NYU Langone Health

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NYU School of Medicine
NYU LANGONE MEDICAL CENTER

A cross-over, single-center study investigating the effects of exercise on reversing diabetic peripheral neuropathy

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Statement of Compliance

This study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), any other applicable US government research regulations, and institutional research policies and procedures. The Principal Investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

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List of Abbreviations

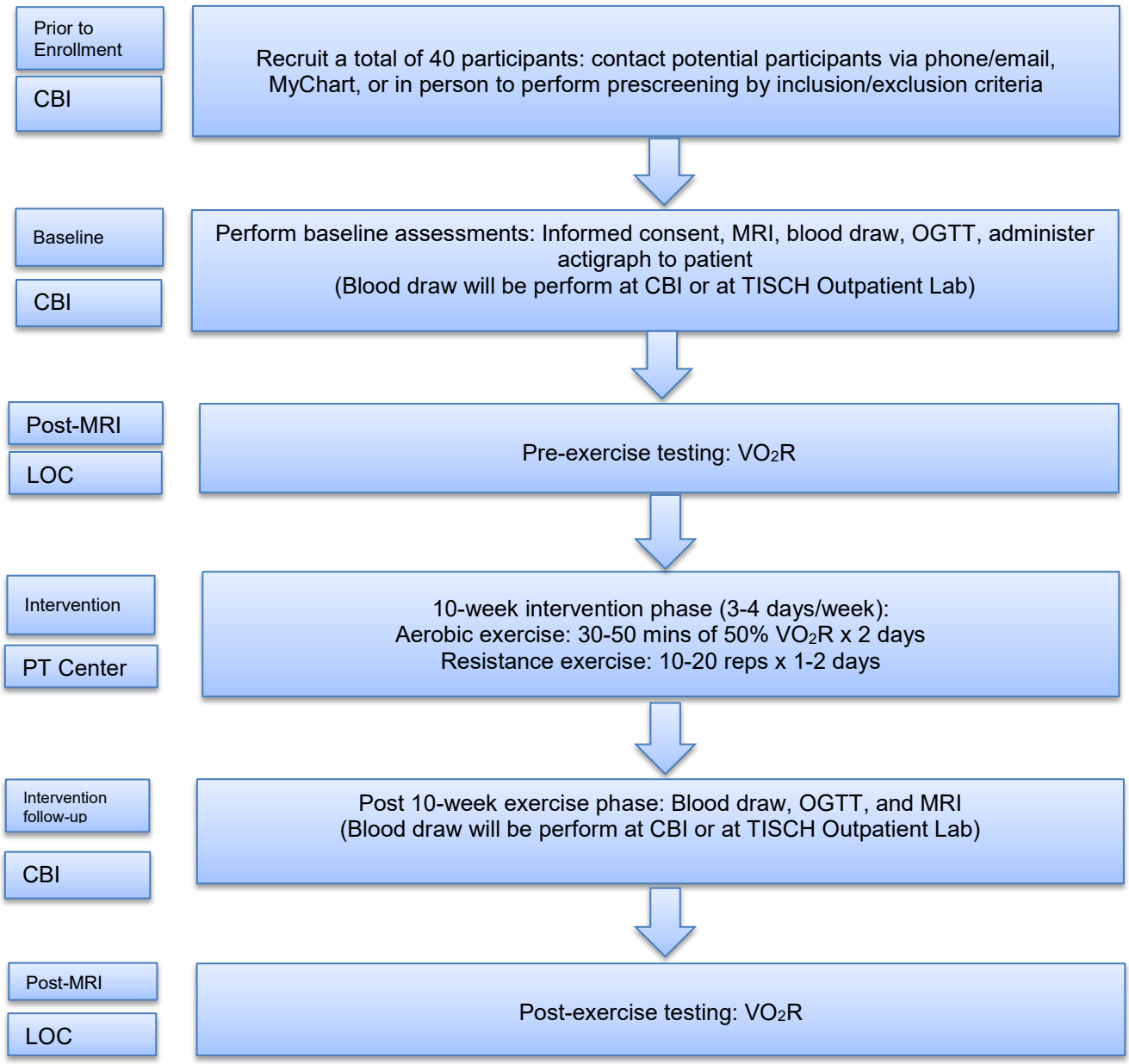
ACSM	American College of Sports Medicine
AE	Adverse Event
BMI	Body Mass Index
CBI	Center for Biomedical Imaging
CFR	Code of Federal Regulations
CRF	Case Report Form
DM	Diabetes Mellitus
DPN	Diabetic Peripheral Neuropathy
DTI	Diffusion Tensor Imaging
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed Consent Form
ITT	Intention to Treat
IRB	Institutional Review Board
MNSI	Michigan Neuropathy Screening Instrument
MRI	Magnetic Resonance Imaging
MV	Microvascular
MVC	Maximum Voluntary Contraction
NIH	National Institutes of Health
OGTT	Oral Glucose Tolerance Test
OHRP	Office for Human Research Protections
PI	Principal Investigator
RPE	Rate of Perceived Exertion
ROS	Reactive Oxidative Species
SAE	Serious Adverse Event/Serious Adverse Experience
T2DM	Type 2 Diabetes Mellitus
US	United States
VO_2R	Maximal Graded Exercise Test

Protocol Summary

Title	*A cross-over, single-center study investigating the effects of exercise on reversing diabetic peripheral neuropathy*
Short Title	*Reversing diabetic peripheral neuropathy through exercise*
Brief Summary	*This project proposes a longitudinal design that uses multinuclear-MRI to evaluate the mechanistic effects of exercise on skeletal muscle function and peripheral nerve integrity in patients with diabetic peripheral neuropathy (DPN), and to determine whether exercise can reverse DPN symptoms. We will prescribe a 10-week exercise program to 40 DPN patients. We will acquire multinuclear-MRI data before and after the intervention that can provide mechanistic insight into the adaptations in lower leg muscle function and peripheral nerve integrity of patients with DPN, and their role in improving DPN symptoms following physical exercise intervention.*
Phase	*N/A*
Objectives	*Objective 1: Determine the effect of exercise on peripheral nerve architecture, function, and symptoms of DPN.* *Objective 2: Determine the effect of exercise on lower leg muscle structure and function in DPN patients.*
Methodology	*Longitudinal design*
Endpoint	*Body mass index (BMI), glycosylated hemoglobin (HbA1c), and C-reactive protein will be assessed before and after the 10-week intervention, along with the following measures of neuropathic symptoms: 1) Michigan Neuropathy Screening Instrument (MNSI) symptom questionnaire and the PROMIS physical function and fatigue questionnaire, 2) MNSI physical exam score, 3) Vibration Perception Threshold (VPT) and Calf Muscle performance, 4) Plasma levels hydrogen peroxide as marker of ROS, 5) Lower leg muscle performance (9-item Physical Performance Test), activity monitor with actigraph, and 6) Multinuclear MRI will assess changes in muscle function and peripheral nerve integrity. 7) Utah Early Neuropathy Scale, which is a physical exam specific to early sensory predominant polyneuropathy.*
Study Duration	*2 years*
Participant Duration	*~10 weeks*
Duration of behavioral intervention	*10 weeks*
Population	*40 patients with Type 2 diabetes and clinical symptoms of DPN (both male and female) between the ages of 40-70 years old*
Study Sites	*Single-center*
Number of participants	*40 participants expected to be enrolled across one site*
Description of Study Intervention/Procedure	*A 10-week exercise program with both aerobic and resistance components, based on the American College of Sports Medicine (2010), will be individually prescribed to 40 DPN patients. Prior to beginning the intervention, subjects will participate in a maximal graded exercise test (VO_2R) using a cycle ergometer with a metabolic cart and integrated ECG.*
Reference Therapy	*N/A*
Key Procedures	*Oral Glucose Tolerance Test (OGTT), multinuclear-MRI, VO_2R, MNSI, physical therapy, activity monitor*

Statistical Analysis	*The analysis will follow the intention-to-treat (ITT) principle and include data from every subject irrespective of whether the subject was compliant with the intervention. A quantitative assessment of compliance with the intervention, ranging from 0 (complete non-compliance) to 1 (full compliance), will be included as a weighting factor to allow more compliant subjects to contribute more to the analysis.*

Flow diagram *(longitudinal design)*



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1 Introduction, Background Information, and Scientific Rationale

This document is a protocol for a human research study. This study is to be conducted in accordance with US government research regulations, applicable international standards of Good Clinical Practice, and institutional research policies and procedures.

