NON-WEIGHT BEARING EXERCISE FOR ACCELERATED HEALING OF DIABETIC FOOT ULCERS

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Background. Up to 25% of patients diagnosed with diabetes will develop a diabetic foot ulcer (DFU) in their lifetime, and foot ulceration is the precursor to ~85% of non-traumatic lower extremity amputation in this population. The longer the wound remains open, the greater the risk of having amputation and the higher the medical costs to heal the wound. Further, five-year mortality rates of between 43% and 55% and up to 74% have been reported in the DFU population. Given the worldwide epidemic of diabetes, the personal and financial burdens associated with DFUs are high. Therefore, it is crucial to implement new effective strategies to shorten the duration of wound healing.

Aerobic exercise (AE) may be an efficacious approach to accelerate diabetic ulcer healing given AE increases blood and oxygen supplies to local vascular beds and AE provides stimuli for tissue remodeling and resolution of metabolic milieu that drives inflammation. The standard care for DFUs is to off-load the wound and thus exercise is contraindicated to prevent further tissue damage. Thus, there are no current recommendations for exercise type, intensity, duration or frequency for improving wound area and/or depth. However, there is a critical need to increase nutritive blood flow to the wound and to prevent cardiovascular and metabolic deconditioning in the diabetic patient. Indeed, many patients with DFUs have concomitant peripheral vascular disease (PAD) which is identified as a major barrier to wound healing and AE in this sub-cohort is likely to have even greater benefit. Recent review of medical records within the University of Michigan Podiatry Clinic indicates ~20% of DFU patients have concomitant PAD.

Rationale. Given the contraindication for DFU patients to engage in weight-bearing exercise, our main objective is to evaluate the feasibility and effectiveness of non-weight bearing, single leg knee extensor exercise on the healing of distal DFUs. Our rationale is that contractile activity of the large muscle mass, proximal to the wound site, will increase blood flow and tissue oxygenation to the wound site while keeping the limb off-loaded. We will accomplish this task by use of a novel exercise device designed for isolation of the quadriceps femoris muscle group of a single leg, coupled with measures of wound plasticity, tissue oxygenation and vascular health. Our central hypothesis is that dynamic single leg knee extension exercise will increase blood and oxygen supply to the foot, thus heal the diabetic foot ulcer faster as compared to standard care only. By way of this exercise paradigm, we will be able to provide patients with a standardized exercise protocol while off-loading the wound. Our rationale and hypothesis are informed by classic experiments in exercise physiology and by our own preliminary data demonstrating that 15 minutes at 50% peak power intensity of isolated knee extension exercise increases blood and oxygen supply to the foot and toe. We plan to test our central hypotheses by pursuing a randomized control trial in DFU patients that pursues the following aims:

Specific Aims and Hypotheses.

Aim 1: Determine the effects of isolated knee extension exercise on DFU healing. We hypothesize that isolated knee extension exercise (30 min/3 days per wk/6 wks.) of the affected leg in subjects with DFU will have a greater decrease in wound size and depth when compared to DFU subjects undergoing standard care alone.

Aim 2: Determine the effects of isolated knee extension exercise on periwound tissue oxygenation and measures of vascular health. We hypothesize that transcutaneous oximetry (tcpO2), a measure of local oxygen perfusion in the periwound area of DFUs, will be improved in the AE group compared to control. We also hypothesize that flow-mediated dilation and posterior tibial artery blood flow will also be improved in the AE group compared to control.

Study Impact. The results of this investigation are expected to have strong research impact and challenge current clinical practice guidelines pertaining to DFU care. Findings of this study will provide direct evidence of controlled and off-loaded exercise on DFU healing. Successful implementation of the ergometer-based exercise will allow us to quantify the dose of exercise. This study protocol will serve as a platform to conduct larger randomized control clinical trials on DFU patients and examine the underlying mechanisms of accelerated wound healing.

Relevance to Diabetes Up to 25% of patients diagnosed with diabetes will develop a diabetic foot ulcer (DFU) in their lifetime. Given the worldwide epidemic of diabetes, the personal and financial burdens associated with DFUs are high (1,2)]. Therefore, it is crucial to implement new effective strategies to shorten the duration of wound healing. Unfortunately, with the exception of wound off-loading, the existing therapies for treatment of DFU have a poor evidence base (3,4). The reasons for poor evidence based are multifactorial. Diabetic foot care has traditionally been neglected and has failed to attract comparable interest as other diabetic complications. With relatively few clinicians engaged in DFU research, poor industrial and pharmaceutical investment and complex DFU pathogenesis, it is not surprising the lack of strong and robust RCTs and advancement in DFU treatment. In order to positively impact clinical practice, there is a critical need for new treatment strategies that shorten DFU duration and improve the quality of life in DFU patients.

