ROLE OF EXERCISE IN THE PREVENTION AND TREATMENT OF RAGE-MEDIATED INFLAMMATION (RECEPTOR) TRIAL

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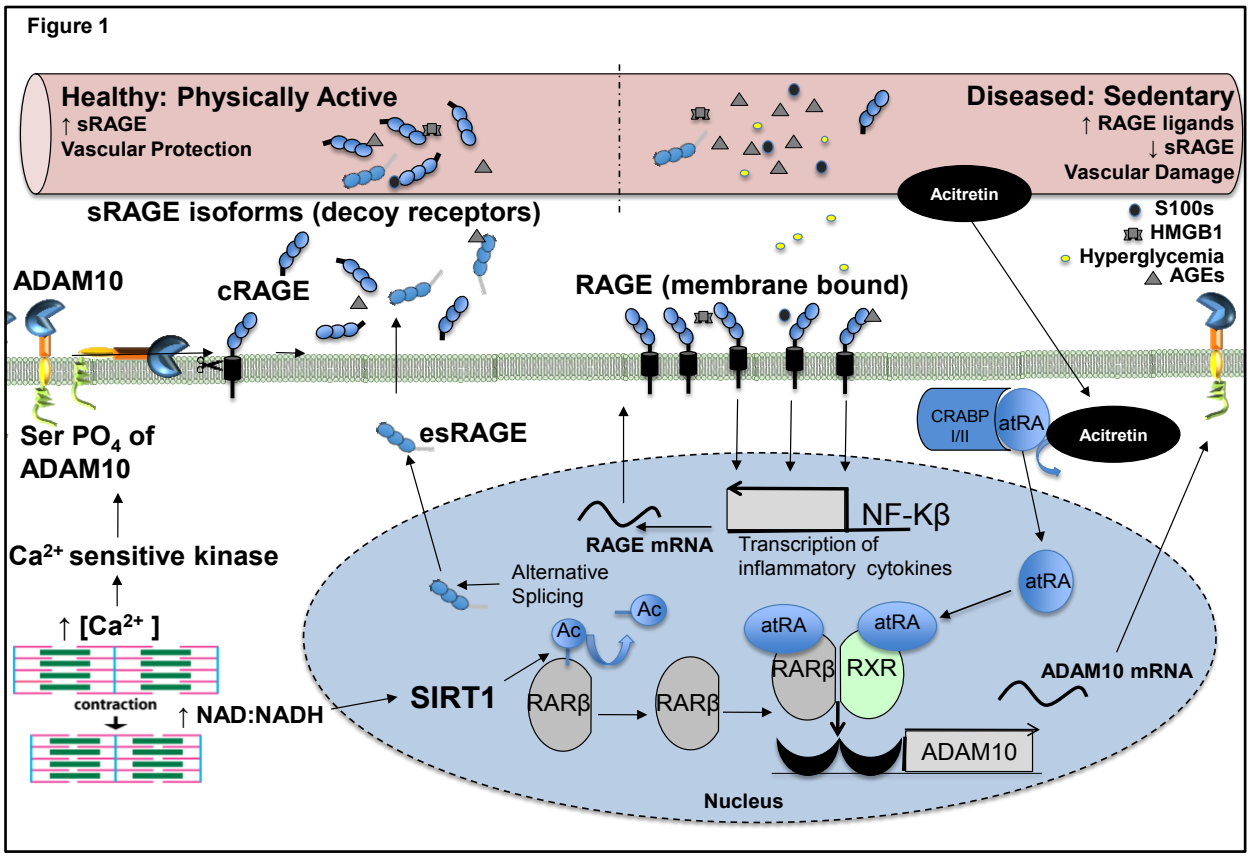
BACKGROUND INFORMATION

Our long-term goal is to identify strategies for the prevention and treatment of diabetes. Exercise is a key factor in mitigating diabetic complications. However, the molecular foundations of the effects of exercise on diabetes are largely unknown (Figure 1). Our lab's specific focus is the receptor for advanced glycation end products (RAGE) and its ligand (advanced glycation end products [AGE]). Activation of RAGE, via binding of AGEs and other ligands, regulates the development and progression of diabetic complications through persistent intercellular signaling (i.e., NF-Kβ) [1]. Targeting RAGE directly as a therapeutic strategy has largely been unsuccessful. However, RAGE signaling can be interrupted, *in vivo,* by ADAM10 (a disintegrin and metalloproteinase 10) [2, 3], thus creating a soluble isoform of RAGE (sRAGE) that is released from the cell and appears into the circulation [3]. Maintaining high levels of circulating sRAGE is advantageous as sRAGE sequesters RAGE ligands and prevent cell signaling [4-8]. Isoforms of sRAGE (cleaved [cRAGE] and endogenous secretory [esRAGE]), are decreased in inflammatory conditions such as type 2 diabetes (T2DM) and coronary artery disease, while treatment with recombinant sRAGE, suppresses atherosclerosis and vascular dysfunction in animal models of diabetic coronary artery disease [4, 9, 10]. Thus, targeting mechanisms that promote sRAGE generation are worth consideration for the prevention and treatment of diabetic complications such as coronary artery disease and microvascular disease [9, 11].



Although the exact mechanisms of ADAM10 mediated RAGE release remain undefined, calcium related [2, 3] and other signaling (SIRT1) impact ADAM10 [12]. Aerobic exercise (AE) presents a unique model for mechanistic study of RAGE release as muscle contraction induces robust calcium signaling [13], activates SIRT1 [14-16], and provides stimuli for tissue remodeling and resolution of the metabolic profile that drives inflammation [17-21]. Our preliminary data show acute AE increases muscle biopsy derived ADAM10 activity in lean healthy individuals. We have also shown acute AE increases plasma cRAGE in obese, normal glucose tolerant, impaired glucose tolerant, and T2DM subjects, while chronic AE (12 weeks) increases skeletal muscle ADAM10 protein expression, and reduces RAGE protein expression in obese impaired glucose tolerant subjects. These findings support the need to explore the effects and molecular foundations of AE on AGE-RAGE biology in humans with T2DM.

Rationale

The rationale for this study was generated using the existing literature base coupled with our preliminary data. Capitalizing on the natural ability of skeletal muscle to turnover following aerobic exercise, we derived an aerobic exercise model to test our central hypothesis that skeletal muscle RAGE release with regular physical activity (via ADAM10) is a quantitatively important source of sRAGE appearance into the circulation. Once the mechanisms of ADAM10 activation and sRAGE generation are elucidated, progress towards the prevention and treatment of diabetic complications may be possible.

Potential Risks and Benefits

Overall, few potential risks exist. These relate to skeletal muscle biopsies, local anesthetic delivery, blood draws, microdialysis of muscle, glucose and insulin infusion, strenuous aerobic exercise, body composition assessment (DEXA scan), and data confidentiality. Specific details on these potential risks and strategies to mitigate their occurrence are discussed in *Assessment of Safety* and *Adverse Events*.

The potential direct benefits include improved health as a result of exercise training and potential educational benefits associated with current health status, healthy eating, and exercise/physical activity. Each participant may gain knowledge of current health status as determined by medical history and physical examination as well as health benefits related to monitoring of their diabetes. The benefit to society will include an improved understanding of the mechanisms of type 2 diabetes (T2DM) development and the treatment of diabetes and reduction of cardiovascular disease risk. Data from this study may also help determine an effective approach to prevent the progression of obesity and obesity-related diseases and inflammatory conditions. The minimal potential risks are outweighed by the benefit of the information that will be acquired from this study.

Study Objectives

1) Test the effects of chronic aerobic exercise versus standard care alone on sRAGE in the circulation of adults with T2DM.

2) Test the effects of chronic aerobic exercise versus standard care alone on RAGE in skeletal muscle of adults with T2DM.

Study Outcomes

The global construct of this study is to provide insight into the role of aerobic exercise on muscle health in the context of RAGE biology. Due to the implication of RAGE signaling, findings of this study may guide the development of therapeutic modalities for T2DM.

Study Hypotheses

Our central hypothesis is that skeletal muscle contributes to sRAGE appearance in the circulation and maintenance of healthy levels of total sRAGE promotes cardiometabolic health. Therefore, two specific working hypotheses have been proposed.

