Activity:* This is a checklist of 60 physical or recreational activities subjects may have participated in over the last 12 months. This takes about 10 minutes to complete.

D. Diet logs and appetite questionnaires

- Subjects will fill out a daily record of eating habits for 3 days at weeks 0, 4, 8, 12 and 16. Subjects will also answer questions that test responses to the food eaten. This takes about 30 minutes to complete.

E. Quality of life questionnaires

- Physical activity enjoyment: We will also provide subjects with a questionnaire to understand feelings about the exercise program at weeks 0, 4, 8, 12 and 16. This takes about 5 minutes to complete
- Sleep History: Subjects will fill out questions to gauge sleep patterns throughout the study at weeks 0, 4, 8, 12 and 16. This takes about 5 minutes to complete

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- Morningness-Eveningness questionnaire: Subjects will answer questions about your daily activity at weeks 0 and 16. This will take about 5 minutes.
- Veteran Rand-36: Subjects will also complete a series of questions related to stress, anxiety, overall happiness, etc. that will have you think objectively about their quality of life at weeks 0, 8 and 16. This takes about 10 minutes to complete.
- Three Factor Eating: Subjects will answer a series of questions related to emotional and behavioral eating patterns. This will be completed at weeks 0, 8, 16, and 24. This takes about 10 minutes to complete.

PHASE 2: TESTING CONDITIONS AND PROCEDURES

Study test Visits 3 and 4

- While subjects are in the study, they will be asked to maintain your normal activity levels.
- Subjects will be provided standardized meals and snacks the day prior to and during all blood test visits (listed below) consisting of 55% carbohydrate, 30% fat, 15% protein.
- **Subjects must not drink alcoholic or caffeinated beverages for at least 24 hours before the study testing visit begins.**
- Subjects must not use allergy, prescription or pain-related medicines (over-the-counter or prescription) or antioxidant dietary supplements for at least 24 hours prior to each testing visit. Prescription meds may be taken after testing.
- Subjects must not perform any vigorous exercise (outside of this training study) for 72 hours prior to each test day.
- **The tests must be performed in the fasted state, so you may not eat or drink anything (except water) after about 9:00 pm the night before.**
- You should not take antibiotics 3-4 week before study testing. If you have been prescribed an antibiotic 3-4 weeks before the study or are prescribed an antibiotic during the study please inform the research team.
- Subjects will be asked to report to the CRC by about 6:30-9:00am on the morning of each test.

Pre and Post Study Testing days:

Blood draws:

- In the morning of the pre and post study testing days in the CRC, an IV catheter will be inserted into a vein in either the forearm or hand. An IV is a small flexible tube that is inserted into a vein guided by a needle. Once the tube is in place the needle is removed and replaced with cap that allows blood to be withdrawn or fluids or medications to be given
- The IV catheter will be in the subjects arm for the remainder of each visit (about 4 hours) and removed before they leave the Clinical Research Center.
- Blood we take will be tested to measure sugars, lipids, and hormones that estimate blood glucose and vessel health.
    - Visits 3 and 55: about 8 tablespoons of blood (40 cc) will be drawn per visit.
    - Visits 4 and 54: about 18 tablespoons of blood (90cc) will be drawn per visit.
    - Visit 58: about 10 tablespoons of blood (50 cc) will be drawn.
    - Additional blood (about 1 tablespoon or 15 cc) may be drawn to verify results of the screening visit at the discretion of the investigator.
- When the blood draws are completed subjects will be fed lunch and discharged to go home.
- The amount of blood collected pre and post study is approximately the same as donating a pint of blood. As a result, subjects are advised not to donate blood while participating in this study.
- Visit 3 and visit 4 tests will be performed about 24 hours or more apart.

Glucose Metabolism test (i.e. blood sugar): Visit 3 or 4, 54 or 55, and 58

Oral Glucose Tolerance Test (OGTT) (approximately 4 hours)

- Subjects will report to the CRC fasted for about 10 hours and participate in an OGTT, and we will put an intravenous catheters (IV) into a vein in the arm and obtain baseline blood samples.

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- The OGTT is used to help determine how quickly sugar is removed from the blood. After the blood sample is taken for the fasting blood sugar test, subjects will drink a sugary solution. Five more blood draws will be taken at 30, 60, 90, 120, and 180 minutes after subjects receive the drink. This procedure will also include a measure of blood flow at 0, 60 and 120 called flow-mediated dilation (see below).
- Participants will complete items on the NIH toolbox for related to working/episodic memory, executive function and attention. The NIH toolbox is an iPad application that consists of cognitive, emotional, motor and sensory function assessments. This will take about 20 minutes and will be completed prior to consuming the 75g and after the 120 minute mark.

Euglycemic-Hyperinsulinemic Clamp (approximately 6 hours)

- Subjects will arrive at the CRC fasted for about 10 hours, and we will put two intravenous catheters (IV) into two vein in the subjects arm and obtain baseline blood samples.
- During this visit, we will perform the 2-hour euglycemic-hyperinsulinemic clamp or "clamp" study. This test is the gold standard for measuring sensitivity to insulin.
- Since the clamp study can be significantly enhanced by the use of glucose tracers, we will inject a glucose labeled with tracer (~2 drops) called dideuterated glucose (Dideuterated glucose is non-radioactive). The tracer contains a very small amount of heavy hydrogen ($^2H_2$), which makes the glucose more identifiable. A small percentage of heavy hydrogen is naturally present in your body (0.015%). What we do is add to this natural pool and measure its concentration in the blood. This allows us to determine how insulin is controlling your rate of liver glucose production vs. skeletal muscle glucose uptake. The infusion of dideuterated glucose through the IV is continued during an entire 4-hour period (2 hour before the clamp and 2 hour for the clamp, i.e. about 4 hour total).
- Subjects will not eat, drink, or take any medications/vitamins on the morning of the test.
- Subjects will be given a meal once the "clamp" study is complete.
- Insulin will be infused at a constant rate to maintain the blood concentration at approximately 100 µU/ml. Subjects blood sugar concentration will be measured every 5 minutes and infused at a variable rate to maintain a normal blood sugar level (90 mg/dl). Blood draws for insulin will occur every 30 minutes up to 90 minutes, and then every 10 minutes until the end of the clamp.
- Subjects will also have a see-through canopy (cover) over their head before and at the end of the clamp. This procedure uses the same machine used during exercise in screening visit 2, part B above. This device allows us to measure how much sugar and fat you are using to create energy. Also, by measuring and analyzing the oxygen and carbon dioxide levels in the subjects breath it is possible to estimate if subjects are storing glucose as glycogen.

Urine Collection:

- Subjects will be provided with a plastic container and asked to collect one urine void on visit 3 and 4. The amount and time of subjects urine collection will be recorded and analyzed for nitrogen, protein and metabolites.

Blood flow to be done at Visit 3 and/or 4, 54 and 58:

Blood pressure:

- Subjects blood pressure will be taken at the beginning of each testing visit. This test takes about 5 minutes.

Large artery flow-mediated dilation (FMD):

- This test allows us to measure the blood flow through the subjects arteries.

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- This procedure uses an ultrasound device to measure blood flow in an artery in the subjects upper arm and leg. Ultrasound is a diagnostic procedure that creates a picture image using sound waves. Unlike X-rays, ultrasound does not involve radiation.
- Just prior to the test subjects will be asked to lie down on a bed quietly for 20 minutes. During this time, subjects will have blood pressure taken, and subjects will be connected to an electrocardiogram (ECG) that will monitor your heart rate. After the 20 minutes have passed, an ultrasound probe will be lightly pressed against the inside of the subjects upper arm and leg.
- Next we will place a blood pressure cuff on the subjects forearm only and pump it up tightly. This portion of the test may cause some discomfort in their forearm and fingers (such as pain, tingling, and numbness).
- We will keep the cuff inflated for 5 minutes, take images, then release it, and measure the increase in blood flow in one of the arteries in your upper arm using the ultrasound probe.
- This procedure takes about 15 minutes. We will perform this procedure just before the glucose beverage during the OGTT, and then again at 1, and 2 hours after you drink the glucose beverage. For the clamp test, we will perform blood flow measures once at the beginning and once at the end.