Off-loading is currently the standard of care for DFU (3). Off-loading refers to the use of devices or surgeries that remove pressure or reduce the load at the site of ulceration to improve healing. DFUs often occur on the sole of the foot at sites of repetitive injury that are unrecognized by patients with diabetic sensory neuropathy. Ulcers are usually at a pressure point on the bottom of the foot where a callus has formed. If a neuropathic patient continues to walk on an ulcer, new tissue that is attempting to organize and fill the soft tissue void is damaged. People without sensory neuropathy find it painful to walk on an open wound and will instinctively avoid any weight-bearing forces on a wounded foot; they alter their gait or limp to protect the injured site. However, in people with sensory neuropathy, ulcers are painless and often go unrecognized. Off-loading devices facilitate healing in several ways. The most effective off-loading strategies reduce pressure and shear forces at the site of ulceration. They reduce motion of the joints of the foot, and they are usually associated with reduced activity. Reducing pressure and shear forces and decreasing the number of steps or loading cycles per day allows healing tissue to bridge the wound without continual damage. Off-loading is one of the most important interventions to facilitate the healing of foot ulcers. *However, long-term off-loading is not without physiological cost.* Patients with DFU tend to have lower step counts than patients with diabetes without foot ulcers (5). In addition, while DFU patients spend similar low amounts of time performing moderate to vigorous daily activity as patients without foot ulcers, they spend significantly more time doing sedentary-intensity activities (6). This fact is exacerbated by long DFU healing times. These data, along with the well know detriments of sedentary lifestyle in T2D individuals, provide strong evidence for the need to engage DFU patients in physical activity and/or structured exercise as well as revisit the recommendations for standard care of DFU.

Efficacy of Physical Activity and Exercise in the Prevention and Treatment of DFUs. Our hypothesis is that aerobic exercise may be an efficacious therapeutic strategy in DFU care. There are numerous benefits to exercise which have been widely documented, especially related to diabetes and glucose metabolism. Importantly, exercise stimulates increases in blood flow and tissue oxygenation to the exercising limb. Further, there are several studies in healthy humans that demonstrate the ability of exercise training to improve cutaneous vasodilation and blood flow (7,8). However, there is limited evidence for sustained vasodilation in non-local vascular beds, especially in states of insulin resistance. Due to local muscle oxygen demand during exercise, circulatory and metabolic adaptations occur so that skeletal muscle are able to accommodate exercise workloads. Repeated exercise over time thus results in increased whole-body fitness. It is well established that physical activity and exercise are highly effective in the prevention and treatment of diabetes and as such are first line diabetes therapies.

Currently, there is strong pre-clinical data demonstrating exercise improves wound healing in mouse models. The sterile skin punch (cutaneous) model of wounding is used as a pre-clinical model to examine wound healing in a number of contexts including diabetes. While these animal models cannot precisely recapitulate the environment in which DFUs develop or exist in humans, they have convincingly demonstrated impaired wound healing in insulin resistant/diabetic states which has been attributed to an exacerbated inflammatory response as well as impaired angiogenesis and blood flow (9,10). Given the ability of exercise training to resolve superfluous inflammation, promote angiogenesis and enhance blood flow, it is perhaps not surprising that several pre-clinical investigations have demonstrated an ability of exercise training to enhance wound healing in various rodent models (11-13). The data examining the effects of exercise on wound healing in diabetic animals are limited with the only one study to our knowledge showing an acceleration of cutaneous wound healing in exercised animals (14). This study demonstrated obese, insulin resistant mice, that were exercise trained, had accelerated wound healing compared to their sedentary counter parts (14). Other studies in healthy rats demonstrated that exercise may be exerting its wound healing effects via the promotion of blood flow to the wounded area via upregulation of angiogenic factors and vasoactive factors such endothelial nitric oxide synthase and increasing nitric oxide (NO) bioavailability (11). These data coupled with other research demonstrating the efficacy of genetic manipulations to improve angiogenesis (15) or low-flow oxygen treatment to promote wound healing in diabetic mice (16), suggest that blood flow and oxygen delivery are important for efficient diabetic wound healing and may explain the beneficial effects of exercise training on wound healing in diabetic conditions. However, given the numerous barriers to exercise in individuals with diabetic wounds these promising therapeutic effects of exercise on accelerating the healing of DFUs have yet to be explored in humans.

How does one exercise while maintaining off-loading of DFU? Non-weightbearing exercise comes in various forms. While arm ergometry is an effective stimulus for cardiac rehabilitation as small muscle contractile activity is effective at increasing heartrate, there is little evidence that arm ergometry would be effective in increasing blood flow and tissue oxygenation to the distal limbs and feet because sustained vasodilation is unlikely to occur in the non-local vascular beds. These and other exercise strategies that rely on the recruitment of small muscles such as local plantar or dorsal flexion may also be limited by rapid development of local fatigue, poor vascular conductance and blood flow. Comparatively, recruitment of large muscle groups during exercise are able to confer a higher metabolic demand and thus a greater cardiovascular and systemic blood flow response which not only acutely promotes a more robust vasodilatory response but has also been demonstrated to promote improvements in cutaneous blood flow as a result of aerobic adaptation following training (8). Exercises such as water immersion may be adapted to recruit large muscles while effectively unloading the wound. However, water exercises are complicated by the difficulty to maintain wound sterility, dressing and moisture balance. Upright and recumbent cycling exercises also target larger muscles such as the quadriceps and gastrocnemius muscles but remain limiting and problematic for DFU patients since

force must thorough pedal and an attempt these some have cycle exercise DFU applying to the foot improved to control