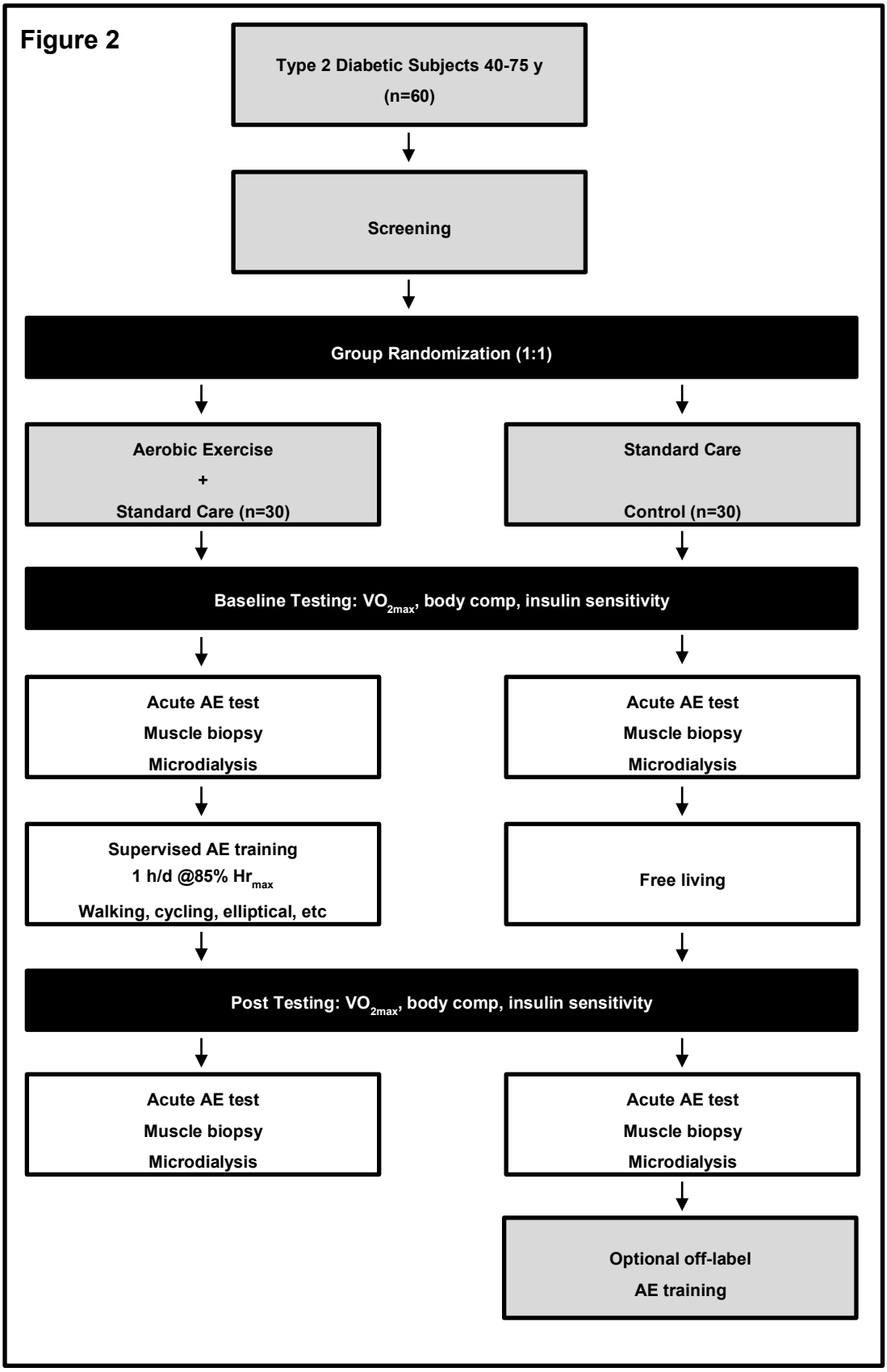
1) Circulating sRAGE levels will increase following 12-weeks of aerobic exercise training and this change will be related to reduced muscle RAGE expression and increases in insulin sensitivity and cardiorespiratory fitness.
2) Skeletal muscle expression of RAGE will decrease following 12-weeks of aerobic exercise training and this change will be related to increases in circulating sRAGE, insulin sensitivity, and cardiorespiratory fitness.

STUDY DESIGN

Figure 2 provides a general framework of the study design. This 12-week clinical study involves the testing and training of middle age to older (40 - 75 y), overweight or obese adults (BMI 26 - 44 kg/m^2) with type 2 diabetes (T2DM). Sixty subjects will be randomized to 12 weeks of aerobic exercise training plus standard care (Aerobic Exercise group; n=30) or standard care only (Control group; n=30). Subjects randomized to the Control group will be given the option of an off-label exercise intervention. Therefore, Control group participants will be allowed to enroll in the AE training group after completion of the original Control group trial period. All subjects will undergo the same baseline and post testing for basal metabolic rate, maximal aerobic capacity (VO_2max), body composition, insulin sensitivity, and microdialysis sampling coupled with muscle biopsies during an acute aerobic exercise trial before.

Due to the complexity of this study, an interdisciplinary research team with experience in clinical trials, pathophysiology of, and research in obesity, insulin resistance and T2DM, vascular function, aerobic exercise testing/training, clinical medicine, biotechnology, and advanced biostatistics has been assembled. The research staff has many years (>10 y) of direct research experience with human subjects and have been trained by experts in the field and University of Michigan protocols and guidelines.

Figure 2


Type 2 Diabetic Subjects 40-75 y
(n=60)
Screening
Group Randomization (1:1)
Aerobic Exercise
+
Standard Care (n=30)
Standard Care
Control (n=30)
Baseline Testing: VO2max, body comp, insulin sensitivity
Acute AE test
Muscle biopsy
Microdialysis
Acute AE test
Muscle biopsy
Microdialysis
Supervised AE training
1 h/d @85% Hrmax
Walking, cycling, elliptical, etc
Free living
Post Testing: VO2max, body comp, insulin sensitivity
Acute AE test
Muscle biopsy
Microdialysis
Acute AE test
Muscle biopsy
Microdialysis
Optional off-label
AE training

Primary Outcome Measure

1) Quantify the change in basal circulating sRAGE after 12-weeks of supervised aerobic exercise training.

Serum will be separated from blood samples collected in vacutainer tubes via centrifugation before and after 12-weeks of aerobic exercise training. sRAGE will be quantified in these serum samples via ELISA (Quantikine, Human RAGE Immunoassay) per manufacture's protocol.

Secondary Outcome Measures

1) Quantify the change in basal muscle RAGE expression after 12-weeks of supervised aerobic exercise training.

Basal biopsy derived skeletal muscle RAGE expression will be determined from the vastus lateralis before and after 12-weeks of aerobic exercise training. RAGE expression will be quantified via Western Blot. Muscle samples (~10 mg) will be homogenized and protein concentration will be determined via BCA assay (Pierce). Samples (20 µg protein) will be diluted in SDS buffer, heated at 85 °C for 5 min, resolved via SDS-PAGE (Bio-Rad Laboratories, Hercules, CA) and transferred to a nitrocellulose or PVDF membrane. Blocking will occur for 1 h and primary antibody (RAGE; Abcam, Cambridge, MA) incubation will occur overnight at 4 °C and quantified vs a standard or total protein.

2) Quantify the change in aerobic capacity (VO_2max) after 12-weeks of supervised aerobic exercise training.

Maximal oxygen consumption (VO_2max) will be established via indirect calorimetry during an incremental treadmill test (Cornell protocol) before and after 12-weeks of aerobic exercise training. Criteria, such as, rating of perceived exertion >18, respiratory exchange ratio >1.10, no further increase in VO_2 despite increasing workloads, heart rate greater than age-predicted maximum, or volitional fatigue will be used to indicate a successful VO_2max was achieved.

3) Quantify the change in insulin sensitivity after 12-weeks of supervised aerobic exercise training.

Insulin sensitivity will be established via hyperinsulinemic-euglycemic clamp before and after 12-weeks of aerobic exercise training. After the initial basal period of this procedure, a primed-continuous infusion (40mU/m^2/min) of human insulin will be initiated and maintained for a period of 120 min. Glucose levels will be clamped at 90 mg/dL by use of a variable glucose infusion (20% dextrose). Blood glucose will be measured every 5 min to monitor levels and used to adjust the variable glucose infusion rate. The clamp procedure will be completed after the 120-min period of hyperinsulinemia or until steady state glucose concentration is achieved.

ELLIGIBILITY AND ENROLLMENT

A total of 60 adults, (approximately 30 males and 30 females) will complete this study. Subjects must be weight stable (±2 kg for ≥6 months), sedentary (<30 min of exercise, 3 times per week), and free of any additional contraindications to participation in exercise testing or the training. Selection criteria will limit confounding influence on the study objectives and exclude subjects with conditions that may have unknown effects on metabolic function and the outcomes proposed or the ability to participate in the aerobic exercise intervention or testing procedures. Criteria will be reviewed by the research team and/or study physician and eligibility to participate will be determined on a case by case basis.

Subject Inclusion Criteria

- Age 40-75 y
- Type 2 diabetes
- Overweight or obese (BMI 26-44 kg/m^2)
- Fluency in English (written and verbal)

Subject Exclusion Criteria

- Age <40 or >75 y
- BMI <26 or >44 kg/m^2
- Existing cardiovascular, cerebrovascular, renal, or hematological disease, cancer, or other metabolic diseases that is suspected to impact study outcomes
- Current use of tobacco
- Pregnant or lactating

Strategies for Recruitment and Retention

Subjects will be recruited from the greater Ann Arbor area through multiple mechanisms. Primarily, study advertisements will be posted and distributed throughout the communities surrounding the University of Michigan. In addition, we will utilize our clinical collaborations within the Division of Endocrinology and Metabolism to strengthen our ability to identify and recruit potential volunteers. We plan to advertise and seek potential volunteers through word of mouth, the Data Office for Clinical and Translational Research, MDRC Diabetes Research Registry, the website of the School of Kinesiology, newspaper, postal mail, and social media outlets. In our previous studies, we have not experienced difficulty recruiting individuals for muscle biopsy or metabolic procedures.