Common carotid artery intima-media thickness (CCA-IMT)

- This test allows us to measure the level of blood vessel thickness in the artery.
- This procedure uses an ultrasound device, similar to FMD above, to measure degree of vascular tissue thickness between the inner 2 layers of the vessel wall in an artery of the upper neck. Ultrasound is a procedure that creates a picture image using sound waves. Unlike X-rays, ultrasound does not involve radiation.
- This procedure takes about 15-20 minutes.
- Prior to the test the subject will be asked to lie down on a bed quietly for about 15 minutes. During this time, blood pressure will be taken, and the subject will be connected to an electrocardiogram (ECG) that will monitor heart rate. Then an ultrasound probe will be lightly pressed against the subjects neck. Images will be taken for about 5 minutes.
- We will perform this procedure before the clamp or OGTT method. If neither test time is possible (e.g. due to personnel or instrument), a separate visit may be used where use of the Cardiology Echo Lab in the hospital is used.

Heart images:

- This procedure takes about 30-40 minutes.
- Prior to the test the subject will be asked to lie down on a bed quietly for 20 minutes. During this time, blood pressure will be taken, and the subject will be connected to an electrocardiogram (ECG) that will monitor heart rate. After the 20 minutes have passed, an ultrasound probe will be lightly pressed against their chest. Images will be taken.
- This procedure uses an ultrasound device to heart size and function
- The images collected use the same approach as described above with FMD.
- We will perform this procedure just before the clamp (or OGTT method). If neither test time is possible (e.g. due to personnel or instrument), a separate visit may be used where use of the Cardiology Echo Lab in the hospital is used.

Augmentation Index (AIx):

- This measures the pulse wave of the vessel and its characteristics that assess the stiffness of the aorta. We will use a waveform analysis from SphygmoCor.
- This is done by placing a blood pressure cuff on the subjects upper arm. The blood pressure cuff will inflate three different times to capture your brachial waveform.
- This will take about 5 minutes. We will perform this procedure before and at 1 as well as 2 hours after the clamp.

Pulse Wave Velocity (PWV):

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- This measures the time difference between the pulse wave at the carotid artery and the pulse wave at the femoral artery. A blood pressure cuff will be placed on the subjects upper thigh to measure your femoral pulse while we measure the subjects carotid pulse with a doppler pen. This will allow us to assess the "stiffness" of the larger vessels. The shorter the time difference, the stiffer or less elastic the vessels are.
- We will use the same SphygmoCor device as described above for AI.
- This will take about 5 minutes. We will perform this procedure before and 2 hours after the clamp technique.

Contrast Enhanced Ultrasound (CEU):

- This is used to look at muscle blood flow at the microcirculation (or closest part of blood flow to muscle/heart).
- We will infuse the microbubbles (brand name is Definity) that are approved by the FDA for imaging of the microvasculature using similar techniques to FMD described above with ultrasound.
- This will take about 10 minutes and will occur before and after the 2 hour clamp technique during.

RANDOMIZATION and STUDY PROCEDURES

Subjects will be randomly assigned (like the flip of a coin) to 1 of 4 study treatment groups. Subjects have an equal chance of being assigned to any one of the groups. Neither the subject nor their doctor can choose which treatment they are assigned. Neither subject nor their doctor will know which study treatment they will get until the study is done. If the subjects doctor needs to know whether metformin or placebo pill is prescribed due to medical reasons that directly affects medical care of the subject, the people doing this study can find out.

PHASE 3: INTEREVENTION PERIOD (Visits 5-53)

Ambulatory Blood Pressure:

- This is used to look at blood pressure throughout the entire day and night for 24 hours.
- This measure occurs using a blood pressure cuff that is wrapped around the subjects arm with a holter monitor secured to their belt that contains a battery and recording device.
- Subjects are instructed to go about their normal activities throughout the day.
- During periods of inflation and deflation, we ask subjects to relax their arm.
- This test will occur before the first training session.
- At the same time, subjects will be given standardized meals to follow for the 24-hr collection period before and after the intervention.

The Ambulatory Blood Pressure will occur before the intervention period and within the last week of the intervention.

Prior to the intervention subjects will be randomized (like the flip of a coin) to receive 1 of 4 treatment options for 16 weeks – all of which expend a matched number of calories per week. *Note, due to COVID-19 if supervised exercise sessions need to vary for safety reasons, virtual sessions will be established within reason to maintain "supervised" sessions.*

- Low Intensity Exercise Training (LoEx) + Placebo
- High Intensity Exercise Training (HiEx) + Placebo
- LoEx + Metformin
- HiEx + Metformin

LoEx + Placebo: If subjects are randomly assigned to this group, subjects will participate in exercise training. Subjects will be asked to attend 3 regularly supervised and scheduled training sessions (e.g. M,

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W, and F) with the exercise physiology staff at the Loree Gymnasium or IFNH on Cook-Douglas Campus. On the remaining 2 days a week (e.g. T and Thr) subjects will be instructed to exercise on their own for half the time that they do in the supervised training sessions. These days are designed to help subjects body recover. Overall, the training program will ask subjects to exercise 5 times per week. Subjects heart rate will be monitored continuously, and subjects will be asked to exercise at an intensity near 50-55% of theiir previously measured fitness level. The amount of time subjects exercise will vary based on capacity to burn 400 kcal on supervised days and 200 kcal on unsupervised days. During the first days of training we will measure breath samples during exercise to ensure proper exercise intensity and determine how many calories from sugar and fat subjects are burning for energy. Every 4 weeks subjects will have their body weight and waist circumference measured prior to a training session. In addition to this training program subjects will be provided a placebo. The Placebo is a harmless substance that looks like the study drug, but which should have no effect. Subjects will not be able to find out if they are on the placebo or metformin (i.e. the real drug) until the study is done. Pills will be provided to the subjects by the research team as prepared by Rutgers Pharmacy in the CRC on a weekly basis (i.e. Elizabeth George, CRC Pharmacy). Unused pills should be returned to our research team. If the subject's doctor needs to know whether metformin or placebo pill is prescribed due to medical reasons that directly affect their medical care, the people doing this study can find out. At weeks 0, 8 and 16, accelerometers will be provided to characterize non-exercise physical activity. Breath samples during exercise will also occur at the end of training.

HiEx + Placebo: If subjects are randomly assigned to this group, subjects will participate in exercise training just as described above. However, here the exercise training intensity based on subjects heart rate will be near 85% of your previously measured fitness level. Exercise duration will vary in order to expend about 400 kcal per session on supervised days and 200 kcal on unsupervised days. In addition to this training program, subjects will be provided a placebo and accelerometer as described above.

LoEx + Metformin: If subjects are assigned to this group, subjects will participate in the same LoEx exercise program as outlined above. But here you will be provided metformin. Metformin is a common medication routinely used to treat high blood sugar and has secondary effects on vascular health. Subjects will not be able to find out if they are on metformin until the study is done. If the subjects doctor needs to know whether metformin or placebo pill is prescribed due to medical reasons that directly affect the subjects medical treatment, the people doing this study can find out.

HiEx + Metformin: If subjects are assigned to this group, subjects will participate in the same HiEx exercise program and receive metformin as outlined above.

