High-intensity exercise to promote accelerated improvements in cardiorespiratory fitness (HI-PACE): Study protocol for a randomized controlled trial

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Abstract

Background

African Americans have a disproportionate prevalence and incidence of type 2 diabetes compared to Caucasians. Recent evidence indicates low cardiorespiratory fitness (CRF) level, an independent risk factor for type 2 diabetes, is also more prevalent in African Americans than Caucasians. Numerous studies in Caucasian populations suggest vigorous exercise intensity may promote greater improvements in CRF and other type 2 diabetes risk factors (e.g. reduction of glucose/insulin levels, pulse wave velocity, body fat, etc.) than moderate intensity. However, current evidence comparing health benefits of different aerobic exercise intensities on type 2 diabetes risk factors in African Americans is negligible. This is clinically important as African Americans have a greater risk for type 2 diabetes and are less likely to meet public health recommendations for physical activity than Caucasians. The purpose of the High-Intensity exercise to Promote Accelerated improvements in Cardiorespiratory fitness (HI-PACE) study is to evaluate whether high-intensity aerobic exercise elicits greater improvements in CRF, insulin action, and arterial stiffness than moderate-intensity exercise in African Americans.

Methods/Design

A randomized controlled trial will be performed on overweight and obese (body mass index: 25-45 kg/m2) African Americans (35-65 years) (n=60). Participants will be randomized to moderate-intensity (MOD-INT) or high-intensity (HIGH-INT) aerobic exercise training, or a non-exercise control group (CON) for 24 weeks. Supervised exercise will be performed at a heart rate associated with 45-55% and 70-80% of VO2 max in the MOD-INT and the HIGH-INT groups, respectively, for an exercise dose of 600 MET-minutes/week (consistent with public health recommendations). The primary outcome is change in CRF. Secondary outcomes include change in insulin sensitivity (measured via an intravenous
glucose tolerance test), skeletal muscle mitochondrial oxidative capacity (via near infrared spectroscopy), skeletal muscle measurements (i.e. citrate synthase, COX IV, GLUT-4, CPT-1, PGC1-α), arterial stiffness (via carotid-femoral pulse wave velocity), body fat, C-reactive protein, and psychological outcomes (quality of life/exercise enjoyment).

Discussion
The anticipated results of the HI-PACE study will provide vital information on the health effects of high-intensity exercise in African Americans. This study will advance health disparity research and has the potential to influence future public health guidelines for physical activity.

Background
The American Diabetes Association (ADA) identifies racial health disparities in type 2 diabetes (T2D) as a major public health concern [1]. T2D prevalence in African Americans (AA) is one of the greatest in the United States with ~1.7-fold higher rates compared to their Caucasian American (CA) counterparts (females: 13.6% vs. 7.4%; males: 14.1% vs. 8.0%, respectively) [2]. A recent ADA position statement [3] recognized the importance of physical activity to prevent T2D, as low levels are associated with greater T2D incidence. The prevalence of AAs meeting physical activity public health recommendations is considerably lower than CA adults (56.5% vs. 67.5%, respectively) [4]. Despite established racial disparities in T2D risk, AAs are under-represented in exercise research (i.e. sample sizes inadequate for sub-group analyses, few randomized clinical trials specifically examining AAs). This lack of data critically limits Federal Physical Activity Guidelines from making accurate conclusions on the effects of physical activity on health outcomes in AAs [5].

Low cardiorespiratory fitness (CRF) is an independent risk factor for T2D incidence [6-11]. However, an incremental dose-response has been observed between CRF level and T2D
incidence [8,12]. Categorically-defined “low CRF” is associated with the greatest risk of T2D and a greater proportion of AAs have “low CRF” than CAs [8,10,13–27]. Higher intensities of aerobic exercise show promise in eliciting a larger magnitude of improvement in insulin sensitivity, compared to moderate. Moreover, arterial stiffness, another racial disparity identified in AAs, has shown a greater decline from high-intensity exercise than moderate [28–30]. An additional contributor to the T2D racial disparities is the lower oxidative characteristics and subsequent reduced insulin sensitivity of skeletal muscle in AAs compared to CAs [13,26,31–33]. AAs tend to have a greater proportion of type-II muscle fibers (i.e. less oxidative, vascularized, lower proportion of GLUT-4 transporters, more insulin-resistant than type-I fibers) compared to CAs [32,34,35]. A major adaptation to aerobic training is the shift in both type-I and -II fibers towards more oxidative properties (e.g. increased mitochondria size, density, and enzymes) and increased sensitivity to insulin (increased GLUT-4 expression) [36,37]. Thus, lower CRF levels in AAs may contribute to the racial health disparities in T2D.

Data examining CAs suggest high-intensity aerobic training results in greater improvements in CRF, insulin action, and arterial stiffness compared to moderate [26,38,39]. Thus, high-intensity exercise may improve the low-CRF, stiffened-artery, and insulin-resistant disposition observed in obese AAs more readily due to greater shear rates in the vasculature, recruitment of type-II fibers, and greater energy expenditure rates than moderate. There are, however, no current randomized controlled trials comparing the health benefits of different exercise intensity training programs in AAs, despite the greater T2D risk in AAs than CAs.

The goal of the High-Intensity exercise to Promote Accelerated improvements in CardiorEspiratory fitness (HI-PACE) study is to evaluate the effects of exercise intensity on CRF, insulin action, and arterial stiffness in AAs at high-risk for T2D. The purpose of the
following paper is to describe the design, rationale, and methodology of the HI-PACE study.