1.1 Background Information and Relevant Literature

Diabetes mellitus (DM) affects 26 million people in the US [1] with 30–50% of Type-2 Diabetes Mellitus (T2DM) patients developing diabetic peripheral neuropathy (DPN) [2-4]. DPN is characterized by impairments of metabolic and microvascular (MV) functions [5], which damage the endoneural capillaries that supply the peripheral nerves [6]. Prolonged DPN can lead to local ischemic conditions in the lower extremities, causing loss of peripheral nerve integrity, neurogenic muscle atrophy, fatty infiltration [7, 8], and loss of muscle endurance [9-12]. These synergistically contribute to altered gait, impaired balance, and increased fall risk, which can lead to bone fractures, poorly healing wounds, and chronic infections [13-15] that often require an amputation [16, 17]. There are no therapies to prevent or reverse the progress of DPN [18-20]. Therefore, it is very important to establish effective treatments for DPN.

A growing body of literature supports the benefits of combining moderate-intensity aerobic exercise with resistance training to improve glycemic control and insulin sensitivity in individuals with DM [21, 22]. However, little is known about the benefits of exercise in individuals with DPN. Preliminary single-group studies conducted by Dr. Kluding (co-investigator) have shown that a moderate intensity supervised exercise significantly improve their cardiorespiratory function, and can also improve DPN symptoms, including nerve function and cutaneous innervation (Fig. 1) [23, 24]. In addition, exercise can improve vascular endothelial function in DPN [25]. Non-invasive exercise intervention is particularly valuable in this population due to its safety, low cost, and potential to augment pharmacological interventions. Our longitudinal study design seeks to understand the effects of exercise in skeletal muscle function and peripheral nerve integrity of DPN patients. We hypothesize that exercise can partially reverse DPN progression because it promotes MV dilation, reduces oxidative stress, and increases the abundance of neurotrophic factors, all of which are compromised in DPN [26]. Exercise will be able to improve lower limb muscle function by inhibiting key metabolic pathways that cause hypoxia. Successful completion of our study will facilitate the development of mechanism-based intervention strategies for individuals with DPN.

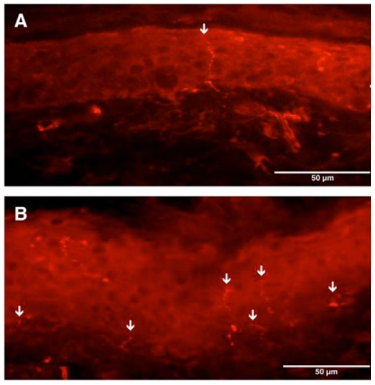

Figure 1. **Effects of Exercise on Cutaneous Innervation in DPN Patients**. Skin biopsy from a DPN patient before (**A**) and after (**B**) 10 weeks of exercise. Intraepidermal nerve fibers are significantly denser (arrows) after the exercise intervention.

1.2 Rationale

Hyperglycemia is an important factor in the development of DPN. However, the pathophysiological processes through which hyperglycemia causes DPN are not fully understood. Furthermore, large clinical studies (ACCORD [27], ADVANCE [28], VADT [29]) have shown that good glucose control is insufficient to reduce the occurrence of foot complications, including DPN and diabetic foot ulcers. There are no Food and Drug Administration (FDA)-approved treatment options that target the underlying pathogenesis of DPN [19, 30]. Therefore, it is important to establish strategies to delay or reverse the effects of DPN.

We hypothesize that DPN patients who undergo 10 weeks of exercise will show improved peripheral nerve integrity and function, and that exercise will improve the structure, local metabolic, and MV functions of skeletal muscle. A major obstacle to assessing how DPN affects skeletal muscle and peripheral nerves is the lack of sensitive, objective, and reproducible tests to detect small changes in muscle and nerve functions [10]. Multinuclear-MRI can bring new insights into how DPN affects skeletal muscle function and peripheral nerve integrity [31-33]. Our group has developed novel phosphorus (31P)-MRI techniques that can reliably assess skeletal muscle metabolic function [34, 35], as well as rapid

techniques to quantify blood oxygenation level dependent (BOLD)-MRI signals, sensitive to MV function [36-38] and its adaptations after exercise interventions [39]. In addition, we will use diffusion tensor imaging (DTI), a powerful MRI technique, to quantitatively assess neuronal integrity and detect nerve injury and monitor reinnervation [40-46]. The use of DTI will allow us to non-invasively assess peripheral nerve adaptations after exercise intervention. Finally, we will use IDEAL-MRI to quantify levels of intramuscular adipose tissue (IMAT), which are elevated in DPN [47-50]. We will prescribe a 10-week exercise program, with both aerobic and resistance components [14, 24], to 40 DPN patients who will receive personal supervision from health professionals. We will acquire multinuclear-MRI data at three time points: 1) baseline, 2) pre-intervention and 3) post-intervention. We will measure plasma levels of reactive oxidative species (ROS) as markers of oxidative stress.

Like all longitudinal studies, exercise intervention studies are at risk of losing participants who choose to discontinue their participation during the intervention. Our team has developed effective strategies to promote high levels of adherence even with this challenging population, with our preliminary studies showing low attrition rates [23, 24]. Our previous experience with intervention studies indicates that the main reason patients discontinue participation is the cost associated with travel (bridge/tunnel tolls, parking, bus, and taxi fare). Therefore, we have budgeted funds to offset transportation expenses for participants (up to $20 per visit). To further convenience our participants, we will offer flexible scheduling for appointments in our research dedicated physical therapy center (380 2nd Ave), including availability 5-days/week and 12-hrs/day (from 8am to 8pm). In the current proposal, data analysis will follow the ITT principle and include data from every subject regardless of whether the subject complied with the intervention.

1.3 Potential Risks & Benefits

1.3.1 Known Potential Risks

There are two categories of risk associated with this study:

1) **<u>The potential risks resulting from physical exercise.</u>**

Qualified, attending medical personnel will categorize the test outcomes (including post-test events) as one of the following, using the criteria that follow:

a) Benign events that fall within the "normal" set of outcomes for the tests;
b) "Abnormal" outcomes that do not, by themselves, warrant termination of the exercise and that do not cause the subject to stop exercise or complain; or
c) Adverse Events or Serious Adverse Events that are not "normal" outcomes, that warrant termination of the exercise, or that cause the subject to complain or terminate the exercise of their own volition.

In *Type c* events, the exercise will be immediately terminated. As needed, participants will receive on-site medical treatment, referral to hospital for immediate follow-up care, or a call will be placed for Emergency Medical Services (EMS), transport to hospital, and emergency treatment. Except where EMS are involved, the subject will be asked to stay at the testing lab for observation for at least half an hour after his or her condition is stable.

In all *Type c* cases, the research staff will attempt to contact the subjects' personal physician (or the person covering his or her practice, as available) by phone, to apprise him or her of the exercise outcome and the treatment given. Except where EMS are involved, this first phone call will be made after the subject is stable and before the subject leaves the testing lab. If no person-to-person contact can be made with the subject's physician (or their substitute) within this time frame, this fact will be documented and repeated attempts will be made and documented until contact is made.

Within 48 hours of the test, for both *Type b* and *Type c* events, the outcomes, treatments, and documentation of phone calls and other correspondence (e.g., with EMS) will be described in a written report to the subject's personal physician. The subject will also receive a copy of the report.

Individuals experiencing *Type a* and *Type b* events will be supervised until their symptoms resolve. The event will be documented. Subsequent exercise sessions will be monitored to prevent recurrence of adverse event.

Criteria for Categorizing Exercise Adverse Events
Type b Indications—"Abnormal" outcomes that do not, by themselves, warrant terminating the exercise test, such as occasional premature beats at rest and which are more frequent with exercise, or the appearance of a new but non-sustained arrhythmia.
Type c Indications—Adverse Events or Serious Adverse Events that should result in terminating the exercise.

Exercise Standards for Testing and Training from the American Heart Association will be used to define absolute and relative contra-indications to exercise testing [51].