PHASE 4: POST INTERVENTION TESTING (Visits 54-56)

After the 16 week intervention subjects will be asked to undergo the same series of testing that they completed in phase 2. Subjects cannot drink alcohol or take medications/supplements 24 hours prior to testing unless approved by the study team. In addition, subjects cannot eat or drink foods that contain caffeine 24 hours prior to testing. We also ask that subjects refrain from any structured exercise for 24 hours prior to testing and limit exercise to your normal everyday activities. All study testing visits highlighted above in Visits 2, 3 and 4 (Body Composition, Fitness, AI, PWV, OGTT, etc.) will be repeated (except physical screening and resting ECG). The order of the tests post-intervention will be:

1. Clamp Study (Visit 54)
2. OGTT (Visit 55)
3. Body Composition and Fitness (Visit 56)

Visits 53, 54 and 55 will be on consecutive days (i.e. 1 day apart).

*Intervention (medication and exercise) may be extended or shortened by approximately one week if scheduling requires to complete visits 54 and 55 of post-intervention testing.

PHASE 5: Unsupervised Exercise Follow-up (Visit 57-58)

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After the post-intervention testing, subjects will be asked to exercise on their own (e.g. purchase a gym membership, etc.) while wearing the polar activity tracker watch and heart rate strap. We ask that subjects exercise at the same intensity that you have been exercising with us during the intervention. We will check in by phone within week 2 to see if they are exercising, or if there are any complications.

Visit 57 *approximately 30 min*
During week 4, subjects will come to Loree Gymnasium/IFNH to check in with the study team. At this visit subjects will receive an accelerometer to wear for 7 days, 3-day diet logs to fill out, and will have waist circumference and weight measured. We will provide subjects a pre-stamped envelope to return the diet log and accelerometer within 7 days.

Visit 58 (about week 8-10) approximately 6 hours
We will provide an accelerometer and diet logs to complete an approximate week prior to the subjects final testing. You will return these items at your testing visit. At this visit, you will also turn in your HR strap.

A. Oral Glucose Tolerance Test (OGTT) (approximately 4 hours):

- Subjects will report to the CRC fasted for about 10 hours and participate in an OGTT.
- Subjects will be provided standardized meals and snacks the day prior to and during all blood test visits (listed below) consisting of 55% carbohydrate, 30% fat, 15% protein.
- Subjects **must not drink alcoholic/ caffeinated drinks for at least 24 hours before the study testing visit begins.**
- Subjects must not use allergy, prescription or pain-related medicines (over-the-counter or prescription) or antioxidant dietary supplements for at least 24 hours prior to each testing visit. Prescription meds may be taken after testing.
- Subjects must not perform any vigorous exercise for 72 hours prior to each test day. In particular, subjects are to complete you typical "supervised" exercise session 48 hours prior to this test.
- **The tests must be performed in the fasted state, so subjects may not eat or drink anything (except water) after about 9:00 pm the night before.**
- About 10 tablespoons of blood is taken during this test through an intravenous catheter.
- The OGTT is used to help determine how quickly sugar is removed from the blood. After the blood sample is taken for the fasting blood sugar test, you will drink a sugary solution. Five more blood draws will be taken at 30, 60, 90, 120, and 180 minutes after you receive the drink. This procedure will also include measurements of blood pressure, heart rate, flow mediated dilation, augmentation index, pulse wave velocity, *common carotid artery intima-media thickness* and resting metabolic rate as described above in Visits 3 and/or 4 (excluding CEU).
- Sleep history, physical activity enjoyment, and vetran rand questionnaires will be given during this visit.
- Subjects will also complete a series of questions related to stress, anxiety, overall happiness, etc. that will have you think objectively about your quality of life. This takes about 10 minutes to complete.
- Subjects will complete the NIH toolbox battery of tests that was collected in pre- and post-intervention testing.
- Subjects will fill out a daily record of your eating habits. You will also answer questions that examine your response to the food you eat. This takes about 30 minutes to complete.
- Subjects will be provided with a plastic container and asked to collect urine.
- Subjects will have heart rate monitoring (as done during training).

B. Treadmill exercise testing for cardiovascular fitness (i.e. VO2max) *(approximately 1 hour)*:

- Subjects will be asked to perform a maximal treadmill exercise test in the CRC or the IFNH lab. The test will begin with low speed on the treadmill, and the resistance will increase gradually every 2 minutes. Subjects will be asked to go as long as you can, that is, until you feel exhausted.
- During the test, subjects may have continuous electrocardiogram (ECG) heart monitoring and blood pressure monitoring if they are considered at risk.

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- During exercise testing we will also measure the subjects metabolic rate, a measure of the amount of energy it takes to burn calories. During exercise subjects will wear a facemask that is connected to a special device (metabolic cart) that measures your exhaled carbon dioxide (CO_2) and oxygen through indirect calorimetry. These measurements are then used to calculate the amount of calories the subject burns.
- During the exercise test, subjects will wear a mask over your nose and mouth. This allows us to measure how much oxygen they are using and will tell us what their fitness level is.
- Visit ends after the subject completes the exercise test. Subjects will receive the results of their test after the entire study.

C. Body Composition and Resting Metabolism (approximately 30 min):

- Subjects must be fasting for at least 4-10 hours before this procedure.
- Subjects weight will be measured without shoes and while wearing minimal clothing.
- Subjects waist and hip circumference ratio will be obtained with a tape measure to determine how much fat is located in the central part of the body.
- Total amount of fat and muscle in the body of subjects will be measured via DEXA in Foran Hall.
- Subjects will be required to wear clothes without metal on them (including zippers and bras with an underwire). If clothes have metal, we will ask that subjects change into a pair of shorts and a t-shirt or wear a lab hospital gown.
- If there are technical issues with the DEXA machine we will measure the total amount of fat and muscle in the subjects body with the BodPod® in the IFNH.
    - We will provide a swimsuit and swim cap for subjects to wear during this procedure to help standardize the results. The swimsuit and swim cap are provided to get accurate results. Subjects will not get wet at any time during this test. Subjects will enter the device and sit down. A door will close while the subject sits in the BodPod®. They are asked to breath normal and be still. The BodPod® looks like a large white egg with a window on the door.

NOTE: in the event of scheduling conflicts, cardiovascular fitness and/or body composition assessments may be completed within 1 week of doing the OGTT during Visit 58.

END OF STUDY:
After subjects have completed Phase 5, the study will be complete.

B. Data Points

We are collecting blood and urine during experimental visits. Vital signs, including blood pressure, temperature, heart rate, as well as aerobic fitness, body composition, resting metabolism, non-exercise physical activity, appetite, as well as quality of life. Please see the study overview (Study Duration) that outlines when outcomes will be collected.

C. Study Duration

Subjects participation in this study will require about **58** study visits over about 7-8 months. Each visit will last between **1-6** hours depending on whether it is for research purposes (e.g. about 3-6 hours) or exercise training (e.g. 1-1.5 hours). There are 2 Screening Visits and 5 Test Visits. Thus, the majority of visits include exercise training (48 visits).

D. Endpoints

The primary outcome for this study will be changes of basal FMD before the insulin clamp pre and post intervention. We will also examine the influence of insulin on FMD as well as metabolic insulin sensitivity, defined as the glucose infusion during the last approximate 30 minutes of the clamp procedure. Together, these findings will help tease out vasculature vs. metabolic insulin sensitivity differences between treatments. Correlations will be conducted to understand the relationship between endothelial function and metabolic insulin sensitivity with substrate oxidation,

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inflammation, blood pressure, glycemic control (OGTT and HbA1c), body composition/appetite as well as aerobic fitness and physical activity/quality of life.

1.4 Preliminary Data

We do have not have preliminary data on endothelial function with metformin combined with exercise. However, our preliminary work in Met/prediabetic individuals shows that when metformin is combined with HiEx there tends to be a blunting effect on skeletal muscle metabolic insulin resistance [4,5,33]. Furthermore, we previously showed that metformin blunted HiEx-induced improvements in blood pressure and hs-CRP vs. HiEx only. Together, along with literature from other investigators, it reasons endothelial function may be impacted by metformin plus exercise.