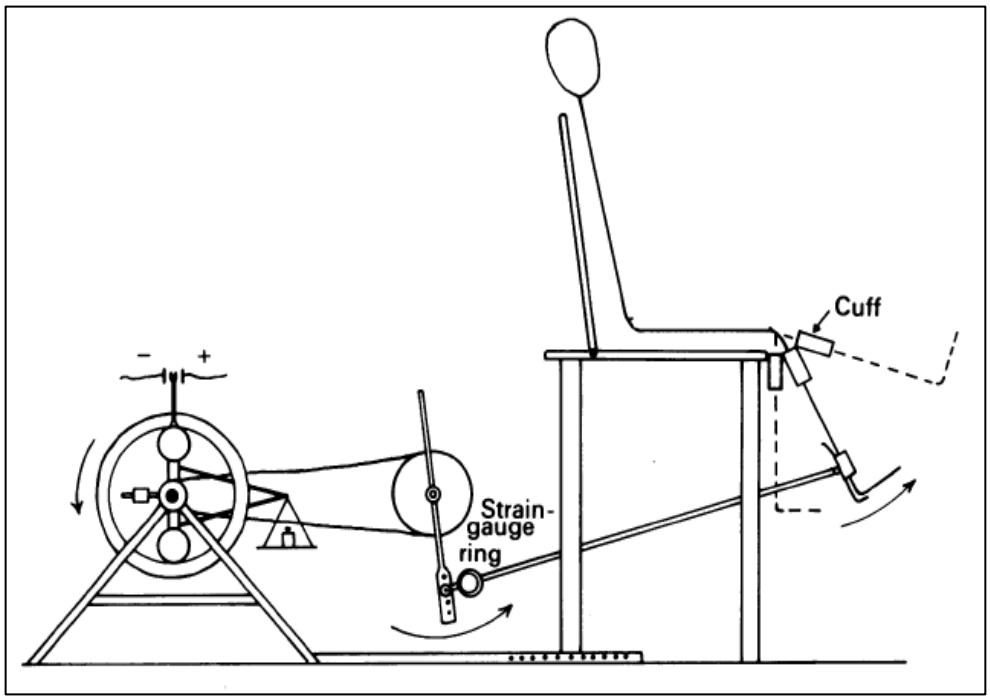


Figure 1. Schematic drawing of the exercise model used. The subject is kept in place by straps around the hip-joint and distal part of the upper leg. A rod attached to the ankle of the subject and to the crank of the Krogh bicycle ergometer is used to transfer the movement of the lower leg to the bicycle. Fly-wheel momentum returns the relaxed leg. Reproduced from Andersen et al. 1985.

still be applied the foot to the crank arms. In to resolve limitations, investigators employed a ergometer protocol for patients by heavy padding and reported DFU compared (17).

Our novel on the extension introduced Andersen as a model isolated quadriceps humans apparatus, approach relies dynamic knee apparatus in 1985, by and colleagues for the study of exercising muscle in (18,19). This depicted in Figure 1, is a modified cycle ergometer with a backward facing seat, and a rod attached on one end to one arm crank of the cycle ergometer and on the other end to an ankle strap or customized foot/ankle orthotic boot worn by the participant. Knee extension by the participant exerts force at the ankle, thus allowing for recruitment of the large musculature of the quadriceps while maintaining off-loading of the foot and any DFU that may exist. In the healthy individuals of this study, the dynamic knee extension model effectively recruited the large quadriceps muscle of the active leg and *increased whole body oxygen consumption up to a value of approximately 0.8 L/min.* Importantly, the authors also directly measured *leg blood flow* of which the average peak (workload of 60 watts) was approximately 4.5 L/min while recruiting only about 2-3 kg of quadriceps muscle mass (18,19).

Table 1. Subject Characteristics

Variable (n=4)	**Mean ± SD (Range)**
Age (y)	57 ± 4 (52-61)
Sex (M/F)	3/1
BMI (kg/m^2)	32 ± 2 (29-34)

Body Fat (%)	40 ± 2 (38-41)
FPG (mg/dL)	156 ± 76 (76-235)
HbA1c (%)	8.6 ± 1.7 (6.6 – 10.8)
ABI (AU)	1.1 ± 0.1 (1.0 – 1.3)
Peak Power (W)	22 ± 6 (15 – 30)
Neuropathy (n)	2
DFU history (n)	4
Current DFU (n)	2

Preliminary work by the study PI.