Absolute Indications
- Acute myocardial infarction (MI), within 2 days
- Ongoing unstable angina
- Uncontrolled cardiac arrhythmia with hemodynamic compromise
- Active endocarditis
- Symptomatic severe aortic stenosis
- Decompensated heart failure
- Acute pulmonary embolism, pulmonary infarction, or deep vein thrombosis
- Acute myocarditis or pericarditis
- Acute aortic dissection
- Physical disability that precludes safe and adequate testing

Relative Indications
- Known obstructive left main coronary artery stenosis
- Moderate to severe aortic stenosis with uncertain relation to symptoms
- Tachyarrhythmia with uncontrolled ventricular rates
- Acquired advanced or complete heart block
- Hypertrophic obstructive cardiomyopathy with severe resting gradient
- Recent stroke or transient ischemic attack
- Mental impairment with limited ability to cooperate
- Resting hypertension with systolic or diastolic blood pressures >200/110 mm Hg
- Uncorrected medical conditions, such as significant anemia, important electrolyte imbalance, and hyperthyroidism

Additionally, to minimize the risk of ulcer development in the “high risk” foot, we will inspect participants’ feet weekly.

2) **Risks that are incurred by the performance of MR imaging. All subjects will be screened for the same potential hazards as routine clinical patients (see below) and those subjects at risk will not be imaged.**

This research study involves the use of newly developed imaging hardware and software at 3T. The human subjects will be scanned using an FDA-approved 3.0 Tesla MRI scanner. A standard MRI safety form will be used for subject eligibility. All new technologies used in this investigation meet or surpass the safety standards of the FDA and are considered non-significant risk as defined in the “Guidance for Industry and FDA Staff – Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices” issued July 14, 2003. According to the Blue Book memorandum entitled “Significant Risk and Non-significant Risk Medical Device Studies”, magnetic resonance devices operating within FDA guidelines up to 8T are considered non-significant risk.

All subjects will be screened for biomedical implants and devices through questioning at the time of recruitment to the study, as well as on the day of the examination. Subjects with potentially MR incompatible devices or other hazards, such as orbital foreign bodies, will be excluded. If pregnancy is a possibility, female subjects will undergo a urine test on the day of the MR examination. If the test is positive, then the subject will be excluded from the study. During the MRI experiments, the MRI scanner will monitor the radiofrequency (RF) power deposition. If it detects any sequence parameters that exceed the allowed RF power limits, it will automatically stop the sequence and a message will be displayed indicating that the sequence cannot be run as it exceeds the power deposition limits. This will eliminate the risk associated with inadvertent errors during scanning time. All subjects will be pre-screened by the study coordinator or research data associate over the phone. The MRI technician will screen all subjects at the time of the MR examination.

Medical Facilities and Data Management: All imaging will be performed at the Department of Radiology. The supervised exercise program will be performed at the Arthur J. Nelson Jr. Human Performance Lab at NYU's Department of Physical Therapy. Both centers are staffed by licensed care providers with CPR certification. All patients enrolled will be required to confirm that they are neither pregnant nor have pacemakers, cerebral aneurysm or other clips. Subjects with pacemakers, clips or metals, or who are claustrophobic will be excluded from the study. To further ensure patients against potential risks, all data will be anonymized as part of standard clinical management. Data specifically derived from all MRI scans will be anonymized, performed on a stand-alone research workstation with no connections to radiologic or hospital records, and correlated independently with appropriate clinical data so as to ensure that they will not interfere with ongoing patient management. All patient MRI scans will be stored electronically and accessed via a NYU MCIT-platform.. All clinical and exercise-related data will be de-identified and stored on a password protected server at the Arthur J. Nelson Jr. Human Performance Lab at the Department of Physical Therapy. All informed consents and paper documents will be stored in a locked file cabinet and room with access given only to authorized study personnel.

1.3.2 Known Potential Benefits

While there are no direct benefits to the patients for participating, the complete medical evaluation will be of general value since previously unknown medical problems may be detected. In addition, individuals who have diminished glucose regulation will be informed of these results, so that they can notify their personal physicians and have this monitored more closely. These individuals will be given advice on measures they can take to improve their peripheral glucose regulation, such as diet. Our licensed physical therapist will offer our subjects nutrition guidelines from the American Diabetes Association. The exercise tests may provide participants with information about their current state of health and physical fitness. The information may also be helpful in developing or altering an exercise program to enhance their physical fitness. The benefit to society will be the establishment of the efficacy of physical exercise on improving DPN symptoms, and by using advanced multinuclear MRI metrics to provide mechanistic insight into the exercise-associated adaptations in MV and metabolic functions.

Risk/Benefit Assessment: Although the potential benefits from participation in this study will be minimal for the individual subjects, the risks are minimal as well. In view of the potential benefits to society of establishment of the efficacy of physical exercise on improving DPN symptoms, the overall risk/benefit is favorable.

2 Objectives and Purpose

2.1 Primary Objective

The primary objective is to assess and compare the interventions (exercise versus control phase) in terms of the baseline to follow-up change in the following outcomes: 1) DTI, plasma ROS level, and the MNSI assessment of DPN symptoms (Aim 1); and 2) leg mitochondrial function, oxidative capacity (31P-MRI), peak MV response (BOLD-MRI), the proportion of fat-free muscle (IDEAL-MRI) and leg muscle strength (Aim 2).

2.2 Secondary Objectives (if applicable)

NA

2.3 Exploratory Objective (if applicable)

The exploratory objective is to identify baseline factors (e.g., age, MNSI, general activity) predictive of improvement, represented as the baseline to follow-up change in each outcome. This objective will be addressed using linear regression and bivariate correlations.

3 Study Design and Endpoints

3.1 Description of Study Design

This single-center, longitudinal design study uses multinuclear-MRI to evaluate the mechanistic effects of exercise on skeletal muscle function and peripheral nerve integrity in patients with DPN, and to determine whether exercise can reverse DPN symptoms. Forty DPN patients will be enrolled and be prescribed a 10-week exercise program following a 4-week non-exercise period. The exercise program will comprise aerobic and resistance components; a moderate level of intensity will be calculated based on results from a maximal graded exercise test (VO_2R) conducted prior to the intervention. Multinuclear-MRI data will be acquired at baseline, as well as before and after the intervention that can provide mechanistic insight into the adaptations in lower leg muscle function and peripheral nerve integrity of patients with DPN, and their role in improving DPN symptoms.

3.2 Study Endpoints

3.2.1 Primary Study Endpoints

Body mass index (BMI), glycosylated hemoglobin (HbA1c), and C-reactive protein will be assessed before and after the 10-week intervention, along with the following measures of neuropathic symptoms: 1) MNSI symptom questionnaire with yes/no responses to 15 items indicating the frequency and severity of neuropathic symptoms and Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Function and Fatigue subscales, which are computer adaptive tools, will be used to assess symptoms related to physical disability and fatigue [52]; 2) MNSI physical exam score, to indicate abnormalities in the appearance of the feet, vibration sense, reflexes, and monofilament sensation; 3) Vibration Perception Threshold (VPT) will be assessed on the plantar aspect of the great toe [53] and Calf Muscle performance will be quantified as peak ankle plantarflexion torque at 60 degrees/sec [54]; 5) Plasma levels hydrogen peroxide as marker of ROS; 6) Lower leg muscle performance will also be assessed using the 9-item Physical Performance Test [54]; and 7) the Utah Early Neuropathy Scale, which is a physical exam specific to early sensory predominant polyneuropathy. Physical activity will be quantified using a wearable activity monitor (actigraph) [55]. Subjects will be required to wear the actigraph for at least a week.

The following outcomes will be assessed through multinuclear-MRI: 1) PCr resynthesis rate in the gastrocnemius (G) and the soleus (S) muscle groups, following a 90-s plantar flexion exercise during which resistance is applied at approximately 40% of the individual's maximum voluntary contraction (MVC); 2) Time-to-peak (TTP) of the BOLD signal in the G and S muscle groups; 3) Peak $\Delta T2^*$ at 100% isometric MVC in the G and S muscle groups; 4) IMAT in the G and S muscle groups using IDEAL MRI; 5) Fractional anisotropy (FA) in the tibial nerve using DTI; 6) Apparent diffusion coefficient (ADC) in the tibial nerve using DTI.