1.5 Sample Size Justification

The primary outcome for **this study** will be changes of basal flow mediated dilation (FMD) before the insulin clamp pre and post intervention. Based on our prior work [47], with 15 subjects per treatment arm we expect to have at least an 80% chance of rejecting the null hypothesis that the mean pre to post-exercise change in FMD is the same for any pair of treatments if the true means of the underlying distributions differ by more than 4.2%. *Details*: In computing the minimum detectable effect size of 4.2%, we assumed that the measurements for pre to post-treatment change in FMD will be normally distributed and that the measurement variability in the pre to post-exercise FMD changes is the same regardless of the treatment. Moreover, we assumed that the standard deviation of the underlying distributions is 3.4% based on our prior work [47], which is notably larger than reported in the metformin literature [48]. To account for a total of 6 pairwise between-treatment comparisons, a Bonferroni corrected experiment-wise type I error of 0.05 was utilized to derive the minimum detectable between-treatment difference of 4.2%. *Limitations*: We do not have data from the literature or from our own studies with exercise to estimate power on changes in MBV, nor with the combination of exercise+metformin. However, considering MBV, we based our estimate of sample size on our study examining the effect of insulin on MBV in both skeletal and cardiac muscle in the control setting and in the setting of elevated plasma FFA. We observed that insulin significantly increased microvascular blood volume (MBV) at both sites ($p<0.01$) in the absence of elevated FFA and this effect was entirely blocked by elevating plasma FFA. These observations were made in 22 subjects, although less would have yielded significance [36, 42]. Moreover, previous work that we conducted with HiEx+metformin demonstrated significant blunting effects on systolic blood pressure vs. exercise alone with only 8 people per group [5]. *Anticipated dropout*: Based on our prior work with this study population, we anticipate that the study participant dropout rate will be 10-15%. Therefore, we intend to enroll 80 subjects with 20 subjects randomized to each treatment intervention.

1.6 Study Variables

A. Independent Variables, Interventions, or Predictor Variables

This is a randomized by sex, double-blind, placebo controlled trial. A placebo is needed to minimize potential behavioral changes with pharmacological intervention. This will isolate the effects of exercise intensity based training on respective outcomes. As a result, exercise at low or high intensity with placebo will serve as standard of care/control, while exercise at low or high intensity with metformin will be the experimental conditions. Pre and post test data will be compared statistically.

Data will be analyzed using R (Vienna, Austria, 2011). A two-way repeated measures analysis of variance (ANOVA) will be used to assess time course differences (group x test). Bonferonni post-hoc analysis will be used to determine group differences and paired t-tests will be used to assess within treatment effects. Linear regression analyses will be used to identify relationships between respective variables. A $P<0.05$ will be considered statistically significant. Data will be expressed as mean ± SEM.

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The same statistical approach for secondary analysis will be conducted as the primary variable analysis.

B. Dependent Variables or Outcome Measures

1. Insulin Sensitivity
2. Fitness (e.g. VO2max, physical activity amount, heart rate, RPE, etc.)
3. Body composition (e.g. weight, fat, FFM, BMD, waist circumference, etc.)
4. Vascular Function (e.g. FMD, CEU, heart, arterial stiffness, etc.)
5. Questionnaires (e.g. Sleep, activity, quality of life, hunger, etc.)
6. Glucose (e.g. substrate, isotope)
7. Lipid Profile (e.g. TG, HDL, FFA, etc.)
8. Blood clinical chemistries (e.g. CBC)
9. C-peptide
10. Insulin
11. Inflammation (e.g. endothelin-1, adiponectin, Microparticles, etc.)
12. Nitrates
13. Gut hormones (e.g. ghrelin, GLP-1, etc.)
14. Pregnancy (in females), blood for screening at each admission.
15. Energy metabolism (e.g. rest, exercise, insulin-stimulation).
16. Gut microbiota
17. Cognitive measures using NIH toolbox

1.7 Drugs/Devices/Biologics

A. Drug/Device Accountability and Storage Methods

All items will be kept in locked rooms in encrypted storage cabinets/refrigerators as appropriate. Access will only be allowed for research personnel. Overall, the PI has responsibility for storage and/or preparation as well as dispensing. Research personnel will assist with distribution/storage. Preparation will be performed by the PI, clinical coordinator and/or Clinical Research Pharmacist accordingly.

Metformin/placebo will be picked up at the Clinical Research Center. Items may be stored in Clinical Research Center and/or Loree for distribution to subject.

Insulin will be stored in the Clinical Research Center.

Definity microbubbles/Dextrose will be stored at Loree and/or Clinical Research Center.

1.8 Specimen Collection

A. <u>Primary</u> Specimen Collection

- **<u>Types of Specimens</u>**: Blood will be collected by trained research personnel in the study. Plasma or serum will then be stored for later use/analysis. Total volume collected will be approximately 125 cc for clamp protocol and 50 cc for OGTT protocol. Blood will be collected in the Clinical Research Center and/or IFNH as described below.
- **<u>Annotation</u>**: Each specimen will be coded with a unique identifier per the study, time point blood was collected (e.g. 0 min, 90 min, etc.) test (e.g. pre or post) and analyte. No personal identifier will be used.
- **<u>Transport</u>**: Specimens will be transported using biohazard containers properly marked on ice by Research Personnel on the IRB.
- **<u>Processing</u>**: Research Personnel.

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- **Storage**: Specimens will be stored in the Clinical Research Center and/or IFNH accordingly. They will be accessed only by individuals with approved access by research personnel approved via the IRB.
- **Disposition**: Samples will be discarded when meeting study completion. Other samples kept will be placed in our freezer Research Repository**.**

B. Secondary Specimen Collection
NA

1.9 Data Collection

A. Primary Data Collection

- **Location**: questionnaire data will be collected in Loree, IFNH and/or Clinical Research Center as well as at home when appropriate. Permission will be granted by the subject.
- **Process of Data Collection**: Research personnel approved to work on this protocol to administer and collect data. The PI will oversee the process.
- **Timing and Frequency**: See below for details.
- **Procedures for Audio/Visual Recording**: NA.
- **Study Instruments**: Questionnaires are widely used and routine for this type of research.
- **Ethnographic Studies, Interviews, Or Observation**: Paper questionnaires will be used.
- **Subject Identifiers**: *E*ach specimen *will be coded with a unique identifier per the study, time point collected (e.g. 0 wk, 4 wk, etc.) and test (e.g. pre or post) will also be included. No personal identifier will be used on the forms.*

B. Secondary Data Collection

- NA

1.10 Timetable/Schedule of Events

Study Screening and Testing Schedule Visit Overview.

Study Visit	Screening Visit 1	Screening Visit 2	Pre-Test OGTT Visit 3 or 4	Pre-Test Clamp Visit 3 or 4	Exercise Training (Intervention Period) 5 d/wk* Visits 5-53	Post-Intervention Clamp Testing Visit 54	Post-Intervention OGTT Testing Visit 55-56	Follow up Week 4 check-in Visit 57	Follow up Week 8 OGTT Testing Visit 58
Informed Consent	X								
Blood Draw	X		X	X		X	X		x
History and Physical		X							
Urine Collection			X	X		X	X		X
VO2peak (Treadmill testing)		X					X		X
Body Fat measurement (Waist and Hip circumference,		X					X		X

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DEXA or Bod Pod)									
Indirect Calorimetry		X		X		X			X
Exercise* Indirect Calorimetry					X*	X			
24 hour Blood Pressure			X		X*				
Blood Pressure, AI, CEU and PWV tests				X		X			X
FMD, CCA-IMT			X	X		X			X
Muscle/Fat Biopsy				X		X			
Diet Record		X			X		X	x	X
Appetite Questions			X		X		X		X
Quality of Life Questions			X				X		X
NIH Toolbox			X				X		X
Accelerometer		X			X			x	x
Heart Rate Monitors					X			x	

Exercise Training will be performed at Loree Gymnasium or IFNH on Cook-Douglas Campus 3 days/week and supervised by an exercise physiologist/research team member. The other 2 days will be non-supervised as you will be asked to wear a heart rate monitor. Accelerometers will be provided for weeks 0, 8, 16, 20, 24. *Exercise Indirect calorimetry will include measures of breath samples the first day of training and within the last weeks of training.