Screening Procedures

Initial screening of participants will occur via a website screening tool or telephone interview. During this time, the study will be described in detail and we will obtain information about age, body mass index (BMI), smoking and physical activity status, current medical conditions, health status and medications, along with other eligibility

criteria. Subjects who meet the initial screening criteria will be scheduled for a complete screening at the Michigan Clinical Research Unit (MCRU), Frankel Cardiovascular Center (CVC), Domino Farms, or the Central Campus Recreation Building (CCRB). At this time, more specific details about the study will be provided along with answers to any questions. Each study procedure and all possible risks related to these procedures will be explained along with sample exercise programs and explanation of the randomization methods of the study. Verbal and written informed consent will be obtained during this initial visit according to the guidelines of our Institutional Review Board. A study team member will perform a comprehensive medical history. Additionally, participants will complete a venipuncture blood draw and urine collection for laboratory assessment of complete blood count (CBCD), comprehensive metabolic panel (COMP), urinary analysis (UA), urinary microalbumin (UMA), hemoglobin A1C (A1C), thyroid stimulating hormone (TSH), and lipid panel. Females will also be asked to provide urine for a pregnancy test (for pre-menopausal women). In addition, participants will complete a supervised 12-lead resting electrocardiogram and graded exercise stress test (with VO_2) at the CVC or Domino Farms. EKG test results will be reviewed by a study physician. If any blood work or EKG results are clinically significant, the participant will be notified and referred to their personal physician for additional evaluation prior to further consideration for participation in this study. Baseline food intake will be assessed from diet history including multiple replicate 24-hour recalls to estimate usual intake, customary eating pattern, and food frequency questionnaires. After confirmation of the participant meeting all inclusion and exclusion criteria, participants will be enrolled into the study intervention.

STUDY INTERVENTION

Intervention Description

This study's intervention includes 12 weeks (±1 week) of supervised aerobic exercise training for the group randomized to Aerobic Exercise. The Control group will not be subject to an intervention as they will receive standard care only (see *Standard Care*). Subjects randomized to the Control group will be given the option of an off-label exercise intervention. Therefore, Control group participants will be allowed to enroll in the AE training group after completion of the original Control group trial period.

Group Assignment Procedure

Following enrollment into the study, participants will be randomized into either the Aerobic Exercise group (n=30) or Control group (n=30. Females and males will be equally distributed between the groups. Regardless of group assignment, each participant will receive standard care for diabetes management.

Aerobic Exercise Training

Every AE training session will be supervised by the research team using established protocols [22-28]. Training sessions will take place at the Central Campus Recreation Building (CCRB), Domino Farms, or other University of Michigan recreation facility (e.g., North Campus Recreation Building) approximately 5 days per week and include treadmill walking, cycling, elliptical, or other related modes of exercise. Briefly, AE training will begin with a 2-week ramp-up, familiarization period of progressing exercise intensity and duration. Training intensity will progress from 55% VO_2max for week 1 (40 min session), to 60-65% VO_2max for week 2 (50 min session), to ~70% VO_2max for all other weeks (50 min session). Subjects will perform a brief warm-up and cool-down (~5 min each) that may include stretching or other light exercises for a total exercise time of 60 min. Subjects will wear heart rate monitors during each training session to provide real-time feedback of target heart rate. Intensity, duration, resting and exercise heart rates, and blood pressures will be recorded for each session. Exercise duration and intensity may be adjusted at the discretion of the PI, Co-I, or study team member. Follow-up VO_2max tests will be performed at approximately weeks 4 and 8 to monitor progress. AE intensity will be adjusted to match the prescribed training intensity [23, 24, 29-32]. Subjects in the AE training group will also conduct foot safety checks with the research team to monitor foot ulcers/blisters. Relevant findings during foot inspections will be addressed with the participant and study physician. Non-weight bearing exercise may be implemented if the subject is limited.

As a contingency, exercise training may be done remotely. At the PI, research team member, or study physician's discretion, participants may conduct exercise training at their residence, local gym, or other appropriate facility. During these training sessions, a study team member will communicate with the participant prior to the session and discuss the goals and training plan. More specifically, a team member will discuss where the session is to occur, the duration of the session, mode of exercise, and exercise intensity. During the session, a team member may monitor exercise via telephone or video chat (e.g., zoom). Exercise intensity is monitored via a HR monitor. Each participant wears a Polar HR monitor watch for the duration of the study and is familiarized with its use. If a remote session is to occur, the participant will be sent home with a heart rate strap that communicates with the watch to give HR feedback (i.e., via mail, during visit to the lab, or meeting a local establishment, etc). HR is digitally recorded and downloaded as described elsewhere (another form of monitoring exercise).

Standard Care

All subjects will receive standard care for diabetes management as directed by their individual physician and diabetes health care team according to American Diabetes Association, Standards of Medical Care in Diabetes position statement, 2018 [33]. All newly diagnosed subjects with type 2 diabetes will be encouraged to receive Diabetes Self-Management Education. In addition, both groups will be given a wrist worn device during the intervention and trained in their use to track physical activity patterns. Subjects will meet approximately twice monthly with the study team to download accelerometer data.

Reasons for Withdrawal

Participants are free to withdraw their consent and discontinue participation at any time. We do not anticipate participant withdrawal due to study-related reasons as similar exercise training interventions have been taking place for decades with high levels of adherence. Additionally, we have extensive experience managing clinical trials in the proposed population [26, 30, 34, 35]. However, we recognize participants interests, health, and schedules may change. The research team's best effort will be made to accommodate the participants needs and minimize attrition. The following are potential reasons for withdrawal:

- **Time Commitment**: Some participants may find the time commitment associated with this study too burdensome for their schedule. The study coordinator will discuss this time commitment prior to enrollment.
- **Schedule Conflicts:** Some participants may find it difficult to determine times they are available to come in for study visits. The study coordinator will also discuss preferred visit start times prior to enrollment to limit potential schedule conflicts.
- **Discomfort:** Participants may experience discomfort during blood and tissue sampling procedures and wish to discontinue the study. Prior to enrollment, the study coordinator will discuss all procedures and answer any questions participants may have concerning sampling procedures.
- **Unforeseen Reasons:** There may be other reasons participants may withdrawal from the research study that are unknown at this time.

Handling of Withdrawals

The participants right to withdraw from the study will be respected and will occur within the timeframe they request. In the event of a participant withdrawal, samples and data collected during their participation will be stored for future use or destroyed, based on the participants request.

Termination of Study

The research staff reserves the right to suspend/terminate subject's participation in this study at any time. This decision will be communicated to the participant and a reasonable effort will be made to mitigate/avoid this outcome. Potential study suspension/termination reasons include:

- **Ineligibility**: The subject's eligibility changes during the study
- **Safety**: The research has become harmful to the participant
- **Health**: The subject's health condition changes and requires treatment which affects the study outcomes
- **Incompliance**: Subject fails to follow the study guidelines
- **Cancelation**: The research study is canceled or suspended

Accountability Procedures for the Study Intervention

The research team will communicate internally weekly to discuss study progress and ensure all objectives and guidelines are met. From a participant perspective, agreement to enroll in the study will necessitate adherence to all study guidelines. The research staff will have regular contact (approximately 5 days/week) with subjects in the Aerobic Exercise group and will meet approximately twice monthly with the control group to monitor habits and study progress. We have found these interactions are indispensable for study adherence.