1. Evidence to support patients with T2D and DFU can safely tolerate maximal and submaximal isolated knee extensor exercise performed on a one-legged ergometer.
In a small group of subjects (n=4), subjects with current DFU or history of DFU performed single-leg peak power tests and demographic information was obtained (Table 1.) These data demonstrate the investigative team's ability to recruit DFU patients for exercise studies and that maximal exercise on a one-legged ergometer (peak power test) was safely tolerated in these cohorts.

2. Evidence that submaximal dynamic knee extensor exercise is able to increase blood flow and tissue oxygenation to the foot of the exercising leg. We hypothesized that large muscle contractile activity, proximal to the foot, would be adequate to increase circulation and tissue oxygenation to the foot during a bout of submaximal (50% peak power) dynamic knee extensor exercise in T2D subjects (n=3). Our data show that both blood flow and tissue oxygenation increased during a 15 min exercise test bout. Average baseline, and peak blood flows, were calculated and compared using a t-test. Peak blood flow increased ~ 3-fold above baseline (131 vs 357 AU, p=0.027

Primary Outcome Measure.

Change in wound size and depth (mm)

Secondary Outcome Measures

Change in periwound tissue oxygenation (TcPO2; mmHg)

Change in posterior tibial artery blood flow (mL/min)

Change in metabolic and blood derived biomarkers

ELIGIBILITY AND ENROLLMENT
Eligibility will be determined by the inclusion/exclusion criteria. Participants must be free of any contraindications to participation in exercise testing or the training. Selection criteria will limit

confounding influence on the study objectives and exclude participants 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. Inclusion/Exclusion criteria will be reviewed by the physician co-investigators and eligibility will be determined in combination with the PI. All participants eligibility will be determined on a case-by-case basis.

Subject Inclusion Criteria

1. Provision of signed and dated informed consent
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male or Female, aged 18 yrs or older
4. Diabetes diagnosed or meets ADA criteria for Type 2 diabetes
5. Foot ulcer of diabetic etiology, with all of the following characteristics:
    - Ulcer size > 0.5cm^2 and < 12cm^2 at least 2 cm from any other ulcer
    - Ulcer with Wagner grade 1 or 2
6. In case of multiple ulcers, select the largest ulcer that meets inclusion criteria.

Subject Exclusion Criteria

1. Patient participating in an interventional clinical trial within 1 month of visit 1
2. Participant has Charcot's foot or other foot deformities that prevent adequate targeted ulcer offloading
3. Participant has active severe infection or osteomyelitis at the time of the screening visit
4. History of cancer within the last 3 years, other than non-melanoma skin cancer
5. Use of adjunctive therapy within previous 30 days
6. Currently receiving medication considered to be a systemic glucocorticoid
7. Plan to perform a vascular intervention, such as surgical bypass, angioplasty or stenting, or < 1 month from a prior ipsilateral (same side) vascular intervention
8. Pregnant or currently lactating
9. Uncontrolled blood glucose with presence of urinary ketones
10. Contraindications for exercise as define by the American Heart Association/American College of Sports Medicine Guidelines for Exercise Testing and Prescription [1]
11. Bilateral wound or ulcer
12. Current infection of COVID19
13. Any history of concomitant medical condition that, in the opinion of the investigator(s), would compromise the participants ability to safely complete the study

Study physicans will use an eligibility checklist to assist in identifying potential subjects for enrollment.

Subject Recruitment. Subjects with existing DFU will primarily be recruited through the Podiatry Clinics and Comprehensive Wound Care Clinic of the University of Michigan Medicine Health System. Recent review of patient records indicates > 200 subjects that meet subject enrollment criteria. Participants will also be recruited from the Greater Ann Arbor and Metro Detroit areas through study advertisements and community outreach such as social media postings and study listing in CT.gov and the University of Michigan websites. All interested participants will be directed to an online qualtrix-based eligibility questionnaire where initial study eligibility will be assessed by the research team. https://umich.qualtrics.com/jfe/form/SV_9nvyprxbvzhhQ21

Screening and Randomization. Subjects meeting initial screening criteria from online screening tool will be scheduled for **VISIT1** which includes informed consent, enrollment and full elgibility screening. After obtaining written informed consent, full screening will consist vitals recording (height, weight, blood pressure, heart rate), and a comprehensive medical and medications history questionnaire which will include history of peripheral and autonomic neuropathy. In addition, a resting 12-lead EKG will be performed. The foot ulcer will be inspected and measured followed by a DEXA scan and 75-gram oral glucose tolerance test (OGTT) to confirm diabetes and degree of glucose tolerance. Participants will also be familiarized to the exercise device to confirm suitability, individualized device settings and fitting to custom boot. Following familiarization, a peak power single-leg exercise test will be performed. Subject eligibility, according to the inclusion/exclusion criteria will be determined prior to proceeding with the study.