2.0 Project Management

2.1 Research Staff and Qualifications

Steven K. Malin, PhD (PI) – is an Associate Professor with extensive experience in implementation of the clinical and exercise procedures proposed herein. He also has numerous publications related to insulin sensitivity, energy metabolism and glycemic control.

Partho Sengupta MD – (Co-I) is the Henry Rutgers Professor of Cardiology and chief of the Division of Cardiology at RWJMS and chief of Cardiology at RWJUH. He has extensive experience in blood flow assessment and cardiac imaging technology.

Peter Kokkinos, PhD - (Co-I) is a Professor in Kinesiology and Health. He has published several papers related to cardiac function and aerobic fitness in clinical populations.

Sue Shapses, PhD - (Co-I) is a Professor in Nutritional Sciences and Director of the Nutrition, Exercise and Metabolism Center in the IFNH. She has extensive experience in assessment of body composition as well as food intake.

Ankit Shah, MD - (Co-I) is an Assistant Professor in Medicine. He has vast experience in stable isotope methodology and glycemic control in patients with obesity.

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Andrea Spaeth, PhD – (Co-I) is an Assistant Professor in Kinesiology and Health. She has extensive experience in sleep assessment and clinical outcomes.

Fredric Wondisford, MD – (Co-I) is as Professor and the Chair in Medicine. He published extensively in stable isotope methodology and glycemic control in patients with obesity..

Sara Campbell, PhD – (Co-I) is an Associate Professor in Kinesiology and Health. She has extensive experience in gut microbiota and clinical outcomes.

Jaclyn Dosik, M.Ed. – is a clinical coordinator in Kinesiology and Health. She has experience with exercise stress tests and management of clinical populations before, during and after exercise related interventions.

Mary-Margaret Remchak, M.S. - is a graduate student in Kinesiology and Health. She has experience with exercise stress tests and clinical outcomes.

Tristan Ragland, Ph.D. - is a post-doctorate fellow in Kinesiology and Health. He has experience with body composition testing, metabolic testing, exercise testing and prescription.

Daniel Battillo, B.S. - is a graduate student in Kinesiology and Health. He has experience with exercise prescription programs and working directly with clinical populations during interventions.

Afsheen Syeda, M.S. - is a graduate student in Nutrition. She has experience in community nutrition research and working with diverse populations.

Habiba Faiz, B.S. - is a graduate student in Nutrition. She has experience in diet preparation in clinical populations and basic science laboratory techniques.

2.2 Research Staff Training

Weekly meetings will be performed with the clinical coordinator and graduate students working with the PI to discuss protocols, data collection and subject adherence. Lab protocols will be implemented with direct oversight by the PI. Students and staff will receive appropriate training for such measurements and require sign off by the PI prior to direct data collection. Faculty with expertise in given areas will also be consultant in the lab protocol and will be communicated with at time of and throughout data collection as appropriate.

Faculty Co-I's will also meet regularly with the PI. For instance, monthly meetings will be held with all investigators during the first quarter of the study to establish/maintain continuity of the research. Thereafter, quarterly meetings with all investigators will be performed to maintain communications and updates on the study.

Individual faculty/staff/students will lastly receive a roles and responsibilities overview for the study, whereby agreements will be had to enhance communication and clarity on said duties.

Nurses and pharmacist of the Clinical Research Center will also be provided with copies of our protocols and the PI will review procedures to ensure communications.

2.3 Resources Available

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NA

2.4 Research Sites

All research will be conducted at Rutgers (e.g. Loree, IFNH and/or CRC) as described.

3.0 Multi-Center Research

NA

4.0 Subject Considerations

4.1 Subject Selection and Enrollment Considerations

A. Method to Identify Potential Subjects

Prospective subjects will be recruited through posted and/or mailed flyers. Please see attached flyer that will be included in newspapers, Instagram, Reddit, Facebook or twitter as well as mailings via coldcontacts. Cold-contacts may be iniatited via use of electronic medical records using inclusion/exclusioncriteria through the Rutgers Robert Wood Johnson Medical School Endocrinology/Cardiology platforms (connected with Dr. Shah and Bhatti). Additionally, ResearchMatch.org will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository (***see IRB #090207***). We will also use Trialfacts, a specialized patient recruitment service that combines extensive marketing and advertising expertise with in-depth clinical trial experience in order to aid research groups in successful study recruitment. Trialfacts uses digital advertising and specialized targeted ads based on eligibility criteria and online questionnaire data collection with specific software.

B. Recruitment Details

Prospective participants will be recruited using listservs and flyers across Rutgers University campus as well the Rutgers Robert Wood Johnson Medical School (125 Paterson St, New Brunswick NJ 08901.

We will also place flyers IRB approved in the New Brunswick and surrounding community at participating stores/libraries/etc. Newspapers (e.g. New Brunswick Today) and social media (Twitter, Facebook) will also be contacted about placing the flyer as an insert or respective info.

Interested individuals will be asked to contact the research team by phone to get a more detailed description of the study. Prospective participants will be scheduled for a screening visit to establish eligibility. Enrollment will be decided after chemistry panel, blood pressure and waist circumference results are confirmed for group assignment. This will be evaluated by the PI, study physician and research team.

C. Subject Screening

Inclusion Criteria for Subjects

- Male or female ≥40 and ≤80 years old.
- Has a body mass index ≥25 and ≤47 kg/m^2.
- Not diagnosed with Type 2 diabetes.
- Not currently engaged in > 150 min/wk of exercise
- At minimum, subject will have abdominal obesity (increased waist circumference as defined below) and may have any additional National Cholesterol Education Adult Treatment Panel III Metabolic Syndrome criteria:
  - Increased waist circumference (≥102 cm in men; ≥88 cm in women)

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- Elevated triglycerides (≥150 mg/dl), or on medication for treating the condition
- Reduced HDL-cholesterol (<40mg/dl in men, <50 mg/dl in women), or on medication for treating the condition
- High blood pressure (≥130 mmHg systolic or ≥85mmHg diastolic), or on medication for treating the condition
- Elevated fasting glucose (≥100 mg/dl), or on medication for treating the condition
- Other major risk factors to be noted based on the Framingham Risk Score
  - HbA1c 5.7-6.4%
  - LDL > 130 mg/dL
  - Family history of type 2 diabetes (immediate family, i.e. parent/sibling)
  - History of gestational diabetes
  - History of Polycystic Ovarian Syndrome
  - Family history of pre-mature cardiovascular disease (immediate family i.e. parent/sibling) before 55 for males or 65 for females that can include heart attack, peripheral arterial disease, abdominal aortic aneurysm, symptomatic carotid artery disease or clinical coronary heart disease)
  - Age (> 45 years old for men; > 55 years old for women)
  - Black/African American, Mexican, Asian, and/or Hispanic

- Subjects currently taking medications that affect heart rate and rhythm (i.e. Ca++ channel blockers, nitrates, alpha- or beta-blockers)