Specific objectives

The objective of the HI-PACE study is to determine whether high-intensity aerobic exercise training results in greater improvements in CRF, insulin action, arterial stiffness, mitochondrial function, adiposity, and quality of life compared to moderate intensity. Thus, the primary outcome of the HI-PACE study is the change in CRF following the intervention. Main secondary outcomes include change in insulin sensitivity (measured via an intravenous glucose tolerance test [IVGTT]), skeletal muscle mitochondrial oxidative capacity (via near infrared spectroscopy [NIRS]), skeletal muscle measurements (i.e. citrate synthase, COX IV, GLUT-4, CPT-1, PGC1-α), arterial stiffness (via carotid-femoral pulse wave velocity [PWV]), body fat, C-reactive protein, and psychological outcomes (quality of life/exercise enjoyment).

Participants will be randomized to one of three groups: 1) moderate-intensity (MOD-INT), 2) high-intensity (HIGH-INT), or 3) a non-exercise control group (CON) for 24 weeks. The exercise volume for both exercise groups will be 600 MET-minutes per week (3-4 sessions per week), which is consistent with the current public health guidelines (500-1,000 MET-minutes) [5]. Participants in the MOD-INT group will exercise at the heart rate associated with 45-55% of maximal oxygen consumption (VO₂ max), and participants in the HIGH-INT group will exercise at the heart rate associated with 70-80% VO₂ max. Fitbit Flex accelerometers (Fitbit Inc., San Francisco, CA) will be worn on the wrist by participants in all randomization groups to objectively monitor non-exercise physical activity during the intervention (devices will be removed during training sessions).

Methods/design
Inclusion/exclusion criteria

Main inclusion and exclusion criteria for HI-PACE are shown in Table 1. The HI-PACE study is designed to intervene in sedentary, overweight and obese AAs at high risk for T2D. Thus, we plan to enroll 60 sedentary, overweight and obese AA adults (body mass index [BMI]: 25.0-45.0 kg/m², age 35-65 years). All participants will be sedentary/low active and not participating in exercise training at time of enrollment (<20 minutes, ≤2 days per week for the last 3 months). Major exclusions for the HI-PACE study include diagnosed type 1 or type 2 diabetes (or fasting glucose >125 mg/dL, or use of diabetes medication), known cardiovascular diseases (e.g. heart failure, serious arrhythmias, peripheral vascular disease), previous stroke or myocardial infarction, excessively high resting systolic (>180 mmHg) or diastolic (>100 mmHg) blood pressure, significant medical conditions, life-threatening conditions, pregnancy or plans to become pregnant, and other medical conditions that are contraindicated for exercise training. Additionally, individuals who plan to diet, engage in weight loss, or demonstrate non-compliance during screening visits will be excluded.

The study protocol has been approved by the East Carolina University (ECU) Institutional Review Board and is registered on Clinicaltrials.gov (NCT02892331). This study protocol was prepared on the basis of the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines. This SPIRIT Checklist is available as Additional file 1.

Recruitment and pre-screening

A detailed summary of the study visits is described in Table 2. Recruitment material will be disseminated through newspaper (general readership and an AA-specific newspaper), targeted social media advertisement (e.g. Facebook, Instagram), email sent through...
company employee listservs (i.e. ECU, Pitt Community College, Greenville government), and local organizational contacts in the Pitt County, North Carolina area (e.g. churches, physician offices, libraries, barbershops, etc.). In addition, a study website will be created to provide basic study information and to serve as a mechanism for web-screening potential participants. Web-screening will be performed using an online survey where basic inclusion/exclusion criteria questions can be completed and subsequently reviewed by study staff. This online survey is created using an online research database, REDCap (Nashville, TN) [40], which is connected to the main study database. Interested individuals can also contact HI-PACE staff by calling the study phone number or by directly emailing the research coordinator. After this, study staff will phone-screen individuals for major aspects of the inclusion/exclusion criteria and provide additional information about study participation. Individuals who are eligible and still interested following phone screening will progress to Screening visit 1.

**Screening visits**

Screening visits will be conducted at the East Carolina Heart Institute by the research coordinator. During Screening visit 1, the research coordinator will describe all properties of study participation, answer questions from individuals, and obtain informed consent. Following consent, the research coordinator will screen participants for the full inclusion/exclusion criteria, collect contact/demographic information, and review prescribed medications (individuals will be required to bring in prescribed medications for verification). For inclusion/exclusion purposes, height and weight (without shoes) will be measured to calculate BMI (kg/m$^2$) and seated resting blood pressure will be assessed via an automated blood pressure monitor (HEM-907XL, Omron Healthcare Co., Kyoto, Japan). Individuals will be screened for ample time to participate in the exercise intervention by
filling out an exercise calendar form, where they will be asked to identify specific days and times (and back-up times) they are available to exercise at our facility (See Appendix A). Staff will also conduct a standardized interview with potential participants in which: a) weekly time commitments, b) responsibilities for family care (i.e. child and elder), c) distances of home and work from our exercise facility, d) personal motivations for exercising, e) levels of familial support, and d) any other barrier(s) that would affect study adherence will be evaluated (See Appendix B). The exercise calendar and interview are intended to screen-out individuals depicting high risk for non-compliance and/or drop-out during the 24-week study. Previous studies utilizing similar methodologies exhibited high exercise training adherence and study retention [41,42].