3.2.2 Secondary Study Endpoints

NA

3.2.3 Exploratory Endpoints

Identification of baseline factors (e.g., age, MNSI [56], general activity) predictive of improvement, represented as the baseline to follow-up change in each outcome.

4 Study Enrollment and Withdrawal

4.1 Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Be 40-70 years old
2. Have a clinical diagnosis of Type 2 diabetes
3. Have clinical symptoms of DPN potentially including lack of monofilament and vibration perception
4. Have a BMI less than 40 kg/m^2 (due to magnet bore restrictions)
5. Be vaccinated for COVID-19

4.2 Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Serious cardiac pathology or musculoskeletal problems that would limit exercise ability
2. MNSI score < 1
3. Current open wound or history of plantar ulcer for the last 3 months
4. Stroke or other central nervous system pathology
5. Stage 2 hypertension (resting blood pressure >160 systolic or >100 diastolic)
6. Contraindications to 3T whole body MRI scanners (e.g., pacemaker, cerebral aneurysm clip, cochlear implant, presence of shrapnel in strategic locations, metal in the eye, claustrophobia, or other problems).
7. Subjects with alcoholism, chronic drug use, chronic gastrointestinal disease, or renal or hepatic impairment
8. Pregnant women and children

4.3 Vulnerable Subjects

Diabetes affects approximately 26 million people in the US and although prevalence may differ among different races and ethnicities, no population is spared. Age and sex-adjusted rates for diabetes by race have been reported as: Asian Americans 8.4%, non-Hispanic whites 7.1%, Hispanic/Latinos 12.6%, non-Hispanic blacks 15.3%, American Indians/Alaska natives 14.2%. From those diagnosed, 49% are women and 51% are men. Our targeted/planned subject enrollment will reflect the diverse racial and ethnic patient population that is served by the NYU Hospitals' system. This system functions as one of the main referral centers for lower Manhattan, Queens, and Brooklyn.

DPN is one of the most common complications of T2DM. Nerve damage in DPN is likely due to combination of factors including high blood glucose, long duration of diabetes, and abnormal fat levels. It is generally accepted that among children with T2DM, DPN is very rare. Therefore, children will be excluded from this study.

If a volunteer is an employee, student, or resident of NYU Langone Health or its affiliates, their decision to participate, decline participation, or withdraw from the study will have no impact on the individual's employment, academic standing, or grades. Record of the participation cannot be linked to an academic record.

The justification for enrolling this population is due to the strict inclusion/exclusion criteria we have for this study. We are not directly targeting the recruitment of NYU students/employees, however, if we come across an eligible candidate who is an NYU student/employee, we want to be able to enroll him/her into our

study. Recruitment is conducted by the PIs, research coordinator(s), and other study team members. Anyone who recruits or consents for this study has no role in grading or evaluating students.

The consent process is conducted in a private room where the study procedures and risks are explained to the subject. A photocopy of the signed consent form is given to the subject. The consent process is appropriate for the research being conducted. Often times an eligible patient's treating physician is called to ask if the physician objects to study participation for the patient. This extra screening step is done for the purpose of maximizing protection against risks and minimizing any coercion.

This study consists of multiple visits which allow the study team the opportunity to frequently discuss with the subject that his/her participation is voluntary. In order to ensure this is done, the study's case report forms include a task to remind the participant that withdrawal from the study is possible at any time. In order to minimize breaches of confidentiality, all participants are assigned a subject ID number upon study enrollment. The ID number is not associated with the subject's name or any other identifying information. Only the subject's ID and initials are used when collecting and recording data. The case report forms and source documentation are kept in a binder that is stored in a locked cabinet, in a locked room. Only Radiology Department staff have the code to the room with the binders.

4.4 Strategies for Recruitment and Retention

Participants who meet our study's criteria will be recruited from collaborating clinics at NYULH, DataCore at NYU, through direct advertising via social media (Craigslist) or brochures/flyers, and from existing relationships with prior research subjects. Interested individuals will complete a structured telephone screening and/or online eligibility screening survey. Eligible individuals will be invited to participate in the study.

Our recruitment methods/materials may include a link to an online eligibility screening survey. The content of the survey will be submitted to the IRB for approval. PHI will be collected as a result of completing the screening form. If the subject is not eligible to participate, PHI will be destroyed immediately.

4.4.1 Use of DataCore/Epic Information for Recruitment Purposes

This study will utilize DataCore and EPIC for recruitment purposes. DataCore will be used to generate a report of potential participants that meet this study's eligibility criteria. Additionally, study team member(s) will perform weekly EPIC screenings of patients at collaborating NYULH clinics until target enrollment is achieved.

The following data points and PHI will be used:

- First and last name
- Contact information
- Clinical diagnoses and medications
- Office visit notes
- Medical Record Number (MRN)
- Treating Physician (TP)

The IRB-approved study personnel who will have access to the EPIC search results are the following:

- Principal Investigator
- Co-Investigator
- Study Coordinator
- Research Coordinator
- Research Data Associate

Once potential subjects have been identified, they will be contacted in one of the following ways:

- The TP will be notified by email or in person that they have an eligible patient. He/she may tell the patient about this research opportunity and may provide recruitment materials. He/she may also obtain the patient's permission to be contacted by study staff (in person or by telephone).
- The study team may access contact information from medical records through DataCore to contact patients directly.
- They may receive an approved recruitment message to their MyChart account. Recipients can respond to indicate whether they are interested in participating.

If a subject requests information regarding opting out of further recruitment for all research, subjects will be directed to contact research-contact-optout@nyumc.org or 1-855-777-7858.

4.4.2 Use of Craigslist for Recruitment Purposes

Craigslist will be used for recruitment. The ad will be posted under "community > volunteers." The language in the ad will be submitted to the IRB for approval.

4.4.3 Direct Advertising

Our team will use brochures, flyers, and other IRB-approved recruitment materials to provide information to interested individuals. We plan to distribute recruitment materials in spaces that we expect our target population to frequent. We will seek approval from the establishment or facilities management prior to posting. Locations that we expect to distribute to include, but are not limited to, collaborating NYULH clinics and physicians, private non-NYULH clinics (i.e. local podiatry clinics), residential buildings, senior centers, libraries, community centers, etc.

4.5 Duration of Study Participation

Participants will be enrolled for a minimum of 10 weeks. We anticipate that some participants will be enrolled for greater than 10 weeks due to challenges associated with scheduling study procedures (limited availability on the MRI scanners). Additionally, we are allowing a one-week grace period during the intervention for patients who express pain/soreness due to the exercise regimen, or for other circumstances (i.e., planned vacation).

4.6 Total Number of Participants and Sites

Recruitment will end when approximately 40 participants are enrolled across one site.

4.7 Participant Withdrawal or Termination

4.7.1 Reasons for Withdrawal or Termination

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate participation in the study if:

- Any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

4.7.2 Handling of Participant Withdrawals or Termination

If a participant misses an intervention session, they will be contacted by study personnel and reasons for missing the session will be documented. We will seek written permission to contact participants and will reach them using their preferred method (phone or email). If attendance drops below 80%, reimbursement will be pro-rated based on the number of sessions completed. Participants will be allowed a grace period/extension of one week. If participants miss more than one consecutive week, participation will be considered terminated.

If a participant chooses to withdraw from the exercise intervention, they will be contacted by study personnel and reasons for withdrawal will be documented. We will seek written permission to contact participants and will reach them using their preferred method (phone or email). Reasons for withdrawal will be classified as related or unrelated to intervention. Participants will be invited to complete endpoint testing even if they choose to withdraw from the intervention.

Participants that cannot complete the study due to incompatible logistics (precipitating from i.e. covid-related study suspension or personal leave of absence) will be encouraged to continue the study at a later date. Participants with long-term absences will be re-consented and will re-perform all study procedures in the longitudinal protocol described in section 6. Such participants will be considered newly enrolled and must meet all inclusion/exclusion criteria laid out in section 4. Long-term absence is defined as: 1) greater than 3-months between Step 2: Blood draw (section 6.4.1.2) and Post-non-exercise phase visit (section 6.4.3) or 2) greater than 4-months between Post-non-exercise phase visit (section 6.4.3) and Post-exercise phase visit (section 6.4.6).

4.7.3 Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the NIH. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension. Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed and satisfy the sponsor and/or IRB.