Exclusion Criteria for Subjects

- Morbidly obese patients (BMI >47 kg/m^2) and overweight/lean patients (BMI <25 kg/m^2)
- Evidence of type 1 diabetes and diabetics requiring insulin therapy.
- Subjects who have not been weight stable (>2 kg weight change in past 3 months)
- Subjects who have been recently active (>30 min of moderate/high intensity exercise, 2 times/week).
- Subjects who are smokers or who have quit smoking <5 years ago
- Subjects prescribed metformin or have taken metformin within 1 year.
- Subjects with abnormal estimated glomerular filtration rate (eGFR).
- Hypertriglyceridemic (>400 mg/dl) and hypercholesterolemic (>260 mg/dl) subjects
- Hypertensive (>160/100 mmHg)
- Subjects with a history of significant metabolic, cardiac, congestive heart failure, cerebrovascular, hematological, pulmonary, gastrointestinal, liver, renal, or endocrine disease or cancer that in the investigator's opinion would interfere with or alter the outcome measures, or impact subject safety.
- Pregnant (as evidenced by positive pregnancy test) or nursing women
- Subjects with contraindications to participation in an exercise training program
- Currently taking active weight suppression medication (e.g. phentermine,orlistat, lorcaserin, naltrexone-bupropion in combination, liraglutide, benzephetamine, diethylpropion, phendimetrazine)
- Known hypersensitivity to perflutren (contained in Definity)
- Subjects who are considered non-English speaking individuals

UPLOAD to e-IRB Section 11.0 Recruitment Materials – *all* recruitment materials, such as: in-person or telephone scripts, emails, flyers, posters, social media posts, and radio or television advertisement scripts, etc. that will be used to recruit individuals to the study.

4.2 Secondary Subjects

NA

4.3 Number of Subjects

A. Total Number of Subjects

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Based on prior work by our group, we anticipate that approximately 25% of subjects who enroll in the study will withdraw/drop out early. Currently the study requires 45 people of 80 given it is a transfer project when our team was at the University of Virginia. In addition, we expect that 70% of individuals who we screen/consent will not qualify. **Thus, we expect to screen 225 subjects of whom about 67 will participate and 45 will complete the study.**

B. Total Number of Subjects If Multicenter Study

NA

C. Feasibility

We are confident that we can recruit the required number of health volunteers given the population in and surrounding Rutgers University. We are also confident we can recruit patients with risk factors for metabolic syndrome in the Clinical Academic Building at RWJMS.

4.4 Consent Procedures

A. Consent Process

- **Location of Consent Process**
  The Clinical Research Center at the Robert Wood John Medical School, Institute for Food Nutrition, and Health or Loree Gymnasium in New Brunswick NJ 08901. Consent process will take place in a private room.

- **Ongoing Consent**
  NA

- **Individual Roles for Researchers Involved in Consent**
  Principal investigator, Steven Malin, PhD, and study team members, e.g. Jacyln Dosik, Mary Remchak, etc. will inform and answer questions of potential participants of all aspects of the study during the consent discussion. Another member from the lab will conduct the consent discussion is neither the principal investigator nor study coordinator are available. Dr Malin, or Jaclyn/Mary/ etc. will sign the consent form.

- **Consent Discussion Duration**
  Consent process is expected to last 60 minutes.

- **Coercion or Undue Influence**
  To minimize the possibility of coercion or undue influence, the consent discussion will not be conducted by laboratory staff who have any known relationship with prospective participants. Individuals will be made aware it is his/her choice whether to take part in the research his/her relationship with the study staff will not change, and he/she may do so without penalty and without loss of benefits to which he/she is otherwise entitled. Participants will also be provided consent forms prior to meeting to allow appropriate decisions without pressure.

- **Subject Understanding**
  Subjects will be made aware of the risks associated with blood draws and the number of blood samples requested during each test day. Subjects will also be made aware of side effects associated with exercise, metformin and research tests. Potential participants will be encouraged to ask questions and be asked questions by staff conducting the consent process to ensure understanding of the study.

B. Waiver or Alteration of Consent Process

- **Waiver or Alteration Details**

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NA

- **Destruction of Identifiers**
  NA

- **Use of Deception/Concealment**
  NA

  a. **Minimal Risk Justification**
     NA

  b. **Alternatives**
     NA

  c. **Subject Debriefing**
     NA

UPLOAD to e-IRB Section 13.17 Consent Form - The consent form(s) you plan to use to inform and consent individuals to take part in the research.

C. Documentation of Consent

- **Documenting Consent**
  Please see consent form attached. The research team will verbally read the consent form with the potential subjects. During this time, all questions will be answered as to increase the participants' comprehension and understanding of the study. The research team and participant will sign the consent prior to any tests. Subjects will receive copies of the consent.

- **Waiver of Documentation Of Consent (i.e., will not obtain subject's signature)**
  NA

4.5 Special Consent/Populations

A. Minors-Subjects Who Are Not Yet Adults

- **Parental Permission**
  NA

- **Non-Parental Permission**
  NA

- **Assent Process**
  NA

- **Documentation of Assent**
  NA

- **Reaching Age of Majority During Study**
  NA

B. Wards of the State

- **Research Outside of NJ Involving Minors**
  NA

C. Non-English-Speaking Subjects

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- **Process for Non-English-Speaking Subjects**
  We will not be recruiting non-English speaking individuals.

- **Short Form Consent for Non-English Speakers**
  NA

D. Adults Unable to Consent / Decisionally Impaired Adults

- **NJ Law-Assessment of Regaining the Capacity to Consent**
  NA

- **Capacity to Consent**
  a. **NJ Law-Selecting A Witness**
  NA

  b. **Removing a Subject**
  NA

4.6 Economic Burden and/or Compensation for Subjects

A. Expenses

There are no expenses incurred by the subject taking part in the research and therefore no reimbursement will be given. We will validate parking at the Robert Wood Johnson University Hospital parking deck for those that may need it. Subjects may lose compensation from their own jobs if they miss work for screening and experimental visits. However, this is a voluntary study and subjects will not be reimbursed for lost wages.

B. Compensation/Incentives

Incentives for this study will be a check for successful completion of protocols at set times (see consent form), food before/after test days and supervised exercise sessions in addition to a heart rate monitor used in the study.

Option 1:

Completion of Phase 3:	$200
Completion of Phase 3(with isotope):	$300
Completion of Phase 4:	$600
Completion of Phase 4 (with isotope):	$700
Completion of Visit 57:	$200
Completion of Visit 58:	$300
Total payment:	**$1,300 (without isotope) or $1500 (with isotope)**

*Will be able to keep Polar HR monitor at the end of the study. (~$150)

Option 2:

(Without isotope) Completion of Phase 3, Phase 4, Visit 57 and Visit 58	**$1,300**
(With isotope) Completion of Phase 3, Phase 4, Visit 57 and Visit 58	**$1,500**

*Will be able to keep Polar HR monitor at the end of the study. (~$150)

C. Compensation Documentation

Subjects will be compensated with a check processed through RU Marketplace. Subjects will have the option to receive compensation either at specified time points during the study or a total sum at the end of the study. Research team members will collect information including name, address,

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telephone number, DOB and social security number to fill out the necessary forms through Rutgers University.

4.7 Risks of Harm/Potential for Benefits to Subjects

A. Description of Risks of Harm to Subjects

- **Reasonably Foreseeable Risks of Harm**

Fitness tests may cause anxiety and/or claustrophobia due to mouthpiece or canopy from indirect calorimetry. Cardiovascular reactions to exercise could include fainting, syncope, chest pain or sudden cardiac death. The later though occurs rarely (risk of cardiac events during exercise testing: 3/5,000 tests; risk of death during exercise: ~1/18,000 individuals per year).

During FMD, subjects may experience mild discomfort (e.g. mild pain, tingling, and/or numbness) in their forearm and/or hand as a result of the prolonged inflation (5 min.) of the blood pressure cuff required for the procedure. This occurs rarely.