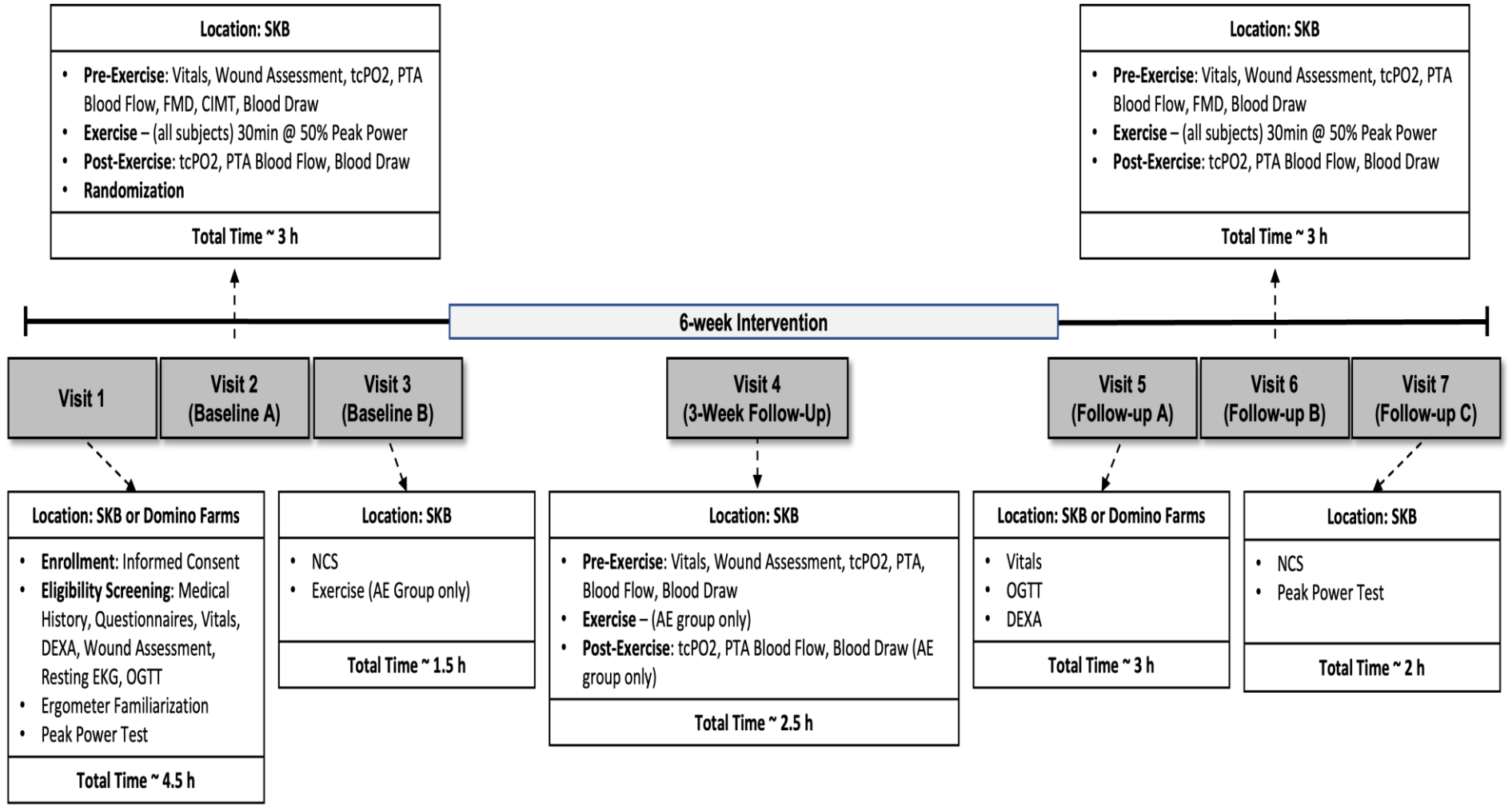
Data from screen failures will be used to help identify patient demographics with DFU and will inform future studies.

Participants fully screened are then eligible for baseline testing and will be randomized at the completion of VISIT2 which includes baseline testing and blood draw, an acute exercise session and post-exercise measures of blood flow and blood draw. Following acquisition of this baseline A data, the subjects will be randomized (2:1; Exercise:control) to 6 weeks of aerobic exercise training plus standard care (Aerobic Exercise group; n=26) or standard care only (Control group; n=14). All participants will undergo the same baseline and follow-up testing.

STUDY DESIGN AND PROCEDURES

The study design is presented in **Figure 2**. This 6-week clinical study involves the prospective study of adults (18-75 yrs) with type 2 diabetes (T2DM) possessing a diabetic foot ulcer/wound. **Fourty** participants will be enrolled, screened for eligibility and then randomized (2:1; Exercise:control) to 6 weeks of aerobic exercise training plus standard care (Aerobic Exercise group; n=26) or standard care only (Control group; n=14). All participants will undergo the same baseline and follow-up testing. The study locations will be the School of Kinesiology Building and Domino Farms at the University of Michigan. No other sites will be used.

Figure 2. Study Design



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.