Concomitant Medications

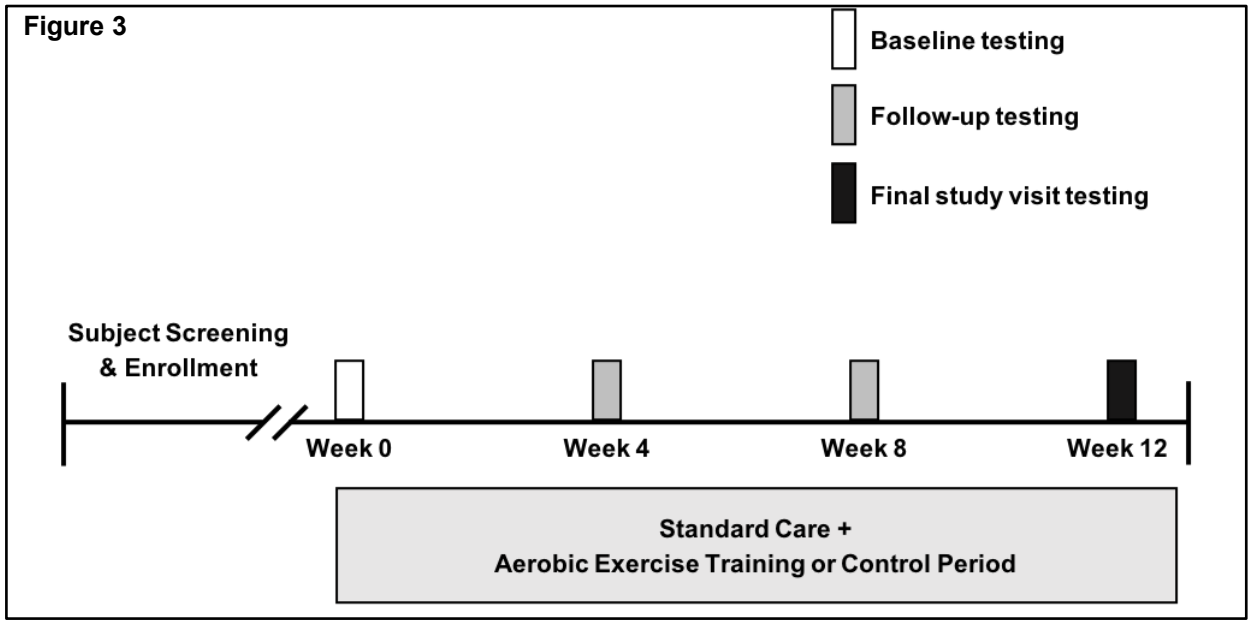
Subjects participating in this study will be diagnosed with T2DM and therefore have unique medical needs. In addition to the screening provided in this study, each participant will be encouraged follow their personal physician's guidance. Prior to and during the intervention period, all medication use, dose and frequency will be documented

Assessment of Subject Compliance with Study Intervention

Subject compliance will be regularly assessed and documented by the research team. Participants enrolled in the AE group will have contact with the research team approximately 5 days per week and the Control group will have contact with the research team approximately twice per month. Compliance will be determined through routine personal communication and primarily evaluated based on exercise training attendance (Aerobic Exercise group only), physical activity tracking, and dietary monitoring. Subjects in the Aerobic Exercise group are expected to complete $\geq$80% of the training sessions over the course of the 12 weeks.

STUDY SCHEDULE AND PROCEDURES

Figure 3 provides a brief study summary of the study schedule. This study is composed of initial subject screening and enrollment based on established inclusion and exclusion criteria and randomization to 12 weeks of Aerobic Exercise training or Control Period (both including established Standard Care practices). Participants will all complete the same baseline testing and final study visit testing. Subjects randomized to the AE group will also undergo follow-up testing at weeks 4 and 8 (±1 week) to adjust exercise training parameters. All testing will occur at the Michigan Clinical Research Unit (MCRU), Michigan Nutrition Obesity Research Center (NORC), Domino Farms, Frankel Cardiovascular Center (CVC), and/or Central Campus Recreation Building (or other University recreation building) and based on subject schedules, will include a total of approximately 7 visits.



Scheduling Flexibility

Due to limitations of the subject's schedule, study team, experimental approach, and/or clinic availability, some procedures may be excluded or adjusted from any given visit. Excluded or adjusted procedures will be at the discretion of the PI or Co-I and will not be considered a protocol deviation.

Subject Screening

Initial screening of participants will occur via a website screening tool or telephone interview. During this time, the study will be described in detail and we will obtain information about age, body mass index (BMI), smoking and physical activity status, current medical conditions, health status and medications. Subjects who meet the initial screening criteria will be scheduled for a complete screening at the MCRU, CVC, Domino Farms, and/or CCRB. Verbal and written informed consent will be obtained during this

initial visit according to the guidelines of our Institutional Review Board. Subjects will be screened based on the inclusion and exclusion criteria. Screening procedures include a comprehensive medical history examination, blood draw, resting electrocardiogram, graded exercise stress test, and urine collection. In addition, pre-menopausal women will have a urine pregnancy test. All screening findings will be reviewed by the research team and study physician(s) (or CVC/Domino Farms physician) when appropriate.

Baseline Testing

Baseline testing procedures will be supervised by the research team and completed at the MCRU, CVC, Domino Farms, CCRB, and/or NORC. Testing includes measurement of basal metabolic rate, maximal aerobic capacity, cognitive assessment questionnaire (NIH ToolBox), body composition, OGTT, and insulin sensitivity. In addition, the Minnesota Leisure Time Physical Activity and Seven Day Physical Activity Recall Questionnaires will be administered to assess habitual physical activity levels. Subjects will also undergo an acute aerobic exercise bout (treadmill walking) coupled with microdialysis and muscle biopsies. Each of these testing procedures are critical for completion of the study objectives and evaluation of the study hypotheses.

Basal Metabolic Rate

Basal metabolic rate will be measured at Domino Farms, MCRU, CCRB, or CVC by the research team and/or medical staff. Subjects will initially rest quietly in their room for ~30 min. We will then measure their basal metabolic rate using a canopy-equipped metabolic cart for ~20 min.

Maximal Aerobic Capacity

Maximal oxygen consumption (VO_2max) will be captured during the GXT and will be used as the criterion measure of aerobic capacity (i.e., physical fitness). The VO_2max test will be performed using a Cornell (or similar) protocol at Domino Farms or the CVC. Measurements of VO_2max and maximal heart rate (HR_{max}) will be performed at baseline and following the 12-week intervention period (interim visit, approximately one week before the final study visit). In the Aerobic Exercise group, baseline VO_2max will used to determine the starting training intensity and VO_2max tests will be repeated at approximately 4 and 8 weeks of aerobic exercise training to adjust target HR. The control group will also return at approximately weeks 4 and 8 as well, as a blood draw (~20 ml) will also be taken for all individuals at these time intervals.

Metabolic Control Period

The metabolically sensitive pre- and post-intervention insulin sensitivity assessment (glucose clamp) will be performed during a 1-night inpatient stay at MCRU (two 1-night stays total over the course of the study). The goal of this overnight stay is to control metabolism to increase outcome validity of the insulin sensitivity assessment.

Participants will be asked to reside in the MCRU for 1 night and will receive a weight-maintenance isocaloric diet (55% carbohydrate, 30% fat, and 15% protein) provided by the study team or MCRU/NORC metabolic kitchen. Subjects will be asked to refrain from physical activity outside of their normal activities of daily living during this control period Additionally, subjects will complete an OGTT, questionnaires, body composition (DEXA scan), and acute exercise test within ~1 week of this overnight visit at the CVC, MCRU, CCRB, and/or Domino Farms. Metabolic measures will be performed ≥24 h after the last exercise bout. This approach has been successfully used by the research team in human metabolic studies to control for effects of diet and physical activity [23-28, 31, 35-37].

Cognitive Assessment

Participants will complete an NIH developed cognitive assessment tool (ToolBox) to evaluate cognition on an iPad. This assessment will be administered by the research team. Dr. Brian Callaghan MD (Neurology) will oversee administration and interpretation of this assessment tool.

Body Composition

Body composition will be assessed by dual-emission X-ray absorptiometry (DEXA). DEXA scans determine components of body composition such as bone mineral density, lean body mass, fat mass, and segmental adiposity such as truncal fat and visceral fat mass. DEXA scans will be performed in the MCRU by the MCRU staff or a member of the study team.

Oral Glucose Tolerance Test (OGTT)

This standard test is utilized to detect and confirm the presence of diabetes. Briefly, a polyethylene catheter will be inserted into an antecubital or dorsal hand vein and the subject will drink a beverage containing 75 g of glucose. If a catheter is not successfully inserted, venipuncture for blood collection may be implemented. In addition, finger-sticks may also be utilized to assess blood glucose levels. A baseline blood draw will precede the consumption of the glucose drink along with blood draws approximately every 30 min for 3 h. Current use of physician confirmed diabetes medication, fasting glucose values ≥ 126 mg/dL and 2 hr OGTT values ≥ 200 mg/dL will be used as the criteria for type 2 diabetes [38]. Blood samples will be collected and immediately transferred to vacutainers, centrifuged, and stored at -80°C until analysis for blood glucose and insulin concentrations. OGTTs will take place at MCRU, CCRB, or Domino Farms and will be supervised by members of the study team after catheter insertion by the clinical staff or qualified research team member.

Measurement of Insulin Sensitivity

Whole body insulin sensitivity will be assessed using a 40 mU/m^2/min hyperinsulinemic-euglycemic (glucose 90 mg/dL) clamp procedure as described

previously and used routinely by the research team [23-27, 31, 34, 35]. Briefly, a polyethylene catheter will be inserted into an antecubital vein for infusion of insulin and glucose. A second catheter will be inserted retrograde into a dorsal hand vein with the hand warmed in a heated box for blood sampling [39]. Once catheters have been inserted, basal blood draws will take place. After the basal period, a primed-continuous infusion (40 mU/m^2/min) of human insulin will be initiated and maintained for a period of approximately 120 min. Glucose levels will be clamped at approximately 90 mg/dL by use of a variable glucose infusion (20% dextrose). Blood glucose will be measured approximately every 5 min to monitor levels and used to adjust the variable glucose infusion rate. The clamp procedure will be completed after once steady state glucose concentration is achieved. Upon completion, insulin infusion will be discontinued while glucose infusion is continued until plasma glucose stabilizes. A comprehensive metabolic panel (COMP) will be assessed before and after the clamp procedure. In addition, the subject will be provided with a post-clamp meal or snack that consists of carbohydrate and protein to further maintain blood glucose (e.g., juice, sandwich). This procedure will take place at MCRU.