IV catheter insertion risk are minimal but could include hematoma, venous thrombosis or infection at the IV site. Subjects may, although rare, feel fain during venipuncture. Also minimal, the risk of isotope tracers include infusion and allergic reaction. Signs and symptoms of such reactions may include low blood pressure, fast heart rate, rash, vomiting, fever, chills, flushing or nausea. Subjects do not need to take any precautionary safety measures before or after infusions. There is no radioactive energy emitted, as they do not spontaneously decay.

Metformin side effects, such as diarrhea, nausea, upset stomach, and abdominal pain may occur. Hypoglycemia is also possible and feelings may include: fatigue, lightheadedness, mood changes, hunger, clammy hands and/or feet. Metallic taste and lactic acidosis also may occur, but are rate. Minimized by following a ramp up protocol over 4 weeks t gradually acclimate the subject. Recommendation ingestion with food will also minimize side effects of low blood glucose and avoiding alcohol intake during the study. People with history of renal/congestive heart failure/liver disease will be excluded to also minimize risk of lactic acidosis, which occurs in ~1/33,000 patients per year).

Definity Microbubbles side effects include: headache, back and chest pain, flushing, dizziness, a metallic taste, vomiting, coughing, and dry mouth. The FDA has issued a black box warning for Definity (a type of warning that appears on the package insert for certain drugs that may cause serious harmful effects) for cardiopulmonary and hypersensitivity reactions. There have been convulsions, arrhythmias, respiratory distress, and death noted in patients with an intracardiac or intrapulmonary shunt so the FDA has suggested using it with extreme caution in patients with known cardiac or cardiopulmonary disease. We will not have any of these types of subjects in our study but should an emergency arise we will have trained personnel and resuscitation equipment available. We have been using Definity in subjects with obesity for over 4 years (~ 100 subjects) and we have had 0 adverse events. Thus, these occur rarely.

Insulin infusion: There is a potential for hypoglycemia (not very often). The doses of insulin used during the euglycemic insulin clamp are low and the blood glucose level will be monitored every five minutes throughout the insulin infusion. The glucose infusion will be adjusted to maintain the subject's glucose level within 10% of normal fasting. This occurs infrequently.

Body composition: Air displacement determination of body composition by Bod Pod may cause anxiety and /or claustrophobia. The DXA measurements will produce a small amount of radiation which is less than that received during two days of background radiation.

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A subject may incur lost wages from his/her job due to time spent at the screening and/or experimental visits. There is also the possibility, though low, for breach of confidentiality with using an outside company, Greenphire, to distribute the ClinCard compensation.

- **Risk of Harm from an Intervention on a Subject with an Existing Condition**
  We expect our subjects to be generally healthy and should not have any severe or decompensated medical conditions. Exclusion criteria and physicals performed should minimize any risks and the exercise/metformin treatment should not bring harm.

- **Other Foreseeable Risks of Harm**
  We do not anticipate any other foreseeable risks given the scope and duration of the study.

- **Observation and Sensitive Information**
  NA

B. Procedures which Risk Harm to Embryo, Fetus, and/or Pregnant Subjects
Pregnancy is an exclusion criterion, and women of childbearing age will undergo a bedside urine pregnancy test prior to treatment.

C. Risks of Harm to Non-Subjects
NA.

D. Assessment of Social Behavior Considerations
Questionnaires used in this study do not assess depression or suicidal ideations. As a result, we do not anticipate these assessments.

E. Minimizing Risks of Harm
All efforts will be made to minimize risk.

General Efforts

- A careful history and physical examination to exclude those subjects with a history or current symptoms of cardio-pulmonary conditions as well as renal, liver or metabolic disease.
- CBC with differential to screen out people with low hematocrit or evidence of infection or other illness
- Glucose and HbA1c to screen undiagnosed diabetes
- Pregnancy test in females
- Liver, kidney, and hematologic function will be assessed from blood samples using standard clinical tests listed below:
  - *CBC, eGFR, and creatinine, tests within normal limits*
  - *Liver function tests no greater than 2-fold within normal limits*
  - *HCT for women > 36%, Men >38%*
- Complications of IV will be minimized by aseptic and skilled techniques
- Each exercise test will be monitored by researchers on the IRB protocol or CRC personnel who have current training in CPR and AED use. An AED and a cell phone available to call 911 if necessary. While a physician is not required to be present during exercise testing in this population (according to AHA/ACC/ACSM guidelines), it is standard CRC/medical practice that the study physician is aware of the test and available by page.

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- If a subject develops leg pain during the exercise treadmill test, the speed/incline on the device will be decreased.

- Fitness testing, DEXA as well as IV placement will be performed by trained personnel

Conditions whereby a subject's participation or study treatment may be stopped or modified:

- If subject has any of the less likely to severe side effects for a particular admission that study will be terminated and depending on the side effect as well as extent may be excluded from all admissions.

- Any significant blood loss is prevented by regular visual inspection of the catheter insertion site and removing a catheter and applying pressure if there is obvious extravasation of blood into the tissue.

- These catheters are in place for a brief time (~5 hours) and careful aseptic technique is used for catheter placement. With these precautions infections secondary to intravenous lines is extremely uncommon. Should an infection occur, we would treat with oral or intravenous antibiotics or if necessary, by local drainage.

- If the subject experiences headache, chest pain, vomiting, flushing, or dizziness.

- If the subject experiences hypoglycemia with a blood glucose of 60 or less the study will be stopped. It may be stopped at a higher value at the discretion of the Principal Investigator if there is loss of IV access.

- A woman with a positive pregnancy test will be excluded.

Exercise Testing (VO2max test)

The ACC/AHA guideline update for exercise testing endorsed by the American College of Sports Medicine (ACSM) is used in the Clinical Research Center and IFNH for all exercise protocols. This document provides guidelines for absolute and relative indications for terminating exercise testing. In addition to D. Shah and Dr. Wondisford, Dr. Sabha Bhatti, the Director of Cardiac Non-Invasive Laboratories and cardiologist, will serve as reference for interpreting ECG work and verify safety in exercise training.

Exercise Termination:

Absolute

1. Drop in systolic blood pressure of >10 mm Hg 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 V1 or aVR.

Relative

1. Drop in systolic blood pressure of >10 mm Hg from baseline blood pressure despite an increase in workload, in the absence of other evidence of ischemia.
2. ST or QRS changes such as excessive ST depression (>2mm horizontal or down-sloping ST-segment depression) or marked axis shift

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3. Arrhythmias other than sustained ventricular tachycardia, including multifocal PVC's triplets of PVC's, supraventricular tachycardia, heart block, or bradyarrhythmias.
4. Fatigue, shortness of breath, wheezing, leg cramps, or claudication
5. Development of bundle-branch block or intraventricular conduction delay that cannot be distinguished from ventricular tachycardia
6. Increasing chest pain
7. Hypertensive response: systolic >250 mm Hg, diastolic >115 mm Hg.

Metformin Treatment

1. People will follow up a 500 mg/d ramp up protocol until 2000 mg/d is met (expected week 4.
2. In the event someone is not able to tolerate a dose, people will be held at that dose for approximately 1 week, thereby increasing the dose at a level tolerable to the subject.

Blood draws

Total of approximately 368 (8 cc, 155 cc x 2 (pre and post test) + 50 cc (follow-up)) cc of blood will be collected during the entire study and tested for:

1. Glucose (e.g. substrate, isotope)
2. Lipid Profile (e.g. TG, HDL, FFA, etc.)
3. Blood chemistries
4. C-peptide
5. Insulin
6. Inflammation (e.g. endothelin-1, adiponectin, Microparticles, etc.)
7. Nitrates
8. Gut hormones (e.g. ghrelin, GLP-1, etc.)

Subjects will be advised to not donate blood during the study to minimize risk of anemia.