Metabolic Control Period. Pre- and post-intervention assessments, as well as the acute AE trial with blood draws will be performed after a metabolic control period. Subjects will be asked to refrain from physical activity outside of their normal activities of daily living. All participants will be encouraged not to alter their dietary intake during the course of the study. Research staff will have regular contact (3 days/wk.) with subjects in the AE group and will meet weekly with the control group participants. This approach has been successfully used by Dr Haus in his human metabolic studies to control for effects of diet and physical activity (24-33).

Body Composition. Body composition will be assessed by Dual-emission X-ray absorptiometry (LUNAR iDXA densitometer, GE, USA). DEXA scans will determine components of body composition such as lean body mass, fat mass, and segmental adiposity such as truncal fat and visceral fat mass (iDXA Core Scan). Identical DEXA machines are located in Domino Farms Lobby M and the School of Kinesiology Building. Dr Haus oversees the use, maintencance and training for both of these instruments. Only trained research staff will operate the DEXA.

Oral Glucose Tolerance Test. This standard test is utilized to detect and confirm the presence of diabetes as well as to interrogate the degree of glucose intolerance and derive robust metrics of insulin sensitivity and insulin secretion. 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. OGTTs will take place at SOK building 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. From glucose and insulin values we will derive a metric of insulin sensitivity via the Matsuda Index (34,35) and calculation of disposition index. Further, with the additional measure of c-peptide from acquired samples, insulin secretion can be calculated using c-peptide deconvolution modeling. Glycemic load and concomitant insulin secretion are physiologically linked to vascular function and the production of vasoactive agents such as nitric oxide and endothelin-1. Thus the OGTT provides both clinical diagnostic value as well as strong research value [47-72, 83-88]. Because we will be collecting samples during the OGTT for the afformentioned metrics, a prior OGTT performed on the research participant can not be accepted. There is no evidence to support an OGTT has any negative effects on DFU healing. The glycemic excursions during an OGTT are physiological and representative of post-prandial metabolism following consumption of Western diet.

Single leg Ergometry. Subjects randomized to AE will complete 30 min of single leg knee extensor ergometry at 50% of their predetermined peak power (PP) while maintaining a cadence of 35 RPM as described by Dr Haus and others (36,37). Subjects randomized to standard care will rest quietly for equal duration. Peak Power will be determined after warm up followed by two-min stages of increased workload of 5 watts until failure. RPM of 35 is maintained throughout the test and PP is determined as the highest workload attained until failure (36,37). Subjects sit on a seat with back support, and the exercise foot is securely strapped in an ankle foot orthotic boot connected to the single-legged ergometer. The boot is customizable so that the wound area is not compressed or experience sheer

Figure 5. Next generation one-legged exercise device.

forces. The non-exercise foot is placed on a stool. One-legged ergometry was designed to isolate the quadriceps femoris for targeted exercise studies (19,38). Subjects will be familiarized to protocol before actual testing. For all exercise sessions, subjects will be instrumented with a heart rate monitor to assess the exercise intensity. A custom-built next generation model of the one-legged exercise device (Figure 5.) will be used. The exercise device is a modified cycle ergometer that is owned by Dr. Haus and was custum built for exercise research studies. The device is not patented by any individual or company.

Aerobic Exercise Training. Subjects randomized to the AE group will be asked to visit the laboratory three days a week for 6 weeks. During each exercise training visit, subjects will be asked to perform 30 minutes of isolated knee extension exercise at 50% peak workload (PP) while maintaining a cadence of 35 rpm (paced by metronome). Blood glucose level will be measured via finger prick and glucometer (Contour Next, Ascencia, Parsippany, NJ) before and after the exercise to monitor for hypoglycemia. If blood glucose is greater than 300 mg/dL then exercise will be rescheduled. Every AE session will be supervised using established protocols for AE testing. Subjects will wear heart rate monitors during each training session to provide feedback of target heart rate equivalent to 50% of PP. Intensity, duration, resting and exercise heart rates, and blood pressures will be recorded for each session.

Standard Care. All subjects will receive standard care for diabetes management and DFU as directed by their individual physician and diabetes health care team according to ADA, Standards of Medical Care in Diabetes and Management of DFU (3). Prior to- and during the intervention period, all medication use, dose and frequency will be documented. Control and AE subjects will meet weekly with the study team to report any changes in medications use, physical activity or changes in diet. Wound size and depth will be measured each week.

Wound assessments and wound dressing. Wound dressings will be removed to expose the wound bed and wound size will be measured by wound scale tool and disposable planimetry transparent film over wound (Figure 6.) Wound depth will be measured using a sterile cotton tipped applicator. A picture of the wound will also be taken using a University of Michigan tagged iPAD to be digitally measured using wound assessment software. No pictures will contain face images or identifiable areas other than the wound. New sterile dressings will be applied to cover and protect the wound after measurements. The wound will be monitored for signs of infection (increase redness and swelling around the wound, increase wound drainage, or foul odor). The study physicians, Dr Haus or a trained research team member will clean the wound and reapply wound dressings before and after wound assessments. Dr Haus has been trained by the study physicians on how to clean and reapply wound dressings. Also, each study participant may clean and dress their own wound with new supplies provided to them.