Individuals who are still eligible at this point will wear a Fitbit Flex (Fitbit Inc., San Francisco, CA) and an activPAL accelerometer (PAL Technologies Ltd., Glasgow, UK) for 7 continuous days to assess baseline non-exercise physical activity level. The Fitbit Flex will be worn on the non-dominant wrist to obtain data on steps, miles, intensity, and calorie expenditure each day of wear (blinded to individual). Study staff will apply the activPAL by rolling a nitrile sleeve over the entire device and wrap an 8x10 cm sheet of transparent medical dressing completely around it to act as a waterproof barrier. Staff will rub an alcohol-based prep pad around the site, place the distal end of the activPAL towards the knee, and apply a separate sheet of medical dressing over the monitor to complete application to the leg (waterproofing method). The activPAL accelerometer will be worn on the individual’s mid-thigh, without removal for the entire 7 days. The activPAL measures postural aspects of time spent sitting/lying down, standing, and walking in hours per day, as well as energy expenditure (MET-hours per day), steps per day, and number of sit-to-stand transitions.

The HI-PACE study will utilize a REDCap database to store all information collected from
screening visits (e.g. contact/demographic information, blood lab results, etc.) and to track physical activity data during the physical activity assessment. During each day the devices are worn, REDCap surveys will be sent automatically to individuals’ email address to ask if the activPAL and Fitbit devices were worn on the previous day and if there were any extended periods of non-wear time. The purpose of the survey is to: 1) increase the accuracy of the physical activity assessment by being able to eliminate days affected by non-wear, and 2) prompt individuals to wear the devices consistently. Since changes in non-exercise physical activity can confound exercise-related changes in outcome measures [43,44], it is necessary to ensure that participants in the HI-PACE study can regularly wear the devices.

Following completion of the baseline physical activity assessment (7 days), individuals will return to the East Carolina Heart Institute for Screening visit 2 in the morning, in the fasted state. The study nurse will perform a fasting blood draw and immediately send the sample to a clinical laboratory (LabCorp Inc., Burlington, NC) for complete metabolic panel, lipid panel, insulin level, and blood chemistries. Pre-menopausal women will also be required to complete a pregnancy test. The Fitbit and activPAL will be retrieved for accelerometer data to be downloaded and recorded in the study database. Upon completion of the screening visits, individuals will be scheduled for the baseline assessment visit.

Assessment visits (baseline, mid-intervention, and follow-up)

A flow chart of the present study is shown in Fig. 1. Primary (i.e. CRF) and secondary outcome measures (i.e. arterial stiffness, mitochondrial measures, insulin sensitivity, skeletal muscle oxidative capacity, quality of life, and food-frequency questionnaires
[FFQ]) will be obtained at baseline and at follow-up (week 24). At mid-intervention (week 12), CRF, resting blood pressures, and anthropometry (i.e. body mass, waist circumference, and BMI) will be re-evaluated. Measurements of arterial stiffness, muscle biopsy, IVGTT, and NIRS will be obtained in this order, during the same visit (baseline and follow-up). Whereas, CRF, anthropometry, and body composition will be obtained during the same visit of a separate week. The primary outcome will be obtained at the Human Performance Laboratory in the Ward Sports Medicine Building, whereas the secondary outcomes will be obtained at the East Carolina Heart Institute. Randomization into a study group will occur upon completion of all baseline assessments. For an overview of the schedule of enrollment, randomization, intervention, and assessments, see Fig. 2 for the completed Standard Protocol Items: Recommendations for Interventional Trials (SPIRITS) figure.

**Primary Outcome – change in maximal oxygen uptake (VO2 max)**

The primary outcome measurement is CRF due to the well-established association between CRF levels and risk of T2D [8,10,11,27]. We will measure CRF as VO2 max (the gold standard) from a maximal exertion treadmill test under supervision of a physician. Maximal exercise testing will be conducted on a treadmill (Cardiac Science TM65, Davis Medical Electronics, Bothell, WA) under a modified Balke protocol. For the warm-up, participants will initially walk at a speed of 2.0 mph at 0% grade for 2 minutes. Subsequently, we will increase treadmill speed to 3.0 mph to begin the treadmill test. During the test, we will increase treadmill grade by 2.5% every 2 minutes until volitional exhaustion. Gas exchange (i.e. VO2, VCO2) and pulmonary ventilation will be measured
continuously using a TrueOne 2400 Metabolic Measurement Cart (Parvo Medics, Salt Lake City, UT). Heart rate, blood pressure, rating of perceived exertion, and electrocardiogram will be monitored and recorded before, during, and after the exercise test. The electrocardiogram will be cleared by the study physician prior to participant randomization. A valid maximal exercise test will meet two of three end criteria: 1) elevated respiratory exchange ratio (RER) (≥1.10); 2) plateauing of VO$_2$; or 3) within ±5 bpm of age-predicted maximal heart rate.

**Secondary outcome measures**

Secondary outcome measures include: change in insulin action, arterial stiffness, mitochondrial function, body fat, C-reactive protein, and psychological surveys.

**Insulin sensitivity**

Insulin sensitivity will be assessed at baseline and follow-up (24 hours following the last exercise session for MOD-INT and HIGH-INT groups) via an intravenous glucose tolerance test (IVGTT). After collection of fasting blood samples, glucose (Dextrose 50%) will be injected into a catheter placed in the antecubital vein, at a dose of 0.3 g/kg body weight. Subsequently, blood samples will be obtained at the following time points: 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 25, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 minutes. Insulin will be injected at minute-20 of the test, at a dose of 0.025 U/kg body weight. Blood samples will be centrifuged and stored at -80°C until sample analysis for glucose and insulin. Insulin sensitivity index will be determined through a minimal model [45]. Follow-up IVGTT will be assessed within 18-24 hours of the MOD-INT and HIGH-INT participants’ last exercise session.