5 Behavioral/Social Intervention

5.1 Study Behavioral or Social Intervention(s) Description

We propose to combine moderate intensity aerobic exercise with lower-extremity specific resistance training, which is expected to reverse DPN symptoms. While current ADA (Americans with Disabilities Act) and American College of Sports Medicine (ACSM) guidelines emphasize the benefits of combination (i.e., aerobic and resistance) training programs, there is significant heterogeneity in the duration, intensity, frequency and specific exercises used in resistance training programs. Since DPN is length-dependent, distal extremities are more severely affected [57]. While most previous studies have assessed thigh skeletal muscle, we will assess the plantar flexors. Previous studies have demonstrated that significant impairments exist in lower extremity muscles in individuals with DPN, most notably lower plantar flexor strength and flexibility [58, 59]. Therefore, we expect an exercise intervention that combines aerobic training and strength training of the lower extremity will further reverse DPN symptoms.

5.1.1 Administration of Intervention

The exercise intervention will be administered in person at NYU's Department of Physical Therapy, 380 2nd Ave. The site PI (Smita Rao) and Research Associate will supervise all procedures. Health professional students may assist Dr. Rao and the Research Associate. The 10-week intervention has been previously published [24] and will include 3 days a week of moderate intensity aerobic and resistance training. Each session will last 60-90 minutes.

5.1.2 Procedures for Training Interventionalists and Monitoring Intervention Fidelity

The exercise intervention will be administered by licensed physical therapists or health professionals with exercise training. Each participant will follow a previously published 10-week moderate intensity aerobic and resistance exercise program [24]. Participants will be allowed to choose their modality (treadmill or recumbent stepper). At each session, modality, repetitions, heart rate, and rate of perceived exertion (RPE) will be recorded. Fidelity will be monitored by tracking the duration, intensity, and frequency of exercise. Additionally, for resistance training, weights and repetitions will be assessed.

The following criteria will be used to signal inadequate fidelity:

1. Aerobic training:
a) Does not complete duration
b) Does not reach prescribed intensity
c) Does not complete prescribed frequency (days per week)

2. Resistance training:
a) Does not complete all exercises
b) Does not complete prescribed number of repetitions
c) Does not reach prescribed intensity (i.e., uses lower or higher resistance)
d) Does not complete prescribed frequency (days per week)

At each session, interventionists will verbally encourage subjects to reach exercise prescription goals. Failure to maintain frequency, duration, or intensity will be documented.

Quarterly review with the research team with be instituted to review intervention fidelity and deploy remedial strategies for interventionists.

6 Study Procedures and Schedule

6.1 Schedule of Events

See Attachment A 'Schedule of Events'.

6.1.1 Standard of Care Study Procedures

N/A

6.2 Laboratory Procedures/Evaluations

6.2.1 Clinical Laboratory Evaluations

All subjects will undergo a 5-sample (10x4 mL of blood) oral glucose tolerance test (OGTT) at NYU CBI, where we carry out all evaluations involving intravenous (IV) catheters. We will ask subjects to abstain from taking oral medications such as metformin for 2 full days prior to all OGTT evaluations in order to obtain results that are unbiased by medication effects, which typically trigger insulin release from the pancreas or reduce glucose production in the liver. The rationale for doing the pre and post-intervention OGTT is to measure the degree of insulin resistance. Exercise has been shown to improve insulin sensitivity in patients with diabetes. We want to see if improvement in insulin sensitivity is correlated with MRI metrics. The OGTT has been used as a safe and standard procedure in our laboratory, and has been approved by our IRB for several years (s15-00271). We will document all those medications and changes and if needed, will account for them statistically. Subjects will be asked to abstain from taking their insulin on the morning of their visit. All diabetes medications will be brought to the visit so that they may be taken at the conclusion of the evaluation for which they need to be fasting for, and around the time they are given food prior to completion of the visit. Studies are performed in the morning after an overnight fast. Baseline bloods are obtained at time 0, prior to the consumption of the 75g glucose drink. We also obtain blood samples to measure inflammation that will help account for variable physiological

conditions such as time of day and other risk factors. For the OGTT, samples are then collected at 30, 60, 90, and 120 minutes post glucose ingestion. We will compute the Matsuda whole body Index of Insulin Sensitivity (ISI) as follows and will be used at the insulin resistance (IR) variable:

$$ISI = \frac{10{,}000}{\sqrt{[fasting\ glucose\ x\ fasting\ insulin]\ x\ [mean\ glucose\ x\ mean\ insulin\ during\ OGTT]}}$$

Baseline bloods obtained at time 0 will also be analyzed for measuring HgbA1C and C-reactive protein (CRP). In addition, we will use baseline bloods to estimate levels of hydrogen peroxide as a marker of ROS. None of these blood biomarkers will be used as an inclusion/exclusion criterion.

6.3 Imaging Procedures

All experiments will be performed on a 3T whole body scanner (Magnetom Prisma; Siemens Medical Solutions, Germany) available in the Department of Radiology at the NYU School of Medicine. We will use our recently developed dual-tuned (31P/1 H) coil array (C.1.6). The MRI scan will include 31P-MRI measurement of muscle oxidative capacity following a 90-second plantar flexion exercise at 40% MVC (Section C.1.1,2), dynamic post-contractile BOLD-measurements following brief 100% MVC every 90 s (Section C.1.3), IDEAL MRI for IMAT calculation, and DTI for assessment of peripheral nerve integrity (FA and ADC). The main MRI sequences and their acquisition parameters are summarized in Table 1. Additional sequences will include localizers and anatomical images for segmentation purposes.

Table 1: MRI Sequences and Imaging Outcomes

Pulse Sequence Description	TA (mm:ss)	N	TTA (mm:ss)	Exercise/Resting	Outcomes
PCr Mapping with ^{31}P-FLORET	1:00	5	05:00	Resting	Resting [PCr]
PCr Resynthesis with ^{31}P-FLORET	0:06	72	7:30	2-min plantar flexion at 40% MVC	PCr Resynthesis Rate
IDEAL-MRI	5:00	1	5:00	Resting	Water Fat Fractions
BOLD-MRI	0:01	600	10:00	1-rep at 100% MVC every 90s	$\Delta T2^*$; TTP
DTI	3:08	2	6:15	Resting	FA, ADC

N: number of acquisition/averages; TA: acquisition time for one average. FA: fractional anisotropy. ADC: apparent diffusion coefficient TTA: Total time of acquisition for experiment: TTP: Time-to-Peak

6.4 Study Schedule

6.4.1 Baseline Visit

6.4.1.1 Step 1: Consent

The PI, Co-Investigator, Research Coordinator, or Research Data Associate will consent individuals at the Center for Biomedical Imaging. They will go over the consent form with the subject and answer any questions that may arise. When the subject has shown that he/she understands the process and has signed the form, he/she will be given a copy of the form and considered “enrolled” in the study.
All subjects will have the option to consent remotely (through send safe) instead of consenting in person. If the subject prefers to sign the consent form electronically, the research coordinator will be able to send the informed consent via email (send safe) to the subject. The research coordinator will go over the consent form with the subject and will give ample time to the subject to ask questions regarding the study,

6.4.1.2 Step 2: Blood draw

After the consent process is completed, participants will be required to have blood tests at the CBI or at the outpatient lab located at TISCH hospital. At CBI a licensed nurse, radiology technician, or study team member with phlebotomy certification will place the IV line and at the outpatient lab at Tisch hospital a

nurse will be performing the blood draw. A total of 14 tubes, approximately 59.2 mL of blood, will be collected. The following blood tests will be performed:

- Insulin (fasted)
- Glucose (fasted)
- Complete Blood Count with Differential
- Comprehensive Metabolic Panel
- Lipid profile
- Fibrinogen
- Highly sensitive CRP
- Thyroid Stimulating Hormone
- Hemoglobin A1C
- OGTT: subject will drink a sweet-tasting drink and then have his/her blood drawn (2 tubes) every 30 minutes for a 2-hour duration.

The following urine test will be performed:

- Microalbumin

Note: The Center of Biomedical Imaging as well as other NYU Health Institutions are taking safety measure necessary to reduce the risk of subjects contacting COVID-19. As per NYU COVID-19 related policies, staff will ensure all rooms or MRI machines are disinfected prior to the subjects scan.