Figure 6. Wound measuring scale and planimetry method of wound size assessment.

Vitals: Height and weight will be obtained by standard measures. After 20 mins of rest in the supine position, blood pressure and heart rate will be taken in triplicate by an automated cuff.

Posterior Tibial Artery Blood Flow Volume: The posterior tibial artery will be examined longitudinally in both legs with the patient still in the supine position using a wide-band, linear transducer with a frequency range of 12-2 MHz or 15-3 MHz (L53K, Hitachi, Ltd., Japan). The posterior tibial arteries will be identified upon detection of a forward flowing color signal vessel wall.

Carotid Intima Media Thickness: Carotid intima-media thickness (CIMT) will be measured in both systole and diastole assessed by distension using M-mode ultrasound performed on the far-wall common carotid artery, prior to the carotid bifurcation. Measurements are assessed in 10mm longitudinal lengths and diameters of far-wall intima to near-wall intima (lumen) are collected for CIMT:Lumen calculation.

tcpO2 measures. $TcPO_2$ determinations will be performed with an oxymonitor. After cleansing the measurement site with alcohol, the probe will be applied, and the skin heated automatically to 44° C after calibration. When a stable steady state is achieved, a $tcPO_2$ is expressed in millimeters of mercury (mm Hg). This value is a measurement of the partial pressure of oxygen on the skin surface. Mean $tcpO_2$ values will be taken over 8-15 minutes while resting in a standardized position of the periwound area and opposing limb.

Flow-mediated dilation: Endothelium-dependent vasodilation will be determined non-invasively using brachial artery flow-mediated dilation (FMD) using a diagnostic ultrasound system. Baseline brachial artery diameter and blood velocities are obtained for 1 minute followed by inflation of a blood pressure cuff on the forearm to 50 mmHg above supine systolic blood pressure for 5 min. After cuff release, brachial artery diameter and velocities are measured for 3 minutes. FMD is calculated as percent change from baseline. In order to minimize error, a custom probe holder is used to hold the probe. All examinations will be performed by the same ultrasound technician, who is an experienced ultrasound operator.

Nerve Conduction Study: Nerve conduction studies (NCS) will be performed by trained neuromonitorists and follow the protocol of the EURODIAB complications study [https://doi.org/10.2337/dc10-0456]. Using the cascade software and surface electrodes, the latencies, amplitudes, and nerve conduction velocities will be measured in the nondominant sural and ulnar sensory nerves, and the peroneal motor nerve. F-wave latencies and amplitudes will also be recorded in the peroneal motor nerve. No data collected during the NCS will be used for diagnosis or treatment of diabetic neuropathy. Data will be used for research purposes only.

Blood Specimen Collection: Blood will be obtained by venipuncture of the antecubital vein or dorsal hand vein using standard procedures by a trained member of the study team.

Total Study Tissue Sampling

Table 2 outlines the approximate amount tissue to be collected during the study. Tissue samples (blood) will be obtained during visits 1, 2, 4, 5 and 6. In addition, participants in this study will likely be > 110 lb. Thus, the proposed blood draws are acceptable by University of Michigan IRBMED guidelines.

Table 2

Visit	Blood volume
Visit 1	
Both groups	160 ml
Visit 2	
Both groups	80 ml
Visit 4	
AE group	80 ml
Control group	40 ml
Visit 5	
Both groups	160 ml
Visit 6	
Both groups	80 ml
AE group total	~560ml
Control group total	~520 ml

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 staff during each procedure. This includes processing of blood samples. Samples will be stored in a -80 °C freezer. Handling and shipping of study samples will only occur by the trained research staff.

Analytical measures. Blood samples taken before and after each exercise session as well as during the time course of the OGTTs will be probed for biomarkers such as nitric oxide and endothelin-1. Additional samples will be reserved for storage and will be available for future exploration of novel biomarkers.

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 3 days/week) with subjects in the Aerobic Exercise group and will meet approximately twice with the control group to monitor habits and study progress.

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 3 days per week and the Control group will have contact with the research team approximately twice or as needed. Compliance will be determined through routine personal communication and primarily evaluated based

on exercise training attendance (Aerobic Exercise group only). Subjects in the Aerobic Exercise group are expected to complete ≥80% of the training sessions over the course of the 12 weeks.

Study Team and Expertise

This application joins the expertise of the PI, Dr. Jacob Haus, PhD (Exercise Physiology, metabolism, vascular function and diabetes lifestyle intervention) with the expertise of Co-Investigators, Drs Michael Munson, MD Crystal Holmes, MD, and Brian Schmidt, MD (podiatric medicine, wound care and limb preservation).

Drs Munson, Holmes and Schmidt will provide medical oversight and recruitment from their podiatry clinics.

Josh Mergos is Director of the Intraoperative Neuromonitoring (IONM) and will provide training and expertise in NCS.

Laura Richardson, PhD is an American College of Sports Medicine Certified Clinical Exercise Physiologist who is an expert in cardiopulmonary exercise testing and EKG.