**Arterial stiffness**

Carotid-to-femoral PWV and aortic blood pressure parameters will be measured using a
SphygmoCor XCEL (AtCor Medical, Itasca, IL). Carotid-femoral PWV, an index of the degree of arterial stiffness, is the gold standard measurement of arterial stiffness [46]. Arterial stiffness measurements will occur during the morning in a quiet, temperature-controlled room at baseline and follow-up. Prior to each measure, participants will refrain from vigorous exercise, tobacco, caffeine, and alcohol for at least 12 hours, as well as large meals for at least 6 hours. Participants will take their prescribed medications which will be logged and repeated at follow-up. The methodology for arterial stiffness measurements will adhere to a position stand by the American Heart Association [47].

For aortic blood pressure and stiffness measurements, participants will be in the seated position for a 5-minute rest period. Following rest, aortic blood pressure (e.g. brachial blood pressures, aortic blood pressures, etc.) and stiffness (e.g. augmentation index, wave reflection, etc.) parameters will be obtained on the basis of acquisition of brachial artery pressure waveforms with application of a generalized transfer function to derive the central aortic pressure waveform, from which estimates of aortic blood pressures are generated. Three measurements will be performed for aortic blood pressure parameters with a 1-minute rest period between each measurement.

Subsequently, PWV will be obtained in the supine position following a 15-minute rest. During rest, body surface measurements will be measured via a Gulick tape measure (Baseline, Fabrication Enterprises, White Plains, NY) in triplicate to determine the distance traveled by the pulse wave between the carotid and femoral artery sites. Study staff will palpate and mark the carotid artery pulse (between the larynx and sternocleidomastoid muscle in the neck), the sternal notch (superficial landmark of aortic arch), and femoral artery pulse (over the ventral thigh halfway between the pubic symphysis and anterior superior iliac spine) [48]. The mean distance for each site will be used for PWV calculation. Pressure waveforms at the carotid arterial site will be acquired via
applanation tonometry and electrocardiographic gating, while the femoral arterial site will be simultaneously acquired using the oscillometric device within the SphygmoCor XCEL. The PWV measurement will be conducted in duplicate, and the mean of these measurements will be the reported value. Both measurements must be within 0.5 meters per second to be considered acceptable for data purposes. If the two measurements differ by more than 0.5 meters per second, a third measurement will be obtained, and the reported value will be the median of the three measurements.

Mitochondrial Function

A percutaneous muscle biopsy (100-200 mg of tissue) will be obtained using sterile techniques at baseline and follow-up from the vastus lateralis with a 5-mm Bergström muscle biopsy cannula with suction (Stille Surgical Instruments, Eskilstuna, Sweden), as previously described [49]. Briefly, participants will lie supine with legs extended (0° flexion) and two operators will spray ethyl chloride on the biopsy site and administer 1% lidocaine at each level of subcutaneous tissue, stopping superficial to the fascia. Following 2-3 minutes to allow for the local anesthetic effects, a 1-cm incision will be made through the skin and subcutaneous tissues, parallel to the femur, until an incision is made through the muscle fascia. The operator will utilize the biopsy cannula to locate the fascia incision site and advance the needle past the fascia, angled downward towards the floor to rapidly clip and collect the muscle sample with suction by the second operator. Following the biopsy, the sample will be trimmed of visible adipose tissue, weighed on a scale (AL54, Mettler Toledo, Columbus, OH), and snap-frozen in liquid nitrogen to be stored at -80°C until analysis at study completion.

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α), COX IV, GLUT-4, and CPT-1 content will be determined; these proteins were selected as they are
downstream of PGC1-α and represent distinct steps in oxidative metabolism [50]. Approximately 50 mg of muscle tissue will be homogenized (T8 Ultra Turrax; IKA, Wilmington, NC) in 20 volumes of cell lysis buffer (50 mM HEPES, 12 mM sodium pyrophosphate, 100 mM sodium fluoride, 100 mM ethylenediaminetetraacetic acid, 10 mM sodium orthovanate, 1% Triton X-100) supplemented with a protease and phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO). Lysates will be sonicated for 5 seconds, rotated for ~1 hour at 4°C, and centrifuged at 13,500 x g for 15 minutes at 4°C. Protein concentration for each sample homogenate will be determined via a commercially available bicinchoninic acid protein assay kit (Pierce, Rockford, IL). Aliquots containing 30 µg of total protein will be diluted in 4x Laemmli Buffer (BioRad, Hercules, CA) with 5% β-mercaptoethanol (βME) at a 3:1 ratio, prior to heating at 70°C for 10 minutes. Denatured samples will be brought to room temperature, loaded onto a 10% polyacrylamide gel, separated by SDS-PAGE, and transferred to nitrocellulose membranes. Membranes will be blocked with Odyssey Blocking Buffer (OBB; Li-Cor, Lincoln, NE) for 1 hour and incubated with primary antibodies. Membranes will be washed with TBST and incubated with an anti-rabbit or anti-mouse fluorophore-conjugated secondary antibody (1:20,000, Li-Cor) in OBB supplemented with 0.1% Tween-20 for 1 hour. The membranes will then be washed with TBST followed by TBS prior to being scanned on the Odyssey CLx Imaging System (Li-Cor) and quantified on Image Studio software (V4.0.21; Li-Cor). GAPDH will be used as a loading control.

Citrate synthase activity will be determined with a colorimetric reaction using reagents in a commercial kit (Sigma CD0720, St. Louis, MO), as in a previous study [51]. A 10- to 15-mg piece of muscle will be diluted 20-fold in a buffer containing 100 mM KH₂PO₄ and 0.05% bovine serum albumin and homogenized at 4°C using the Ultra Turrax. Homogenates will undergo four freeze-thaw cycles before experimentation. Protein
content will be measured using the bicinchoninic acid assay and citrate synthase activity will be assessed with reagents provided in the commercial kit (Sigma CS0720), which uses a colorimetric reaction to measure the reaction rate of acetyl coenzyme A and oxaloacetic acid.