6.4.1.3 Step 3: MRI Session

The MRIs will be performed on the day of consenting and blood tests, also at the CBI on the FDA-approved 3T whole body scanner.

All MRIs are reviewed by the licensed clinical radiologist on duty immediately as they are done. A report is issued and faxed to the PI the same day with the radiologist's interpretation of the scan as well as recommendations based on any incidental findings. In the case of an incidental finding, either the PI or another investigator on the study will speak to the subject in person at their next visit or via phone regarding the new information. Types of incidental findings would be a bone infarct, meniscal tear, or meniscal destruction found on the MRI. A copy of the original image report will also be provided to the patient in person and they will be encouraged to follow up on the discovery with their treating physician. We will ask subjects to abstain for 2 full days prior to their MRI scan from taking oral diabetes medications such as metformin. Subjects will be asked to fast the night prior to visit. Subjects will be asked to not take their insulin on the morning of their visit. All diabetes medications will be brought to the visit so that they may be taken at the conclusion of the evaluation for which they need to be fasting for.

6.4.2 Maximal Graded Exercise Testing (VO_2R Assessment)

Maximal graded exercise testing (VO_2R assessment) will be performed prior to the 10-week exercise intervention by an exercise physiologist at Langone Orthopedic Center, 333 East 38th St. A standardized exercise protocol with a recumbent stepper/treadmill will be used (Trackmaster, USA). We will use a ramping protocol with increases in workload equivalent to 0.5 METS per 1 minute. Continuous monitoring of heart rate will be performed. Additionally, blood pressure and RPE are measured at the end of each stage. Criteria for a "maximal" effort during exercise testing include: reaching a plateau in VO2 with increased workload, a respiratory exchange ratio of greater than or equal to 1.15, a peak HR within 85% of the age-predicted maximal HR (in absence of beta blockage), and an RPE of greater than or equal to 18 (ACSM Guidelines for Exercise Testing and Prescription 10th edition, 2017.) The test will be stopped before maximal effort was achieved if any of the following occur: angina, dyspnea, fatigue (voluntary exhaustion or inability to maintain a speed/cadency of 115 on the stepper), hypertension (250 mm Hg systolic or 115 mm Hg diastolic), and hypotension (>10mmHg drop in blood pressure with workload increase), or ischemic electrocardiographic abnormalities. The same exercise physiologist will administer tests (before and after intervention). In addition to standardized protocols, the following procedures will be used to minimize bias:
1) The use of timed encouragement scripts
2) No review of pre-intervention test results before post intervention tests

6.4.3 Exercise Phase (10 weeks)

All procedures related to exercise intervention and its safety have been previously published in the peer-reviewed literature [15, 24, 60-62]. Each participant will be prescribed an individualized, 10-week exercise program (after the 4-week non-exercise phase) with both aerobic and resistance components, based on the ACSM Guidelines [15, 24]. Exercise training will be performed under the supervision of a licensed physical therapist at the Department of Physical Therapy, 380 2nd Ave. Participants will have the choice of using a treadmill or a recumbent stepper. The physical therapist will monitor blood glucose level, blood pressure, heart rate, and RPE during each exercise session.
Additionally, in light of the COVID-19 pandemic, all subjects will be required to complete the PCR Covid test at a site that is convenient for them.

Note: Due to COVID-19 one of the requirement policy for the Department of Physical Therapy is to complete the PCR test within 5 days prior to patient's appointment. It is required that all subjects complete this testing as a way to reduce the risk of patients contacting COVID-19.

Aerobic Exercise: The maximal workload obtained from the VO2R assessment will be used to calculate a moderate level of intensity (50–70% of VO_2 reserve) for the aerobic training program. A brief overview of the program is provided in Table 2.

Table 2

Week	Day 1	Aerobic Exercise	Resistance Exercise
1	1	30 mins, 50% VO_2R	Determine 1 RM max, 10 reps x 1 day, Record medication use, Utah Early Neuropathy Scale, Give Actigraph
	2	30 mins, 50% VO_2R	PROMIS Physical Function and Fatigue, Calf muscle performance
	3	35 mins, 50% VO_2R	9 item Physical Performance Test
2	1	35 mins, 50% VO_2R	Get Actigraph, 10 reps
	2	35 mins, 50% VO_2R	15 reps
	3	40 mins, 50% VO_2R	
3	1	40 mins, 50% VO_2R	15 reps
	2	40 mins, 50% VO_2R	15 reps
	3	45 mins, 50% VO_2R	
4	1	45 mins, 50% VO_2R	15 reps
	2	45 mins, 50% VO_2R	15 reps
	3	45 mins, 50% VO_2R	
5	1	45 mins, 60% VO_2R	15 reps
	2	45 mins, 60% VO_2R	15 reps
	3	45 mins, 60% VO_2R	
6	1	45 mins, 70% VO_2R	15 reps
	2	45 mins, 70% VO_2R	15 reps
	3	45 mins, 70% VO_2R	
7	1	50 mins, 70% VO_2R	20 reps
	2	50 mins, 70% VO_2R	20 reps
	3	50 mins, 70% VO_2R	
8	1	50 mins, 70% VO_2R	20 reps
	2	50 mins, 70% VO_2R	20 reps
	3	50 mins, 70% VO_2R	
9	1	50 mins, 70% VO_2R	20 reps
	2	50 mins, 70% VO_2R	20 reps

	3	50 mins, 70% VO_2R	Give Actigraph
10	1	50 mins, 70% VO_2R	20 reps
	2	50 mins, 70% VO_2R	PROMIS Physical Function and Fatigue, Calf muscle performance
	3	50 mins, 70% VO_2R	9 item Physical Performance Tests, Get Actigraph

Resistance Training: will include the following exercises: biceps/triceps curls, seated row, squats, and heel raises. Dumbbells will be used for strength training, with resistance levels gradually increased to maintain RPE in a moderate range (7–8 out of 10) for each participant. The weight of the dumbbells will be based on RPE 5-7, on a scale of 0-10, and 50-70% of 1 repetition maximum weight. There will be 1 to 5 minutes of rest between each exercise. We will use the following sequence of exercises in this study: Blood pressure, blood sugar check, warm-up, aerobic exercise, resistance exercise, and stretch.

Prior to each exercise session, the following will be assessed, consistent with guidelines set forth by the American Physical Therapy Association and American Diabetes Association.

- Foot: a visual inspection for cuts, blisters, cracked skin, and/or ulcer development
- Finger stick blood glucose:
    - If hypoglycemic (less than 100 mg/dL), carbohydrate snacks will be provided, and glucose testing will be repeated in 15-20 min.
    - If hyperglycemic (more than 300 mg/dL) and the participant *was not taking insulin*, exercise will be permitted with close monitoring of blood glucose every 10 to 15 minutes to ensure that the blood glucose level does not increase with activity.
    - If hyperglycemic (more than 300 mg/dL) and the participant *was taking insulin*, urine will be checked for ketosis. If the ketone test result is positive, then exercise will be postponed. If the ketone test result was negative, then exercise will be permitted with close monitoring of blood glucose every 10 to 15 minutes.
- Blood pressure: participants will not be permitted to exercise if blood pressure is higher than 200/110 mm Hg
- RPE will be assessed at baseline and every 20 minutes. A moderate RPE level (11–13 on the 17-point scale) should be achieved at each session.

Adverse event tracking: Adverse events will be tracked at each session. Events will be classified as related to exercise intervention or unrelated to exercise intervention. Severity will be classified using the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Aaron Chidakel, M.D. will serve as the safety monitor for this study and review adverse events as necessary.

6.4.4 Post-Exercise Phase Visit

Participants will undergo blood tests, VO_2R, and a final MRI to compare with results collected prior to the exercise intervention. The final visit should occur within two weeks after the last exercise session. This will allow for feasible scheduling, data collection, and study completion considerations.

6.4.5 Withdrawal Visit

Participants will be invited to complete endpoint testing even if they choose to withdraw from the intervention. Endpoint testing will include:

- BMI
- HbA1c and C-reactive protein
- MNSI symptom questionnaire and the PROMIS physical function and fatigue questionnaire
- MNSI physical exam
- VPT and Calf Muscle performance
- Plasma levels of hydrogen peroxide
- Lower leg muscle performance & physical activity

- MRI

6.5 Concomitant Medications, Treatments, and Procedures

All concomitant prescription medications, over-the-counter medications, and non-prescription medications taken during study participation will be recorded on the CRF. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

6.5.1 Precautionary Medications, Treatments, and Procedures

N/A

6.6 Prohibited Medications, Treatments, and Procedures

Oral medications such as Metformin are prohibited two full days prior to all OGTT evaluations. Participants will be asked not to take their insulin on the morning of their visit at CBI.