James Shadiow and Jacob Haus are trained in phlebotomy and ultrasound

Assessment of Safety

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

Adverse events will be reviewed by a physician co-investigator in combination with the study PI and assessments will be made as to whether the adverse event changes the risks to participants or in the study overall. Adverse events will be mitigated by members of the research team being 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.

STUDY RISKS

- **Blood Draw**: The risks of drawing blood from a vein includes discomfort at the site of the needle stick, possible bruising and swelling around the site of the needle stick, rarely an infection, and uncommonly feeling faint from the procedure.

- **EKG**: Men may need to have a small amount of chest hair shaved off for the EKG 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.

- **Blood Pressure:** Bruising and discomfort may occur from the constriction of the blood pressure cuff.

- **tcPO2***:* There is little known complications and side effects from oximetry collection. However, the application of sensors that use double sided adhesive pads may cause irritation.

- **Ultrasound measures***:* Ultrasound measurements may cause heat or vibrational damage to the measured site. Ultrasound gel has also been noted to cause allergic reactions in rare instances.

- **Wounds Complicated by Infection after Enrollment***:* Infection at the site of the wound/ulcer may occur. Immediate action may be needed at the discretion of the supervising physician.

- **Oral Glucose Tolerance Test**: Reaction to the ingestion of the glucose drink is possible. These reactions could include nausea, low blood sugar, an increase in blood pressure, flushing, and/or sweating.

- **Study Diet and Dietary restrictions**: Subjects may find it burdensome to adhere to a special diet, abstain from eating or drinking food or drink items containing caffeine or alcohol during the study periods for a prolonged period of time.

- **Exercise capacity test/high intensity exercise**: With a high-intensity effort exercise tests, such as a the single-leg peak power testing there is an inherent risk of complications such as chest pain, shortness of breath, dizziness, and rarely heart attack. Subjects may experience tiredness or shortness of breath and palpitations, syncope or angina. Subjects will be constantly monitored by exercise professionals who are CPR certified and will terminate any of the exercise trials at any time if they should feel it necessary. Further, a resting EKG will be used to identify any underlying heart arrythmias or conditions at screening which will be assessed by a certified clinical exercise physiologist.

- **Arrythmia:** There is a small chance strenuous exercise may result in an irregular heartbeat.

- **Exercise training**: Subjects may find the exercise training program or returning to train with the research team fatiguing or burdensome.

- **Radiation Exposure from DEXA scan**: Subjects will be exposed to radiation in the form of a DEXA scan. Radiation exposure in this study will amount to less than 0.03 rems.

- **Nerve Conductance Study (NCS)**: There may be minor discomfort of patches being placed on the skin and the adhesive used to say on the skin. There also might be discomfort at the site of study by the electrical signal which may be described as a small shock.

- **Pregnant women, fertile females/males**: There may be unforeseen risks to an unborn child associated with some of the study testing. Pregnancy tests will be performed on all women of child-bearing potential before beginning the study and before each DEXA scan.

- **Disclosure of medical history.** Disclosure of medical information may be uncomfortable such as timing of female menstrual cycles or disclosure of medical diagnoses and past medical history.

- **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.

- **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, 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. All Adverse events will be managed as described above.

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 study physicians and the PI. The study principal investigator, Dr. Jacob Haus PhD, and the study physicians, will ensure the University of Michigan Institutional Review Board is notified within a timely manner.

Safety Oversight

Dr. Jacob Haus, PhD and Drs. Holmes, Munson and Schmidt will provide safety oversight for the study. Dr Haus is responsible for the overall execution and management of the study.

STATISTICAL CONSIDERATIONS

Statistical Analyses and Power Calculations. The study involves collecting data at pre-AE training (T1) and then collecting 4 repeated measures at 1-day, acute AE trial (T2), 3 weeks (T3) and 6 wks. (T4) of AE training. Comparisons of trajectories across groups (AE training vs. Control) over time are made with appropriate adjustments for fixed (age, gender, medications, diabetes duration and severity) and time-varying covariates (i.e. weight, BMI, etc.). Other fixed baseline variables will be documented, including demographic information (age, gender, BMI), medical history, diabetic foot evaluation. Because of the anticipated nonlinear response curves of outcome variables (wound size, tcpO2), we will use nonlinear mixed effects models to analyze our data. Using PROC NLMIXED in SAS (SAS Inc.) we will fit the nonlinear models using two likelihood-based methods: adaptive Gaussian quadrature and a first-order Taylor series approximation (44-46). 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 AE training group (AE vs. Control) 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 AE training on the outcome variables across time during the AE intervention. Our human sample size of was determined based upon

anticipated subject enrollment and feasibility within the pilot project time period. Our sample sizes were also informed by Flahr et al, one of very few reports on DFU and exercise (17). From these calcualtions we have derived that a 2:1 randomization with 26 Exercise and 14 controls subjects will meet the expected power of 80%.

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 in person in private room or via a website screening tool or telephone interview. During this time, the study will be described in detail and 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 andstudy 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 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.

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