Urine Collection

Subjects will be asked to collect their urine starting at approximately 4:00am on the morning of the hyperinsulinemic-euglycemic procedure. Urine samples will be collected in standard urine collection vessel. Total volume and time of collection will be recorded.

Skeletal Muscle Biopsy

The modified muscle biopsy technique was introduced nearly 60 years ago [40] and is a fundamental component of muscle health research (e.g., insulin sensitivity, inflammation). This technique is used commonly in the medical research field (as indicated by large scale consortiums [e.g., NIH funded MoTrPAC]) and is currently utilized at the University of Michigan. Members of the research team have been involved with muscle biopsies for ~15 y, totaling >500 muscle biopsies in populations including athletes, elderly, obese, type 2 diabetics [23, 35, 41-46]. Dr. Jacob Haus, PhD and Dr. Jeffrey Horowitz, PhD will perform the muscle biopsies which are critical to completion of this study's objectives.

Muscle biopsies will be obtained before and within 30 minutes after the acute exercise bout. These exercise trials will take place before and after 12 weeks of the aerobic exercise intervention or standard care periods (4 total muscle biopsies per subject). Muscle samples (~200 mg; approximately the size of a pea) will be obtained from the *vastus lateralis* muscle (thigh muscle) following local anesthetic delivery using the modified muscle biopsy technique [40, 41, 44, 47]. Muscle tissue will be quickly trimmed of excess connective tissue and fat, blotted with gauze to remove blood and processed specific to future analysis. Subjects will be instructed on how to care for the wound and supplied with ice and pressure bandages after each biopsy to reduce

inflammation and any soreness. A member of the research team will follow-up with each participant the day after a muscle biopsy.

SOP for skeletal muscle biopsy

- Anti-coagulant medication (e.g., Coumadin, Rivaroxaban) and Lidocaine allergy/sensitivity are exclusion criteria for the biopsy procedure

Before performing the tissue biopsies, Dr. Haus (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)

Skeletal Muscle Biopsy Procedure

- Confirm that the participant does not have any known allergies/sensitivity to local anesthetic (e.g., Lidocaine/Xylocaine/bupivacaine) – 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 or chlorohexadine
- Put on sterile gloves and all the following procedures must be performed using aseptic technique
- Create sterile field on tray top and around the antiseptic covered area of the participant's thigh
- Infiltrate skin and underlying tissue with approve analgesic using a 25g x 1.5" or similar needle (For example, if using lidocaine ~5-10 ml will be administered: total dose of lidocaine not to exceed 4.5mg/kg)
- Make an incision above the vastus lateralis with a #10 or #11 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 or similar 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 to syringe (or similar) 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 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 and gently dabs the sample on the absorbent pad to remove excess liquid
- The sample is processed according to PI discretion
- While research team member 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 or equivalent and an overlaying transparent dressing
- A pressure dressing is applied to the thigh until bedtime the night of the biopsy procedure
- Participant is instructed to avoid water submersion for 3 days and to keep the bandages on for 3-5 days

Upon discharge, provide participant with pre-printed post-procedure care instructions with emergency contact information.

Acute Aerobic Exercise Bout: Treadmill Walking

Subjects will complete an acute aerobic exercise bout coupled with muscle biopsies and adipose microdialysis at baseline and following the 12-week intervention period. This exercise bout includes 60 min of treadmill walking at ~65-70% of their predetermined VO_2max (VO_2max will be measured approximately one week prior to the acute exercise bout). The acute exercise bout is considered “moderate” intensity, given the relatively low heart rate maintained and muscles utilized. Subjects will be familiarized to the protocol before testing. For all acute exercise trial sessions, heart rate and oxygen consumption will be monitored to assess the exercise intensity. The acute aerobic exercise bouts will be performed at MCRU or Domino Farms and supervised by the research team which will include a study physician on-call.

Microdialysis

Our lab has experience with the microdialysis technique and has published muscle specific microdialysis data with acute aerobic exercise in human subjects [48]. We have also enlisted the expertise of Dr. Robert Hickner, PhD (Florida State University) to provide consultation on microdialysis best practices and interpretation of data generated. Dr. Hickner is a microdialysis expert who is one of the original developers of the microdialysis methodology in skeletal muscle nearly 25 years ago [49].

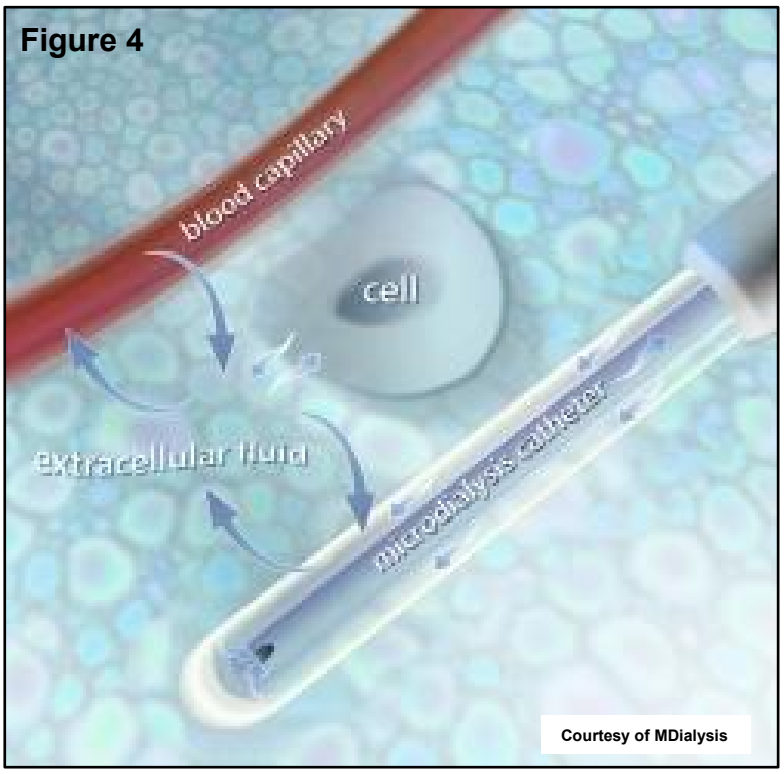
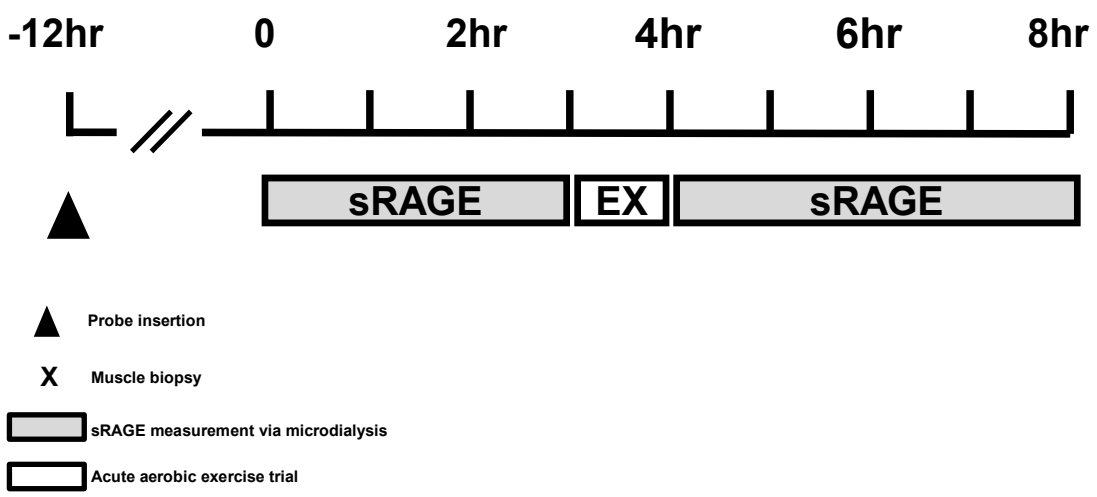


Figure 4

Microdialysis catheters mimic blood capillaries (Figure 4). Substances from the extracellular space can be collected into the catheter. The timeline of microdialysis events are portrayed in Figure 5. Two, small, M Dialysis 71 high cut-off microdialysis catheters will be inserted into the abdominal subcutaneous adipose bed (no closer than 3 in to the umbilicus). The microdialysis probes will be perfused with sterile water at 0.3 µl/min for 10 minutes before and immediately after probe insertion with a calibrated micro infusion pump. The probes will then be disconnected from the perfusion pump and left in place with the inlet and outlet tubing capped, which is secured to the leg overnight. The timing of the probe insertion (approximately 12 hr before the resting measurements) is to ensure that the resting measurements are not influenced by the probe insertion [50, 51]. The following morning each subject will undergo the baseline measurement of resting adipose tissue sRAGE collected by the microdialysis probe. The microdialysis probes are perfused with a sterile solution supplemented with 37 g/L dextran-70. Dextran is used to assure there is no net fluid transport across the microdialysis membrane [52, 53]. Therefore, the subjects do not come into contact with the dextran solution. Samples will be collected in sealed microvials that are pre-weighed before and after each collection period to determine actual dialysate weight and calculate dialysate volume. At the start of each microdialysis measurement, each probe is reconnected to the perfusion pump and perfused with sterile water for 10 minutes at 0.3 µl/min and then at 0.3 µl/min for 3 hr while the subjects rest quietly or 4 hrs post-acute aerobic exercise. Subjects will be instructed on how to care for the wound and supplied with ice and pressure bandages after to reduce inflammation and any soreness.