6.7 Prophylactic Medications, Treatments, and Procedures

- If hypoglycemic (less than 100 mg/dL), carbohydrate snacks will be provided and glucose testing will be repeated in 15-20 min
- No other prophylactic medications will be provided by study personnel

6.8 Participant Access to Study Intervention at Study Closure

Participants will be encouraged to continue exercising upon completion of the intervention as this will be beneficial to their health.

7 Assessment of Safety

7.1 Specification of Safety Parameters

7.1.1 Definition of Adverse Events (AE)

An *adverse event* (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Inter-current illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

7.1.2 Definition of Serious Adverse Events (SAE)

Serious Adverse Event

Adverse events are classified as serious or non-serious. A *serious adverse event* (SAE) is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result

in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as *non-serious adverse events*.

7.1.3 Definition of Unanticipated Problems (UP)

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

7.2 Classification of an Adverse Event

7.2.1 Severity of Event

For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

7.2.2 Relationship to Study Intervention

The clinician's assessment of an AE's relationship to study intervention is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study intervention assessed. In a clinical trial, the study intervention must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the AE, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the AE.
- **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

7.2.3 Expectedness

Aaron Chidakel, MD, the study's safety officer, will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

Adverse events will be collected at each study visit by the technologist performing the MRI scan.

7.3 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care. All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate CRF. Information to be collected includes event description, date of onset, clinician's assessment of severity, relationship to study intervention (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. UPs will be recorded in the data collection system throughout the study.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

The PI/research coordinator will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit.

All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study.

7.4 Reporting Procedures – Notifying the IRB

7.4.1 Adverse Event Reporting

Severe AEs will be reported to the IRB within 5 working days. Other adverse events will not be reported to the IRB.

7.4.2 Unanticipated Problem Reporting

Incidents or events that meet the OHRP criteria for UPs require the creation and completion of an UP report form. It is the site investigator's responsibility to report UPs to their IRB and to the NIH. The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are SAEs will be reported to the IRB and to the NIH within 24 hours of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the NIH within 5 working days of the investigator becoming aware of the problem.

- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within 5 working days of the IR's receipt of the report of the problem from the investigator.

7.4.3 Reporting of Pregnancy

Should a participant get pregnant during the course of the study, he/she will be discontinued immediately. The pregnancy will be documented and reported to the IRB.

7.5 Safety Oversight

Safety oversight will be under the direction of the safety officer, Dr. Aaron Chidakel. Dr. Chidakel's study responsibilities include the following:
1) Review all AEs/SAEs on a regular basis throughout the trial
2) Be available to advise the investigators on trial-related medical questions or problems
3) Evaluate cumulative participant safety data and make recommendations regarding the safe continuation of the study.

8 Data Safety Monitoring Plan

Meetings among members of the research team will be held quarterly to discuss the preliminary report and to ensure that the study is being conducted according to the protocol. Additionally, members will discuss whether any protocol amendments are necessary and whether the safety/privacy of subjects who are participating is being respected. The following events/data points will be reviewed:

- Subject attrition
- Occurrence of adverse events
- Subject compliance
- Subject complaints
- Site issues (i.e. scheduling, recruiting, staffing, study flow, etc.)

There are no predefined stopping rules for the entire study.

Responsibilities and roles for gathering, evaluating and monitoring the data:

- PI will gather data regarding recruitment rate. This will be reviewed with members of the research team every month.
- PI and co-investigators overseeing the MRI scan will gather data regarding unanticipated adverse events. The PI and co-investigators will report any serious adverse event immediately to the NYU IRB.

Information about the monitoring entity:

- The Individual Data Monitor will be the PI, Ryan Brown, NYU Langone Health, Department of Radiology, 660 First Avenue, 4th floor, New York NY 10016, 212-263- 3396. The PI will follow the policies and procedures of the IRB for monitoring this study for safety concerns, with ongoing updates from the co-investigators on a continuous basis.

Assessments:

- The PI and Co-PI will assess the risk to benefit ratio and determine whether the level of risk and patient safety were accurately outlined and accounted for in study application, protocol and consent. These meetings will occur annually when the study is being prepared for re-approval. The IRB will be informed if any reasons warrant "holding" the study.

Procedures for Communicating – Dissemination of safety information

As appropriate:

- The PI will notify in writing the outcomes of the monitoring events. Any significant deviation will be reported immediately to the IRB using the IRB Reportable New Information form of Research Navigator.

- New information that would affect the safety of the subject or his/her decision to participate in the study will be provided by the PI or his co-investigators.

9 Statistical Considerations

9.1 Statistical and Analytical Plans

The primary objective is to assess and compare the interventions (exercise versus control phase in the same individual) in terms of the baseline to follow-up change in the following outcomes: 1) DTI, plasma ROS level, and the MNSI assessment of DPN symptoms (Aim 1); and 2) leg mitochondrial function, oxidative capacity (31P-MRI), peak MV response (BOLD-MRI), the proportion of fat-free muscle (IDEAL-MRI) and leg muscle strength (Aim 2).

9.2 Statistical Hypotheses

Hypothesis 1a: Exercise will improve peripheral nerve integrity (DTI fractional anisotropy will increase, and the apparent diffusion coefficient will decrease).
Hypothesis 1b: Exercise will decrease plasma ROS levels.
Hypothesis 1c: Exercise will improve DPN symptoms, assessed with the MNSI.
Hypothesis 2a: Exercise will increase leg mitochondrial function, as defined by an increase in oxidative capacity (measured using ^{31}P-MRI).
Hypothesis 2b: Exercise will improve leg MV function, as defined by an increase in peak MV response (measured using BOLD-MRI)**.**
Hypothesis 2c: Exercise will decrease intramuscular fat and increase levels of fat-free muscle (measured using IDEAL-MRI)**.**

9.3 Description of Statistical Methods

A weighted paired sample *t*-test will be used to assess whether each outcome changed from baseline to follow-up as a result of the exercise intervention. Weighted analysis of covariance (ANCOVA) will be used to compare the time points in terms of the change in each outcome. The change in each outcome from baseline to follow-up will be the dependent variable and the ANCOVA model will include the matching factors (age, baseline MNSI) and the measure of general activity level as numeric covariates and the binary indicator of intervention as a classification factor. The secondary objective is to assess the extent to which exercise induced improvements in DTI outcomes are mediated or explained by reduction in ROS. After testing the effect of the intervention on the change in DTI and the correlation between changes in DTI and ROS, an incomplete causal pathway meditational analysis will be conducted using the method of Baron and Kenny[63] with a bootstrap based test of the indirect (mediation) effect of ROS. The exploratory objective to identify baseline factors (e.g., age, MNSI, general activity) predictive of improvement, represented as the baseline to follow-up change in each outcome, will be addressed using linear regression and bivariate correlations. This analysis will be stratified by intervention and then conducted using data from both intervention groups combined. In each case, the assessment of compliance will be included as a weighting factor.

9.3.1 General Approach

In this longitudinal, single-center study we will recruit 40 T2DM patients. Each participant will be imaged two times: upon enrollment (visit 1) and after completion of the 10 week exercise program (visit 3).

9.3.2 Analysis of the Primary Efficacy Endpoint(s)

A matched-pairs Wilcoxon signed rank test will be applied to the within-subject change in each outcome among subjects demonstrating full compliance with the exercise program. Additionally, weighted analysis of covariance (ANCOVA) will be used to test the change in each outcome adjusting for the measured level of compliance using data from all subjects. The values of each outcome at

baseline and follow-up for all subjects will be the dependent variable and the ANCOVA model will include time (baseline versus follow-up) as the within-subject factor of interest covariates, the measure of compliance as numeric covariate and an anonymized subject ID as a classification factor so that the comparison of times will be based on their within-subject difference. The secondary objective is to assess the extent to which exercise induced improvements in DTI outcomes are can be at least partially explained by reduction in ROS will be addressed using linear regression, The baseline to follow-up change in each outcome will be the dependent variable and the model will include the measure of compliance as a numeric factor and the within-subject change in ROS as the numeric factor of interest. A second regression will be conducted with baseline factors (e.g., age, MNSI, general activity) added to the model in order to identify factors predictive of improvement in each outcome.