In vivo skeletal muscle mitochondrial oxidative capacity

As an additional measure of mitochondrial function, in vivo skeletal muscle mitochondrial oxidative capacity will be measured non-invasively via near-infrared spectroscopy (NIRS) at baseline and follow-up. This NIRS approach measures the recovery kinetics of skeletal muscle oxygen consumption (mVO$_2$) following brief exercise and has demonstrated strong correlations with current in vivo and ex vivo gold standard measurements of mitochondrial function (i.e. magnetic resonance spectroscopy and muscle biopsy) [52,53]. We will implement a NIRS testing protocol similar to Ryan et al. [53]. NIRS data will be obtained using an OxiplexTS (ISS, Champaign, IL), a frequency-domain tissue oximeter. Briefly, the OxiplexTS is equipped with 2 independent data acquisition channels, with 8 infrared diode lasers (four emitting at 691 nm and four at 830 nm) and a detector within each (emitter-detector distances 2.0-4.0 cm). The absolute values of oxygenated hemoglobin (O$_2$Hb) and deoxygenated hemoglobin (HHb) will be calculated in micromoles according to manufacturer instructions. Data will be collected at 4 Hz. Both NIRS probes will be calibrated prior to each test using a phantom with known optical properties, once the device warms-up for at least 20 minutes.

For each NIRS measurement, participants will be supine on a padded table with both legs extended (0° flexion). A skinfold caliper (Lange, Beta Technology, Santa Cruz, CA) will be used to measure subcutaneous adipose tissue thickness at the probe site (~10 cm above
the patella). The NIRS probe will be secured to the skin at the vastus lateralis site with double-sided adhesive tape and Velcro straps. Additionally, a blood pressure cuff (Hokanson SC-10D or SC-10L, D.E. Hokanson, Inc., Bellevue, WA) will be placed proximal to the NIRS probe, as high as anatomically possible to prevent unwanted signal noise from cuff inflation. A 15-gallon air compressor (Model D55168, Dewalt, Baltimore, MD) set to 30 psi will power a rapid-inflation system (Hokanson E20, D.E. Hokanson) to control the blood pressure cuff.

Upon securement of the probe and cuff, participants will complete a short-duration (~10-30 seconds), submaximal, repeated knee extension and/or isometric quadricep exercise to increase mVO$_2$. Following exercise, the recovery kinetics of mVO$_2$ will be determined from a series of repeated arterial occlusions (275-300 mmHg) for ~5-7 minutes in duration using the following inflation/deflation timing: 5 seconds inflated/5 seconds deflated for ~90 seconds, then 10 seconds inflated/10 seconds deflated for remainder of test. The beginning and end of each occlusion will be marked for calculations of mVO$_2$ from the deoxygenated hemoglobin/myoglobin signal (i.e. slope during occlusion). The post-exercise mVO$_2$ data will then be fit to a mono-exponential function to calculate the rate constant, which is directly related to the mitochondrial respiratory capacity [52]. Three trials of exercise and occlusion procedures will be performed, and results will be averaged. Baseline and follow-up NIRS data will be analyzed via custom-written routines in MATLAB R2017b (MathWorks, Natick, MA).

**Blood sample collection**

A venous blood sample will be drawn with a 21-gauge needle with the participant in the fasted state at baseline and follow-up. A total of 21 mL of blood will be drawn by the study nurse and will immediately be sent to a clinical laboratory (LabCorp Inc., Burlington, NC).
for a complete metabolic panel, lipid panel, insulin level, and blood chemistries. Prior to glucose injection during the baseline and follow-up IVGTT, we will collect vials of archive plasma, serum, and red blood cells to be stored at -80°C for future analysis. We also will send an additional serum separator tube to LabCorp for measurement of C-reactive protein.

**Anthropometry and body composition**

Body weight will be measured in the fasted state via a calibrated scale (DigiTol 8510, Mettler Toledo, Columbus, OH) (recorded to the nearest 1/10 of a kg). Dual Energy X-ray Absorptiometry (DEXA) (GE Lunar Prodigy Advance, Fairfield, CT) will be used to measure body composition (fat and fat-free mass) at baseline and follow-up. Waist circumference will be measured via a Gulick tape measure at the natural waist (halfway point from the inferior border of the rib cage and the superior point of the iliac crest). Participants will be instructed to stand straight and upright, with their feet together, and arms to their side. Study staff will mark each landmark and measure the distance to determine the proper measurement site. For each measure, study staff will confirm: 1) the tape is parallel to the floor; 2) the tape touches the entire circumference of the participant; 3) the tape is not compressing any abdominal tissue; 4) the tape is not within abdominal folds; and 5) the measurement is recorded following a normal exhalation by the participant. Duplicate waist circumference measurements will be obtained. If measurements are ±0.5 cm, the reported value will be the average of the two. If measurements differ >0.5 cm, a third measurement will be assessed, and the reported value will be the average of the three measurements. Waist circumference will be evaluated at baseline, mid-intervention, and follow-up.
Non-exercise physical activity levels

Non-exercise physical activity data will be monitored in all randomization groups using a Fitbit Flex activity tracker throughout the intervention period. Each group will be blinded to the number of steps accrued and instructed to not change their non-exercise physical activity levels from baseline. Prior to each exercise session, the Fitbit device will be removed from the participant (to not mix exercise and non-exercise physical activity data) and synced to the Fitbit software to upload their non-exercise physical activity levels. Participants in the CON group will sync their data at home using the Fitbit software and be monitored by study staff to assure compliance. Automated REDCap surveys will be emailed 3 times per week to all participants to inquire about Fitbit wear. Participants will be instructed to fill-out these surveys to validate consistent device wear as this will allow staff to input non-exercise physical activity data on a weekly basis. This process helps to ensure consistent daily wearing of the device and to determine if the participant did not wear the Fitbit for extended periods of time.