Figure 5



Initial Exercise Bout (moderate treadmill walking)

Upon completion of the baseline testing period, all subjects will be asked to undergo a single, modest treadmill aerobic exercise bout (65% VO_2max for 30 minutes). Prior to this trial, each participant will have been medically cleared for exercise based on screening criteria. This treadmill bout will be supervised by a member of the research team and may be completed at CCRB, Domino Farms, or MCRU (or other appropriate University facility). Participants will be familiarized with the protocol, In addition to intermittent oxygen consumption evaluation, subjects will be fitted with a heart rate monitor to track exercise intensity. Each subject will have a simple blood draw before and after the trial (~80 ml total). This exercise will count as the first exercise training session for subjects randomized to the training group and subjects randomized to the Control group will be advised to resume normal daily activity and standard of care for the duration of the study.

Follow-up Testing

All subjects will return for follow-up testing at approximately weeks 4 and 8. Each subject will have a blood draw (~20 ml) and the AE group will also complete a VO_2max test to adjust target HR for the training protocol.

Final Study Visit Testing

The final study visit at MCRU or Domino Farms will include the same testing procedures for both groups that were completed during baseline testing (i.e., body

composition assessment, cognitive assessment, OGTT, measurement of insulin sensitivity). In addition, all subjects will undergo an acute aerobic exercise bout (treadmill walking) coupled with microdialysis and muscle biopsies.

Total Study Tissue Sampling

Table 1 outlines the approximate amount tissue to be collected during the study. Tissue samples (blood, urine, and muscle) will be obtained during the screening and/or baseline (before the 3 month AE intervention or control period), follow-up visits, and final study visits (after the 3 month AE intervention or control period) time points. Therefore, these time points will be separated by >3 months. In addition, participants in this study will be overweight or obese and have a body weight > 110 lb. Thus, the proposed blood draws are acceptable by University of Michigan IRBMED guidelines.

Table 1

Time point	**Blood**	**Muscle**
Screening Visit		
Both groups	20 ml	-
Baseline Visit		
AE group	280 ml	400 mg
Control group	280 ml	400 mg
Initial Exercise Bout		
AE group	80 ml	-
Control group	80 ml	-
Follow-up Visits		
Both groups	40 ml	-
Final Visit		
AE group	280 ml	400 mg
Control group	280 ml	400 mg
AE group total	~700 ml	~800 mg
Control group total	~700 ml	~800 mg

Sample Preparation, Storage, and Shipping

Study samples and resultant data will not contain personal identifying information. Each subject will receive a unique study identifier and samples will be appropriately labeled based on the testing procedure and sample type. All source document data will be kept in a locked file cabinet and converted to electronic data. All electronic data will be safe-guarded by password protection on a University of Michigan server. Sample preparation will be completed by the research team and/or MCRU/NORC staff during each procedure. This includes processing of muscle biopsy, blood, and microdialysis

samples. Samples will be stored in a -80 °C freezer. Handling and shipping of study samples will only occur by the trained research staff as directed by Michigan Occupational Safety and Health Act (MIOSHA) and Occupational Safety and Health Administration (OSHA) guidelines.

Assessment of Safety

The risk to benefit ratio for this study is excellent. Overall, the risks are minor with the information gained on the impact of exercise on diabetes invaluable. Safety will be regularly monitored through personal communication, observation, and documentation. Each individual involved in this study will be encouraged to communicate needs and any potential issues that arise.

Adverse Events

Members of the research team will be present for all components of the study to monitor safety. Additionally, the research team will communicate weekly for review of safety and data collection. In the situation of an adverse event, the event will be immediately managed an IRBMED Adverse Event Report Form will be sent to the appropriate clinical site and Institutional Review Board in a timely manner. This report will include a full description of the event, including the relationship of the incident to the test procedure.

Potential Adverse Events and Mitigation Strategies

Breach of Confidentiality: There is a potential for breach of confidentiality. Study samples and resultant data will not contain personal identifying information. Each sample will be stored with a unique code. All source document data will be kept in a locked file cabinet and converted to electronic data. All electronic data will be safe-guarded by password protection and encryption on a University of Michigan server.

Muscle Biopsy: Risks include discomfort, infection, or excessive bleeding at the biopsy site. These complications will be minimized using sterile techniques and pressure by an experienced team member. Subjects will be instructed that they should report any bleeding or opening of the wound immediately for follow up care. Subjects will be supplied with ice and pressure bandages after each biopsy to reduce inflammation and any soreness after the biopsy. A member of the research team will follow-up with participants the day after each muscle biopsy.

Lidocaine: Lidocaine is a local anesthetic administered during the muscle biopsy technique. It is possible to develop an allergic reaction to lidocaine, which could range from minor itching and a rash to severe respiratory arrest and death. This is a possibility with all medications and we will include subjects in the study only if they are without a history of an allergic reaction. Medical treatment will be provided if an allergic reaction is evident.

Bupivacaine: Bupivacaine is a local anesthetic delivered during the muscle biopsy technique. It is possible to develop an allergic reaction to bupivacaine, which could range from minor itching and a rash to severe respiratory or cardiac arrest and death. This is a possibility with all medications and we will include subjects in the study only if they are without a history of an allergic reaction. Medical treatment will be provided if an allergic reaction is evident.

DEXA: There is a risk of x-ray exposure with DEXA. The duration of each scan will be less than 10 min and the subject will be exposed to a radiation dose of ≤ 0.3 mrem based upon manufacturer's specifications and calculations from Stanford Dosimetry, LLC RADAR Medical Procedure Radiation Dose Calculator. This amount of radiation exposure is considered "general public" exposure by the Nuclear Regulatory Commission.

Blood Samples: Inserting a needle for blood sampling may be associated with some discomfort, bruising, and on a very rare occasion, with inflammation. Strict sterile procedures will be utilized during blood draws to minimize risks.

Urine Collection: There are no known risks to collecting urine samples. However, some people may find it uncomfortable or embarrassing to collect samples.

EKG: Men may need to have a small amount of chest hair shaved off for the ECG pads to stick correctly. A small amount of adhesive might remain on the skin when the pads are removed or the adhesive might pull on hair when removed.

Exercise: There is a risk of physical injury or fatigue due to the cardiorespiratory fitness test and participation in an exercise intervention. However, these risks are generally considered rare and minor. Our investigation team has extensive experience conducting graded exercise tests in a number of special populations (including elderly and diseased populations) and is well versed in how best to minimize risk during these tests. In addition, exercise testing and training will be performed in a facility equipped with AED devices and staff trained in CPR.

Insulin Clamp Procedure: There is a risk that the subject may react to the infusion of glucose or insulin. These reactions could include low blood sugar, an increase in blood pressure, flushing and/or sweating. In the event one of these reactions occur, medical staff will be notified and appropriate measures will be taken.

Heated Hand Box: There is a minor risk of skin discomfort or burn. If the temperature is too warm it can be reduced to improve comfort.

Oral Glucose Tolerance Test: There is a minor potential risk of reaction to the ingestion of glucose. These reactions could include nausea, low blood sugar, an increase in blood pressure, flushing and/or sweating. Continuous blood glucose monitoring will take place during this procedure to provide real time feedback of glucose levels.

Microdialysis: Inserting the microdialysis probes may be uncomfortable or painful.

Study Diet: It may be burdensome for subjects to adhere to a special diet for an extended period of time. The metabolic kitchen staff at MCRU will work to accommodate participant needs.

Unforeseeable Risks: There may be risks or side effects related to the study that are unknown at this time. Continuous monitoring of all participants will facilitate appropriate action in the event of an unforeseeable risk.

Expected Adverse Reactions

There are no expected adverse events.

Serious Adverse Events and Unanticipated Problems

Subjects will be closely monitored by the research team, MCRU, CVC, and/or Domino Farms staff during all testing procedures. Should a serious event occur, it will be managed promptly by the research team and appropriate medical staff.

Abnormal Laboratory Test Values or Abnormal Clinical Finding Procedure

Should an abnormal test value or finding be identified, the subject will be promptly notified and encouraged to communicate the finding with their personal physician.

Reporting Procedures

All adverse events will be reported to the research team for awareness. The study principal investigator, Dr. Jacob Haus PhD, and a study physician, will ensure the University of Michigan Institutional Review Board is notified within a timely manner.