Clamp Protocol

With each study protocol there will be documentation of study records of drug administration and the person who administers. For microbubbles, isotope and insulin, administration is always intravenous. All invasive training protocols are under supervision of an MD. However, an MD will not be required to be present in the room when performing them, as all research personnel (e.g. Dr. Malin, Ms Dosik) are trained to implement the protocol and will be under the supervision of the MD.

Dr. Ankit Shah and Dr. Frederick Wondisford will serve as primary endocrinologists on the study. Dr. Shah and Wondiisford have reviewed and approved all aspects of monitoring patient safety, drug preparation and administration (including preparation of infusates for insulin clamp studies and administration of Definity microbubbles for microvascular imaging). The team has written protocols for preparation and administration of pharmaceuticals, which are prepared by Elizabeth George, a pharmacist in the CRC.

The team is aware how to treat adverse events, should they arise. Potential adverse events include: 1) hyper or hypoglycemia secondary to infusion of either glucose or insulin; 2) back pain secondary to infusion of microbubbles which is treated by discontinuing the infusion and resolves spontaneously in 3-5 minutes; 3) idiosyncratic reactions (e.g. vasovagal syncope from phlebotomy or venipuncture) which is treated with supine posture, cold compresses and if necessary saline infusion; 4) allergic reactions which is treated by stopping all infusions, administering Benadryl if needed. In the event of any of the above, either Dr. Ankit Shah or another designated licensed practitioner on the IRB protocol will be available for immediate consultation.

Emergency Response in CRC

The CRC has stock medications on site for emergency use (i.e. diphenyhydramine, Epipen), as well as IV fluids and oxygen. An AED is located in the hallway outside of the CRC. If a subject becomes unresponsive or hypotensive, nursing and other staff will be summoned to the study area for immediate assessment. If the subject is thought to be having a severe allergic reaction and/or is hypotensive, any study infusions will

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be stopped, and treatments will be administered either per a licensed MD present in the room during the procedure or according to the established CRU standing order for an allergic reaction. This will include the use of IV fluids, Epipen, diphenyhydramine and oxygen as applicable. Other personnel will immediately call the RWJMS Emergency Services line (732-828-3000) and call the study physician if not already in the room.

- **Certificate of Confidentiality**
  This study is funded by NIH RO1 5R01HL130296 and a Certificate of Confidentiality was automatically issued.

- **Provisions to Protect the Privacy Interests of Subjects**
  Subjects will only interact with research team members. If a subject does not feel comfortable sharing personal health information, she/he does not need to participate in the study.

  All research records are kept strictly confidential and in a locked cabinet and a locked computer that requires a password; which is available only to persons conducting the study and who have passed the Human Certification (CITI) training. Samples sent to the laboratory (screening/analytes for analysis) do not have any names on them and are only identified with ID #code. The de-identified blood and urine samples are kept in a locked freezer, and the cabinets, computers and freezers are within the principle investigator's oversight to ensure participant confidentiality.

F. Potential Benefits to Subjects
There will be no immediate benefits from participation in this study. The procedures done for this study are investigational and have no immediate health benefits.

5.0 Special Considerations

5.1 Health Insurance Portability and Accountability Act (HIPAA)
NA

5.2 Family Educational Rights and Privacy Act (FERPA)
NA

5.3 Code of Federal Regulations Title 45 Part 46 (Vulnerable Populations)
NA

A. Special Populations
- Prisoners: NA
- Neonates: NA
- Neonates of Uncertain Viability: NA
- Children: NA
- Individuals with Impaired Decision-Making Capacity: NA

5.4 General Data Protection Regulation (GDPR)
NA

5.5 NJ Access to Medical Research Act (Surrogate Consent)
NA

6.0 Data Management Plan

6.1 Data Analysis
Describe the data analysis plan. Include any statistical procedures and power analysis, if applicable to the research.

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6.2 Data Security

Describe the steps that will be taken to secure the data through all phases of the research—from the time of its collection, to its storage, use and study closure— such as staff training, transporting or transmitting data from the study site or 'field' to Rutgers, methods to restrict access, password protection, encryption, use of key codes to separate identifiers from the data, and state how and when identifiers will be deleted from the data. Identify who will be responsible for each of these tasks.

6.3 Data and Safety Monitoring

The PI, research team and CRC research staff will review data, events and charts after any adverse event to do a root cause analysis. If we determine a correctable error, we will make the necessary changes to our experimental protocol and inform the IRB.

A. Data/Safety Monitoring Plan

The PI and research team will generate a safety report for any adverse events (hospitalizations, infusion reactions, and intravenous line site issues). These reports will be classified as serious/non-serious. We will report all serious adverse events (hospitalizations, death) and poor outcomes to the IRB, CRC and NIH. The PI will evaluate the relatedness of the adverse events to the study as:

Related:	AE is clearly related to the intervention
Possibly related:	AE may be related to the intervention
Unrelated:	AE is clearly not related to intervention

B. Data/Safety Monitoring Board Details

NA

6.4 Reporting Results

A. Individual Subjects' Results

Subjects will be provided a report at the end of completion that provides routine clinical labs, aerobic fitness and body composition along with resting metabolism (i.e. calories expended per day). Results are for research purposes only as indicated in the consent. In event any data is suggestive of disease (e.g. type 2 diabetes or liver/renal dysfunction), then individuals will be recommended to seek consult from their health care provider. They will not be able to participate in the current study unless cleared by their own health care team.

B. Aggregate Results

We will not share aggregate results with the study subjects of physicians, as data are for research purposes.

C. Professional Reporting

We plan to present data generated from this study at regional, national or international conferences and publish the results in a timely manner. All final peer-reviewed manuscripts that arise from this study will be submitted upon acceptances to the digital archive NIH National Library of Medicine PubMed Central (PMC) database. Any data released for publication will be for research purposes only and will not include identifiable data on any of the participants.

D. Clinical Trials Registration, Results Reporting and Consent Posting

Yes. This study will be registered as a clinical trial.

6.5 Secondary Use of the Data

Data will be stored in a -80 freezer for future use. Biospecimens will be de-identified and this info is included in the consent process. Specimens stored for yet to be identified future research will be labeled

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with a unique identifying code specifying the admission number (i.e. study ID), test, sample type, condition, date and collection time point. No name, subject initials, or medical record # will be included on the specimen.

Dr. Malin will have oversight of biospecimens only relevant data (e.g. age, sex, etc.) may be shared with other Rutgers and non-Rutgers faculty to complete analysis. Only a unique code specific to the study will accompany the specimen. That code will be kept by the PI using a password protected database to link identifiers. Only study personnel will have access to the password. Identification of subjects (name, DOB, etc.) will not be shared.

7.0 Research Repositories – Specimens and/or Data

Blood and urine will be stored in room 250 of the Institute for Food, Nutrition and Health, which is at: 61 Dudley Rd, New Brunswick, NJ 08901-8525 (Cook Campus). The -80 freezer is approved for storage of human samples (IBC Registration #: 18-044 (v1.00)) by Dr. Sara Campbell. The purpose of a specimen bank is to process, and store samples until researchers at this University and other Universities need them for future research. Dr. Steven K. Malin will not give your name to other researchers who want to use your sample but will only give them information like your age and what disease/condition you have.
The long-term goals of the samples collected in this bank will be mainly used for research on diabetes and cardiovascular disease prevention/treatment. It is not possible, however, to list every research project that will include the samples because we cannot predict all of the research questions that will be important over the coming years. As we learn more, new research questions and new types of research may be done. There is no set limit to the number of individuals that may provide biospecimens to the repository. The more biospecimens available, the more useful the repository will be for scientific research.
This information is within the consent form/addendum.

8.0 Approvals/Authorizations

See attached Material Transfer Agreement Radiation Safety Approval and Bio-Safety Approval.

9.0 Bibliography

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