Study staff will use a database program (Fitabase, Small Steps Labs, San Diego, CA) to centralize all non-exercise physical activity data (i.e. total daily steps, minutes of light, moderate and vigorous physical activity, miles traveled, and estimated kilocalorie energy expenditure). All non-exercise physical activity data synced to Fitabase will be stored in a custom-made REDCap database. Study staff will compile and inp daily steps, intensity, miles, and energy expenditure from each participant on a weekly basis.

Dietary composition

Dietary intake will be tracked at baseline and follow-up via the Block food frequency questionnaire (FFQ) [54]. The FFQ consists of 105 categorized items and assesses both
frequency of consumption and portion size selections, in which participants will recall their typical eating habits within the previous 3 months at baseline and follow-up timepoints. The questionnaire estimates daily intake values of kilocalories, select macronutrients and micronutrients, and also calculates servings by food group. The research coordinator will instruct participants at screening visit 1 to maintain current dietary habits and not to begin intentional dieting for the entirety of the study. Additionally, study staff will remind all participants on a weekly basis to not change their eating habits during the intervention. The FFQ serves as a semi-quantitative measure to ensure dietary habits are not changed throughout the intervention.

Psychological parameters

The short form health survey (SF-36) [55] will be utilized to measure quality of life at both baseline and follow-up. Exercise enjoyment will be assessed via the Physical Activity Enjoyment (PACE) Scale [56] and the Feeling Scale [57]. As secondary outcomes, the impact of exercise intensity on these affective responses (i.e. feelings of overall pleasure/displeasure and enjoyment) play an important role in physical activity participation and adherence [58–60]. The PACE Scale is comprised of 8 items rated on a 7-point semantic differential scale with “4” representing a neutral position. The PACE Scale will be collected every 4 weeks during the exercise intervention. Affective responses to exercise will be assessed by having participants complete the Feeling Scale. The Feeling Scale is a single-item, 11-point scale which assesses how individuals feel at a specific moment in time, ranging from -5 (very bad) to +5 (very good) with 0 representing neutral feelings. Study staff will collect Feeling Scale data every 5 minutes during the first exercise session of every week of the exercise intervention.

Randomization
Participants will be randomized to either the non-exercise control (CON), moderate-intensity (MOD-INT), or high-intensity (HIGH-INT) group upon completion of all baseline assessments and approval by the study physician. The study biostatistician will generate a randomization list to allocate participants in a 1:1:1 ratio to study groups. The randomization process will be performed by an individual separate from the research team, who has no interaction with the study participants or access to HI-PACE study data. All other research staff will not have access to the randomization list (including the principal investigator). Once a participant has completed all baseline assessments, study staff will email the participant’s identification number and gender to the individual. The participant will be assigned to the next group on the randomization list. Upon randomization, the intervention period will begin the following week. A study flow chart is shown in Fig. 1.

**Aerobic exercise training**

All exercise sessions will be supervised by study staff and performed on a treadmill (Precor TRM 885, Precor Inc., Woodinville, WA) to sustain control of energy expenditure from exercise. Participants in the MOD-INT group will exercise at a target heart rate associated with 45-55% VO$_2$ max, and participants in the HIGH-INT group will exercise at a target heart rate associated with 70-80% VO$_2$ max. The heart rate range for each participant will be determined based on the maximal exercise test (baseline and mid-intervention). The full exercise dose for both groups will be 600 MET-minutes per week, which is consistent with current public health guidelines [5]. Since participants will be sedentary at baseline, we will increase exercise dose incrementally throughout the study to avoid potential adverse events during exercise. Initially, the exercise dose will be 300 MET-minutes during week 1 and will increase by 50 MET-minutes per week until the
maximum exercise volume of 600 MET-minutes is reached at week 9. The exercise dose will remain at 600 MET-minutes until conclusion of the intervention (see Fig. 3). We will calculate the number of MET-minutes exercised based on treadmill speed/grade and the participants’ weight, using the standard American College of Sports Medicine (ACSM) walking equation [61]. Custom-made Excel spreadsheets will be utilized to determine exercise time for each session, based on 1) the required weekly MET-minutes, 2) the participants’ weight, 3) exercise speed/grade, and 4) the amount of expected sessions per week (3-4 sessions per week).

At the first exercise session of each week, study staff will weigh participants (without shoes) on a calibrated scale and remind participants to not alter their diet or engage in an exercise program outside of the study. Additionally, we will ask participants about any changes to their prescribed medications on a weekly basis.

Prior to starting exercise, participants will rest for 5 minutes in the seated position, after which study staff will measure systolic/diastolic blood pressures using a mercury sphygmomanometer and record resting heart rate via a Zephyr Bioharness 3 monitor (Medtronic, Annapolis, MD). Each participant will be instructed to complete a 5-minute warm-up on the treadmill at a low speed (~2.0 miles per hour) at 0% grade. Following completion of the warm-up, participants will begin their prescribed exercise by adjusting speed and/or grade of the treadmill. During the supervised exercise, heart rate will be monitored continuously using the Bioharness monitor to confirm exercise intensity and participants will be required to remain within their target heart range (MOD-INT: heart rate associated with 45-55% VO₂ max; HIGH-INT: heart rate associated with 70-80% VO₂ max). Heart rate will be recorded every 5 minutes, along with participants’ subjective rating of perceived exertion (RPE) via the Borg scale [62]. The Feeling Scale will also be recorded every 5 minutes on the first exercise session of each week. Study staff will keep
mobile laptop carts nearby the exercising participant(s) and will use custom-made Excel spreadsheets to: 1) quantify the number of MET-minutes accumulated during exercise and number of MET-minutes remaining in the session, 2) mean heart rate and RPE of the session, and 3) calculate amount of time remaining in the current session. The spreadsheet calculates these variables in real-time and can compensate for potential adjustments during the exercise session, such as increasing or decreasing treadmill speed and/or grade.