9.3.3 Analysis of the Secondary Endpoint(s)

NA

9.3.4 Adherence and Retention Analyses

The analysis will follow the intention-to-treat (ITT) principle and include data from every subject irrespective of whether the subject was compliant with the assigned intervention. A quantitative assessment of compliance with the intervention, ranging from 0 (complete non-compliance) to 1 (full compliance), will be included as a weighting factor to allow more compliant subjects to contribute more to the analysis. The weight for the control phase will be set to 1 since subjects are not required to exercise.

9.3.5 Baseline Descriptive Statistics

In this longitudinal study, each participant will participate in both an exercise phase. Therefore, there will be no comparison between groups.

9.3.6 Exploratory Analyses

The exploratory objective to identify baseline factors (e.g., age, MNSI, general activity) predictive of improvement, represented as the baseline to follow-up change in each outcome, will be addressed using linear regression and bivariate correlations. This analysis will be stratified by intervention and then conducted using data from both phases combined. In each case, the assessment of compliance will be included as a weighting factor

9.4 Sample Size

Assessment of statistical power was based on tests conducted at the two-sided 5% significance level and the preliminary data in Table 3. Under the conservative assumption of a moderate within-subject correlation (ρ=0.5) between baseline and follow-up measures, the expected mean and SD of the within-subject change in the outcomes is given in Table 4. It is noted that statistical power will be higher if ρ>0.5 as observed in the majority of longitudinal imaging studies. With data from 40 subjects included in the analysis according to the ITT principal the study will have 80% power to detect a mean within-subject change in each outcome among subjects in each intervention group provided the change is between 10% and 18% when expressed as a percentage of the mean at baseline. The mean percentage change observed in the preliminary data among subjects

Table 3. The mean and standard deviation (SD) of the BOLD response, PCr resynthesis rate and MNSI score before (baseline) and after (follow-up) a control or exercise intervention observed in preliminary data.

	Exercise				Control			
	Baseline		Follow-up		Baseline		Follow-up	
Outcome	Mean	SD						
BOLD respone	3.24	1.04						
PCr resynthesis	41.16	14.1						
MNSI	5.2	1.4						

Table 4. Expected mean and standard deviation (SD) of the within-subject change in BOLD response, PCr resynthesis rate, and MNSI score from baseline to after a control or exercise intervention based on the preliminary data in Table 3 and the conservative assumption of moderate within-subject correlation (ρ=0.5) between baseline and follow-up measures.

	Exercise		Control	
Outcome	Mean	SD	Mean	SD
BOLD respone	1.11	1.26	0.18	1.42
PCr resynthesis	10.61	12.85	0.06	8.53
MNSI	1.20	1.78	NA	

in the exercise group ranged from 23% to 34% across outcomes, implying that the study will have >80% power to detect anticipated changes resulting from exercise. The minimum difference in terms of the mean within-subject change in a given outcome that is detectable with 80% power is 4.5% for BOLD response and 120% for PCr resynthesis where each difference is expressed as a percentage of the mean within-subject change. The difference in terms of the mean within subject change observed in the preliminary data was 5.2% for BOLD response and 176% for PCr resynthesis, respectively. Since the observed differences exceed the minimum detectable differences, the study will have >80% power to detect anticipated group differences.

10 Source Documents and Access to Source Data/Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The study CRF is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Access to study records will be limited to IRB-approved members of the study team. The investigator will permit study-related monitoring, audits, and inspections by the IRB/EC, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethics/Protection of Human Subjects

11.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 CFR Part 46.

11.2 Institutional Review Board

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

11.3 Informed Consent Process

11.3.1 Consent/Assent and Other Informational Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention.

11.3.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the signed informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

A copy of the signed informed consent document will be stored in the subject's research record. The consent process, including the name of the individual obtaining consent, will be thoroughly documented in the subject's research record. Any alteration to the standard consent process (e.g. use of a translator, consent from a legally authorized representative, consent document presented orally, etc.) and the justification for such alteration will likewise be documented.

11.4 Participant and Data Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

All the MRI data will be archived into the research image database in the Department of Radiology at the New York University Medical Center. Dr. Brown (PI) will monitor all the MRI data and save the data on the NYU Radiology password-protected server. A copy of the data will be kept on the P.I.'s workstation, which has a secure password protection. Every attempt will be made by Dr. Brown to maintain all information collected in this study strictly confidential, except as may be required by court order or by law. All clinical and exercise-related data will be de-identified and stored at the Arthur J. Nelson Jr. Human Performance Lab at the Department of Physical Therapy. All digital materials will be stored on a secure password protected server with access given only to authorized study personnel. All paper documents will be stored for three years in a locked file cabinet and lab, with access given only to authorized study personnel. Dr. Rao will work with the Data Services and Data Management Planning Team

(http://guides.nyu.edu/data_management) to develop and implement this plan. Authorized representatives of the New York University Institutional Review Board (IRB), a committee charged with protecting the rights and welfare of research subjects, may be provided access to medical or research records that identify subjects by name. In any publication or presentation resulting from this research, the subjects will not be identified by name. Any serious adverse effects to the participants will be immediately communicated to the IRB as well as our departmental research committee that oversees clinical research

11.5 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site PI. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Black ink is required to ensure clarity of reproduced copies. When making changes or corrections, cross out the original entry with a single line, and initial and date the change. DO NOT ERASE, OVERWRITE, OR USE CORRECTION FLUID OR TAPE ON THE ORIGINAL.

11.6 Study Records Retention

Study documents will be retained for the longer of 3 years after close out or 5 years after final reporting/publication. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the NIH, if applicable. It is the responsibility of the NIH to inform the investigator when these documents no longer need to be retained.

11.7 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation. Protocol deviations must be reported to the local IRB per their guidelines. The site PI/study staff is responsible for knowing and adhering to their IRB requirements.

12 Study Finances

12.1 Funding Source

This study is financed through a grant R01DK114428 from the National Institute of Diabetes and Kidney Disease (NIDDK)

12.2 Costs to the Participant

There will be no cost to the participant for enrolling in this study.

12.3 Participant Reimbursements or Payments

Each participant will be offered $500 upon completion of the 10-week supervised exercise intervention. They will also receive a clincard (similar to a debit/credit card) of $50 for undergoing each MRI scan (two in total) and up to $20 reimbursement for travel to our facilities.

If attendance during the exercise phase drops below 80%, reimbursement will be prorated based on the number of sessions completed. Participants will receive $17 per session.

13 Conflict of Interest Policy

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by the NYU Langone Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All NYULMC investigators will follow the applicable conflict of interest policies.

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Attachments

These documents are relevant to the protocol, but they are not considered part of the protocol. They are stored and modified separately. As such, modifications to these documents do not require protocol amendments.

Attachment A

Schedule of Events

Activity	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9
Consent		X								
MNSI	X	X								
Medical History	X									
Height/Weight		X					X			
OGTT		X								
Biweekly Health Assessment				X		X				
VO_2R Assessment							X			
Plantar flexion training		X								
Bloodwork		X								
Actigraph**		X	X					X	X	
MRI		X				X				
Aerobic exercise								X	X	X
Resistance exercise								X	X	X
PROMIS								X		
Calf muscle performance								X		
9-item physical performance test								X	X	X
Blood Pressure (BP)								X	X	X

**Actigraph to be worn for at least one week.

Attachment A

Schedule of Events Cont….

Activity	Week 10	Week 11	Week 12	Week 13	Week 14	Week 15	Week 16	Week 17	Week 18
Consent									
MNSI									X
Medical History									
Height/Weight								X	
OGTT									X
Biweekly Health Assessment									
VO_2R Assessment								X	
Plantar flexion training									
Bloodwork									X
Actigraph						X	X		
MRI									X
Aerobic exercise	X	X	X	X	X	X	X		
Resistance exercise	X	X	X	X	X	X	X		
PROMIS							X		
Calf muscle performance							X		
9-item physical performance test	X	X	X	X	X	X	X		
Blood Pressure (BP)	X	X	X	X	X	X	X		