Safety Oversight

Dr. Jacob Haus, PhD and Dr. Elif Oral, MD (the physician-of-record) will provide safety oversight for the study. Dr. Jeffery Horowitz, PhD (School of Kinesiology) will serve as our data safety monitor.

STATISTICAL CONSIDERATIONS

Study Hypotheses

Our central hypothesis is that skeletal muscle contributes to sRAGE appearance in the circulation and maintenance of healthy levels of total sRAGE promotes cardiometabolic health. Therefore, two specific working hypotheses have been proposed.

1) Skeletal muscle expression of RAGE will decrease following 12-weeks of aerobic exercise training and this change will be related to increases in insulin sensitivity and cardiorespiratory fitness.
2) Circulating sRAGE levels will increase following 12-weeks of aerobic exercise training and this change will be related to reduced muscle RAGE expression and increases in insulin sensitivity and cardiorespiratory fitness.

Statistics and Sample Size Considerations

The primary outcomes of this study involve collecting data at pre-aerobic exercise training and 12 weeks of training. Comparisons of trajectories across groups (training vs. Control) over time are made with appropriate adjustments for fixed (age, sex, medications, diabetes duration and severity) and time-varying covariates (i.e., weight, BMI, etc). Because of the anticipated nonlinear response curves of outcome variables we will use nonlinear mixed effects models to analyze our data. Using PROC NLMIXED in SAS 9.2 (SAS Inc.) we will fit the nonlinear models using two likelihood-based methods: adaptive Gaussian quadrature and a first-order Taylor series approximation [54-56]. The nonlinear mixed model is: $y = f(x_{ij}, \beta, u_i) + e_{ij}$, where f is some nonlinear function of known vector covariates ($x$ij) for the jth observation on the ith subject, unknown fixed effect parameters (β), an unknown vector of random effect parameters (u_i), and unknown random errors (e_{ij}). For this study, we will treat training group as main fixed effect, intercept as random effects, and all outcomes will be used as responsive variables. Using nonlinear mixed effects models described above, we will reject null hypothesis if significant ($p<.05$) interaction effect between group and time is observed. Post-hoc analysis will examine differences between groups at different time points to assess the effect of exercise training on the outcome variables across time during the intervention. Our human sample size was determined using data from preliminary studies to provide adequate power after attrition to test the hypothesized effect of training on skeletal muscle RAGE and sRAGE concentrations. We will enroll 75 subjects with the anticipation that there will be 20-25% attrition at 12 weeks. We anticipate that the final N after attrition will be 60 (n= 30 per group). Using the two-sided alpha level of 0.05, we estimate that a total sample size of 60 will enable us to detect a pre-post, between-group difference of 1.24 standard units for RAGE between Aerobic Exercise training and Control groups with a target of 90% statistical power.

Safety and Efficacy Review

Our team has a Michigan Occupational Safety and Health Act (MIOSHA) approved chemical hygiene plan in place. This hygiene plan requires extensive training in the University of Michigan's Responsibility Conduct of Research training program, known as PEERRS (Program for Education and Evaluation in Responsible Research and Scholarship), first aid, and CPR. The research team will be meet weekly to discuss study progress, training, and ensure study objectives and guidelines are met.

Analysis Plan

Due to nature of this study, data will be analyzed on an on-going basis. Participant enrollment will be rolling with sample collection occurring intermittently. Samples will be batched and analyzed as appropriate. Study samples and resultant data will not contain personal identifying information (only a unique study code). All source document data will be kept in a locked file cabinet and converted to electronic data. All electronic data will be safe-guarded by password protection on a University of Michigan server.

QUALITY CONTROL AND QUALITY ASSURANCE

Ethics and Protection of Human Subjects

Coupled with extensive experience testing human subjects in a variety of settings, the training provided by the University of Michigan's Responsibility Conduct of Research training program, known as PEERRS (Program for Education and Evaluation in Responsible Research and Scholarship) will guide the protection of the research team and study participants.

Informed Consent Process

As explained in detail elsewhere, initial screening of participants will occur via a website screening tool or telephone interview. During this time, the study will be described in detail and we will obtain information about age, body mass index (BMI), smoking and physical activity status, current medical conditions, health status and medications. Subjects who meet the initial screening criteria are scheduled for a medical screening. These tests will be performed in the Michigan Clinical Research Unit (MCRU), CVC, Domino Farms, and/or the CCRB (or other University recreation building). Verbal and written informed consent will be obtained during this initial visit according to the guidelines of our Institutional Review Board. Subjects are screened based on the inclusion/exclusion criteria.

Informed Consent/Assent Process (in Case of a Minor)

Minors will not participate in this study.

Subject Confidentiality

The screening process utilized in this study was selected to minimize study risk. All conversations between the research team and participants are considered confidential. Screening will take place in a private environment with only the research team and/or Michigan Clinical Research Unit (MCRU), CVC, or Domino Farms staff present. Study samples and resultant data will not contain personal identifying information. All source document data will be kept in a locked file cabinet and converted

to electronic data. All electronic data will be safe-guarded by password protection on a University of Michigan server.

Study Discontinuation

Participants are free to withdraw their consent and discontinue participation at any time. We do not anticipate participant withdrawal due to study-related reasons as similar exercise training interventions have been taking place for decades with high levels of success. Additionally, we have experience managing clinical trials in the proposed population [26, 30, 34, 35]. However, we recognize participants interests, health and schedules may change. The research team's best effort will be made to accommodate the participants needs and minimize attrition.

Future Use of Stored Specimens

Participants will be asked to consent for future use of their samples. We expect that the data and tissue and blood samples obtained from the proposed study will have lasting research value to maximize productivity for the research team. We intend to use these resources to explore future mechanisms and the data obtained that can be mined for new and impactful opportunities.

DATA HANDLING AND RECORD KEEPING

Data Management Responsibilities

Data will be managed internally by the research team. Source study documents will be converted to electronic files as needed and interpreted by the research team on an on-going basis. The source study documents will be safe-guarded in a locked file cabinet. Weekly study team meetings will address data management needs.

Data Collection Process

Data collection will be conducted by the research team, MCRU, CVC, and/or Domino Farms staff at the University of Michigan. Specifically designed protocol documentation forms will guide the data collection process.

Study Records Retention

Similar to the future use of stored samples, we intend to use study records as resources to explore future mechanisms and the data obtained that can be mined for new and impactful opportunities. Study records provide important clinical context and interpretation of study findings.

Protocol Deviations

Study protocol deviations are not anticipated. In the event a deviation from study protocol becomes necessary, documentation and dissemination within the research team and to the Institutional Review Board (as needed) will occur in a timely fashion.

References

1. Ramasamy, R., S.F. Yan, and A.M. Schmidt, *The diverse ligand repertoire of the receptor for advanced glycation endproducts and pathways to the complications of diabetes.* Vascul Pharmacol, 2012. **57**(5-6): p. 160-7.
2. Galichet, A., M. Weibel, and C.W. Heizmann, *Calcium-regulated intramembrane proteolysis of the RAGE receptor.* Biochem Biophys Res Commun, 2008. **370**(1): p. 1-5.
3. Metz, V.V., et al., *Induction of RAGE shedding by activation of G protein-coupled receptors.* PLoS One, 2012. **7**(7): p. e41823.
4. Falcone, C., et al., *Plasma levels of soluble receptor for advanced glycation end products and coronary artery disease in nondiabetic men.* Arterioscler Thromb Vasc Biol, 2005. **25**(5): p. 1032-7.
5. Grossin, N., et al., *Severity of diabetic microvascular complications is associated with a low soluble RAGE level.* Diabetes Metab, 2008. **34**(4 Pt 1): p. 392-5.
6. Ha, C.H., et al., *Inhibitory effect of soluble RAGE in disturbed flow-induced atherogenesis.* Int J Mol Med, 2013. **32**(2): p. 373-80.
7. Lin, J., et al., *Inflammation and progressive nephropathy in type 1 diabetes in the diabetes control and complications trial.* Diabetes Care, 2008. **31**(12): p. 2338-43.
8. Thomas, M.C., et al., *Soluble receptor for AGE (RAGE) is a novel independent predictor of all-cause and cardiovascular mortality in type 1 diabetes.* Diabetologia, 2011. **54**(10): p. 2669-77.
9. Park, L., et al., *Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts.* Nat Med, 1998. **4**(9): p. 1025-31.
10. Basta, G., et al., *Circulating soluble receptor for advanced glycation end products is inversely associated with glycemic control and S100A12 protein.* J Clin Endocrinol Metab, 2006. **91**(11): p. 4628-34.
11. Endres, K. and F. Fahrenholz, *Upregulation of the alpha-secretase ADAM10--risk or reason for hope?* FEBS J, 2010. **277**(7): p. 1585-96.
12. Tippmann, F., et al., *Up-regulation of the alpha-secretase ADAM10 by retinoic acid receptors and acitretin.* Faseb J, 2009. **23**(6): p. 1643-54.
13. Rose, A.J. and M. Hargreaves, *Exercise increases Ca2+-calmodulin-dependent protein kinase II activity in human skeletal muscle.* J Physiol, 2003. **553**(Pt 1): p. 303-9.
14. Kang, M.R., et al., *Reciprocal roles of SIRT1 and SKIP in the regulation of RAR activity: implication in the retinoic acid-induced neuronal differentiation of P19 cells.* Nucleic Acids Res, 2010. **38**(3): p. 822-31.
15. Lee, H.R., et al., *Cilostazol suppresses beta-amyloid production by activating a disintegrin and metalloproteinase 10 via the upregulation of SIRT1-coupled retinoic acid receptor-beta.* J Neurosci Res, 2014. **92**(11): p. 1581-90.
16. Tang, S., et al., *SIRT1-mediated deacetylation of CRABPII regulates cellular retinoic acid signaling and modulates embryonic stem cell differentiation.* Mol Cell, 2014. **55**(6): p. 843-55.
17. Irimia, J.M., et al., *Muscle Glycogen Remodeling and Glycogen Phosphate Metabolism following Exhaustive Exercise of Wild Type and Laforin Knockout Mice.* J Biol Chem, 2015. **290**(37): p. 22686-98.