Once completed with the exercise session, participants will perform a 5-minute cool-down at a similar intensity as the warm-up. Subsequently, participants will rest for 5 minutes in the seated position for recording of post-exercise heart rate and systolic/diastolic blood pressures. Lastly, study staff will input all exercise session data (i.e. total exercise duration, MET-minutes, caloric expenditure, miles traveled, average speed, grade, heart rate, and RPE, and percentage of time participant exercised within target heart rate range) into the database.

For each exercise session the mean heart rate will be calculated using Omnisense Analysis version 5.0 software (Medtronic, Annapolis, MD). The Bioharness monitors continuously record heart rate data on a second-by-second basis. Thus, to collect mean heart rate for each session, study staff will analyze heart rate data only during exercise by creating a time-specific sub-session annotation within the Omnisense software to exclude non-exercise heart rate data from calculation. This process will more accurately calculate exercise intensities during training sessions since heart rate will be measured continuously as opposed to intervals (e.g. every 5 minutes).

Exercise economy

To address potential variability in exercise economy at a given workload, study staff will
directly measure energy expenditure (EE) rate via indirect calorimetry (TrueOne 2400) at
the participant’s prescribed exercise speed and grade on a treadmill [42,63,64]. This
exercise economy test will be performed on week-1, -3, -5, and then monthly until the
conclusion of the intervention. The rate of EE determined through indirect calorimetry will
be divided by estimated EE determined from the ACSM walking equation to develop a
correction factor (i.e. actual EE rate/predicted EE rate). This correction factor will be used
to: 1) adjust the EE calculated from the ACSM equation to more accurately implement the
exercise prescription which corresponds to 600 MET-minutes per week, 2) adjust
participants’ exercise session time according to potential changes in metabolic and/or
biomechanical efficiency from exercise training, and (3) verify required MET-minutes
exercised by increasing or decreasing exercise session time accordingly.

Training data management

Exercise volume adherence will be defined as MET-minutes exercised divided by required
MET-minutes. Exercise intensity adherence will be quantified as time within the required
target heart rate range divided by total exercise time. Exercise compliance will be defined
as the amount of sessions attended divided by the amount of sessions required. The
research team will actively monitor exercise volume/intensity adherence, compliance, and
other indicators of intervention fidelity (e.g. target heart rate compliance, wear rate of
accelerometer, participant morale, progression rate of speed/grade) on a weekly basis in
study meetings. In all randomization groups, Fitbit wear compliance will be monitored
throughout the 6-month intervention. Weekly reports will be compiled from the study
databases to monitor and review the compliance and adherence rates of all participants.

Statistical considerations

The results of the current pilot study will be used to advise the design (effect
size/statistical power) of a larger prospective intervention. The response variable for the primary outcome is change in VO\textsubscript{2} max. The three treatment groups, CON, MOD-INT, and HIGH-INT will be compared in terms of baseline VO\textsubscript{2} max using side-by-side boxplots and the corresponding numeric summaries along with mean and standard deviation. This will be repeated for post treatment values of VO\textsubscript{2} max as well as for VO\textsubscript{2} max differences of post-treatment and baseline. If there are distributional concerns, log transformation of VO\textsubscript{2} max will be considered. Unless there are extreme outliers or severe heteroscedasticity, one-way ANOVA will be used for inference regarding the primary outcome. The two sample t-test (without assuming equal variances) along with the associated confidence intervals will be used for differences in group means; confidence intervals for differences in group means obtained from the one-way ANOVA will also be reported.

The above steps used for VO\textsubscript{2} max (the primary outcome) will be repeated for each of the numeric variables used for secondary outcomes. These variables are change in insulin sensitivity, mitochondrial protein content, citrate synthase activity, skeletal muscle mitochondrial oxidative capacity, arterial stiffness parameters, body fat %, and C-reactive protein. Data will be analyzed on an intention to treat basis.

The ordinal variables, exercise enjoyment and quality of life, will be dichotomized into “no improvement” and “improvement.” Fisher’s exact test will be used to obtain an overall p-value and restriction to two of the treatment groups will provide estimated odds ratios and the associated confidence intervals.

The three groups will be compared using the following demographic and other variables that may be related to exercise, change in VO\textsubscript{2} max, or one of the other response variables: age, sex, body weight, BMI, waist circumference, body fat %, fat mass, fat-free
mass, cholesterol, triglycerides, and blood pressures. If differences among the treatment groups in terms of one or more of these variables are deemed important, adjustments to the above comparisons will be made using higher order ANOVA, ANCOVA, and/or linear regression.

Power ranges from .85 for 15 participants per group to .96 for 20 participants per group using -.112, .124, and .200 L/min as the means for the three groups based on previous data [66], a common standard deviation of .250 L/min, and significance level = .05. We expect attrition to be approximately 10-15% so that enrollment of 60 participants (20 per group) will be sufficient for the primary outcome.

All statistical analyses will be performed using statistical software in R version 3.5.1. [65]. The resultant mean change and standard deviation of the change of outcome measures (if indicative of enhance cardiometabolic improvements in the HIGH-INT compared to the MOD-INT and CON groups) will be used for power calculations to determine the necessary sample size of a larger study.