18. Ferraro, E., et al., *Exercise-induced skeletal muscle remodeling and metabolic adaptation: redox signaling and role of autophagy.* Antioxid Redox Signal, 2014. **21**(1): p. 154-76.
19. Neubauer, O., et al., *Time course-dependent changes in the transcriptome of human skeletal muscle during recovery from endurance exercise: from inflammation to adaptive remodeling.* J Appl Physiol (1985), 2014. **116**(3): p. 274-87.
20. Feng, Z., et al., *Mitochondrial dynamic remodeling in strenuous exercise-induced muscle and mitochondrial dysfunction: regulatory effects of hydroxytyrosol.* Free Radic Biol Med, 2011. **50**(10): p. 1437-46.
21. Marzetti, E., et al., *Modulation of age-induced apoptotic signaling and cellular remodeling by exercise and calorie restriction in skeletal muscle.* Free Radic Biol Med, 2008. **44**(2): p. 160-8.
22. Younk, L.M., et al., *Exercise-related hypoglycemia in diabetes mellitus.* Expert Rev Endocrinol Metab, 2011. **6**(1): p. 93-108.
23. Haus, J.M., et al., *Intramyocellular lipid content and insulin sensitivity are increased following a short-term low-glycemic index diet and exercise intervention.* Am J Physiol Endocrinol Metab, 2011. **301**(3): p. E511-6.
24. Solomon, T.P., et al., *A low-glycemic index diet combined with exercise reduces insulin resistance, postprandial hyperinsulinemia, and glucose-dependent insulinotropic polypeptide responses in obese, prediabetic humans.* Am J Clin Nutr, 2010. **92**(6): p. 1359-68.
25. Solomon, T.P., et al., *Randomized trial on the effects of a 7-d low-glycemic diet and exercise intervention on insulin resistance in older obese humans.* Am J Clin Nutr, 2009. **90**(5): p. 1222-9.
26. Solomon, T.P., et al., *Improved pancreatic beta-cell function in type 2 diabetic patients after lifestyle-induced weight loss is related to glucose-dependent insulinotropic polypeptide.* Diabetes Care, 2010. **33**(7): p. 1561-6.
27. Solomon, T.P., et al., *Effects of exercise training and diet on lipid kinetics during free fatty acid-induced insulin resistance in older obese humans with impaired glucose tolerance.* Am J Physiol Endocrinol Metab, 2009. **297**(2): p. E552-9.
28. Kelly, K.R., et al., *A 7-Day Exercise Program Increases High Molecular Weight Adiponectin in Obese Adults.* Med Sci Sports Exerc, 2011.
29. O'Leary, V.B., et al., *Exercise-induced reversal of insulin resistance in obese elderly is associated with reduced visceral fat.* J Appl Physiol, 2006. **100**(5): p. 1584-9.
30. Ahmed, N., et al., *Increased protein glycation in cirrhosis and therapeutic strategies to prevent it.* Ann N Y Acad Sci, 2005. **1043**: p. 718-24.
31. Haus, J.M., et al., *Free fatty acid-induced hepatic insulin resistance is attenuated following lifestyle intervention in obese individuals with impaired glucose tolerance.* J Clin Endocrinol Metab, 2010. **95**(1): p. 323-7.
32. Hickson, R.C., et al., *Time course of the adaptive responses of aerobic power and heart rate to training.* Med Sci Sports Exerc, 1981. **13**(1): p. 17-20.
33. Association, A.D., *Standards of Medical Care in Diabetes - 2018.* Diabetes Care, 2018. **41**(Suppl 1): p. S1-S156.

34. Haus, J.M., et al., *Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance.* Diabetes, 2009. **58**(2): p. 337-43.
35. Solomon, T.P., et al., *Progressive hyperglycemia across the glucose tolerance continuum in older obese adults is related to skeletal muscle capillarization and nitric oxide bioavailability.* J Clin Endocrinol Metab, 2011. **96**(5): p. 1377-84.
36. Haus, J.M., et al., *Decreased visfatin after exercise training correlates with improved glucose tolerance.* Med Sci Sports Exerc, 2009. **41**(6): p. 1255-60.
37. Kelly, K.R., et al., *A low-glycemic index diet and exercise intervention reduces TNF(alpha) in isolated mononuclear cells of older, obese adults.* J Nutr, 2011. **141**(6): p. 1089-94.
38. Association, A.D., *Diagnosis and classification of diabetes mellitus.* Diabetes Care, 2010. **33 Suppl 1**: p. S62-9.
39. McQuire, E.A.H., et al., *Effects of arterial versus venous sampling on analysis of glucose kinetics in man.* J Appl Physiol, 1976. **41**: p. 565-73.
40. Bergstrom, J., *Muscle electrolytes in man.* Scand. J. Clin. Lab Invest. Suppl., 1962. **68**: p. 1-110.
41. Haus, J.M., et al., *Contractile and connective tissue protein content of human skeletal muscle: effects of 35 and 90 days of simulated microgravity and exercise countermeasures.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(4): p. R1722-7.
42. Haus, J.M., et al., *Collagen, cross-linking, and advanced glycation end products in aging human skeletal muscle.* J Appl Physiol, 2007. **103**(6): p. 2068-76.
43. Kasumov, T., et al., *Improved insulin sensitivity after exercise training is linked to reduced plasma C14:0 ceramide in obesity and type 2 diabetes.* Obesity (Silver Spring), 2015. **23**(7): p. 1414-21.
44. Lemoine, J.K., et al., *Muscle proteins during 60-day bedrest in women: impact of exercise or nutrition.* Muscle Nerve, 2009. **39**(4): p. 463-71.
45. Weinheimer, E.M., et al., *Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis.* Am J Physiol Regul Integr Comp Physiol, 2007. **292**(6): p. R2241-8.
46. Williamson, D.L., et al., *Aberrant REDD1-mTORC1 responses to insulin in skeletal muscle from type 2 diabetics.* Am J Physiol Regul Integr Comp Physiol, 2015: p. ajpregu 00285 2015.
47. Burd, N.A., et al., *Effect of a cyclooxygenase-2 inhibitor on postexercise muscle protein synthesis in humans.* Am J Physiol Endocrinol Metab, 2010. **298**(2): p. E354-61.
48. Haus, J.M., et al., *The effect of strenuous aerobic exercise on skeletal muscle myofibrillar proteolysis in humans.* Scand J Med Sci Sports, 2007. **17**(3): p. 260-6.
49. Pierce, J.R., J.M. Maples, and R.C. Hickner, *IL-15 concentrations in skeletal muscle and subcutaneous adipose tissue in lean and obese humans: local effects of IL-15 on adipose tissue lipolysis.* Am J Physiol Endocrinol Metab, 2015. **308**(12): p. E1131-9.

50. Langberg, H., et al., *Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans.* J Physiol, 1999. **521 Pt 1**: p. 299-306.
51. Gutierrez, A., B. Anderstam, and A. Alvestrand, *Amino acid concentration in the interstitium of human skeletal muscle: a microdialysis study.* Eur J Clin Invest, 1999. **29**(11): p. 947-52.
52. Hamrin, K., et al., *Microdialysis in human skeletal muscle: effects of adding a colloid to the perfusate.* J Appl Physiol, 2002. **92**(1): p. 385-93.
53. Stahl, M., et al., *Human microdialysis.* Curr Pharm Biotechnol, 2002. **3**(2): p. 165-78.
54. Pinheiro, J. and D. Bates, *Approximations to the log-likelihood function in the nonlinear mixed-effects model.* Journal of Computational & Graphical Statistics, 1995. **4**: p. 12-35.
55. Davidian, M. and R. Gallant, *The nonlinear mixed effects model with a smooth random effects density.* Biometrika, 1993. **80**: p. 475-488.
56. Zhang, D. and M. Davidian, *Linear mixed models with flexible distributions of random effects for longitudinal data.* Biometrics, 2001. **57**(3): p. 795-802.