Discussion

The HI-PACE study has high public health relevance due to the increased disease burden of T2D and the lack of exercise training studies in AAs. HI-PACE will be the first study to compare two exercise training programs on multiple T2D and cardiovascular risk factors in overweight and obese AAs. HI-PACE has the potential to influence future physical activity recommendations and advance health disparity research. Additionally, valuable psychological parameters of quality of life and enjoyment of exercise will be obtained, which will help determine whether high-intensity aerobic exercise is a feasible strategy over a six-month period to improve health outcomes in AAs. We anticipate the results of the HI-PACE study will provide clear evidence of health benefits from high-intensity exercise, successful recruitment tactics, and favorable exercise adherence data in at-risk
AAs. The pilot data will be necessary to conduct a larger sample sized exercise intensity study in AAs with adequately powered primary and secondary variables.

**Trial status**

Participant recruitment for this study initiated in November 2016 and is ongoing. The study recruitment is expected to complete October 2019.

**Abbreviations**

AA, African American; ADA, American Diabetes Association; BMI, body mass index; CA, Caucasian American; CRF, cardiorespiratory fitness; DEXA, dual energy X-ray absorptiometry; EE, energy expenditure; FFQ, food frequency questionnaire; HHb, deoxygenated hemoglobin; IVGTT, intravenous glucose tolerance test; MET, metabolic equivalent; mVO₂, skeletal muscle oxygen consumption; NIRS, near infrared spectroscopy; O₂Hb, oxygenated hemoglobin; PACE, physical activity enjoyment scale; PWV, pulse wave velocity; RER, respiratory exchange ratio; RPE, rating of perceived exertion; SF-36, short form health survey; T2D, type 2 diabetes; VO₂ max, maximal oxygen consumption.

**Declarations**

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**Ethics approval and consent to participate**

Written consent will be obtained from every participant. The current study was performed in accordance with the Declaration of Helsinki. The East Carolina University & Medical
Center Institutional Review Board approved the study protocol.

Consent for publication
Not applicable.

Availability of data and material
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Authors’ contributions
JEM was responsible for recruitment, data collection and analysis, and manuscript writing. SGB, NRG, and PMB were responsible for recruitment, management of the study, data collection and analysis, and critical revision of the manuscript. AC and GSD were responsible for data collection and critical revision of the manuscript. TER and JAH were responsible for design of the study, data collection and analysis, and critical revision of the manuscript. PV and DLS calculated the power, the sample size, developed the statistical analysis plan for outcomes, and will conduct final analyses. TDR was responsible for design of the psychological aspects of the study and critical revision of the manuscript. DLS conceived, designed, and managed the study, and was responsible for recruitment, data collection and analysis, and manuscript writing. All of the authors reviewed and approved the final manuscript.
References

1. American Diabetes Association. 2018 State and Federal Legislative & Regulatory Priorities: Diabetes Research and Programs. 2018.

2. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, et al. Heart Disease and Stroke Statistics—2018 Update: A Report From the American Heart Association. Circulation [Internet]. 2018;137:e67–492. Available from: http://circ.ahajournals.org/content/circulationaha/137/12/e67.full.pdf

3. Colberg SR, Sigal RJ, Yardley JE, Riddell MC, Dunstan DW, Dempsey PC, et al. Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. Diabetes Care. 2016;39:2065–79.

4. CDC. Prevalence of Self-reported physically active adults--United States. MMWR. 2007;57:1297–300.

5. Physical Activity Guidelines Advisory Committee Department of Health and Human Services. Physical Activity Guidelines Advisory Committee Report. Washington, DC; 2008.

6. Wei M, Gibbons LW, Mitchell TL, Kampert JB, Lee CD, Blair SN. The Association between Cardiorespiratory Fitness and Impaired Fasting Glucose and Type 2 Diabetes Mellitus in Men. Ann Intern Med [Internet]. 1999;130:89–96. Available from: http://dx.doi.org/10.7326/0003-4819-130-2-199901190-00002

7. Boulé NG, Kenny GP, Haddad E, Wells GA, Sigal RJ. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in Type 2 diabetes mellitus. Diabetologia [Internet]. Springer-Verlag; 2003;46:1071–81. Available from: http://dx.doi.org/10.1007/s00125-003-1160-2

8. Sui X, Hooker SP, Lee I-M, Church TS, Colabianchi N, Lee C-D, et al. A Prospective Study of Cardiorespiratory Fitness and Risk of Type 2 Diabetes in Women. Diabetes Care [Internet]. 2008;31:550-5. Available from:
9. Sawada SS, Lee I-M, Naito H, Noguchi J, Tsukamoto K, Muto T, et al. Long-Term Trends in Cardiorespiratory Fitness and the Incidence of Type 2 Diabetes. Diabetes Care [Internet]. 2010;33:1353–7. Available from: http://care.diabetesjournals.org/content/33/6/1353.abstract

10. Carnethon MR, Sternfeld B, Schreiner PJ, Jacobs DR, Lewis CE, Liu K, et al. Association of 20-Year Changes in Cardiorespiratory Fitness With Incident Type 2 Diabetes. Diabetes Care [Internet]. 2009;32:1284–8. Available from: http://care.diabetesjournals.org/content/32/7/1284.abstract

11. Lynch J, Helmrich SP, Lakka TA, Kaplan GA, Cohen RD, Salonen R, et al. Moderately Intense Physical Activities and High Levels of Cardiorespiratory Fitness Reduce the Risk of Non-Insulin-Dependent Diabetes Mellitus in Middle-aged Men. Arch Intern Med [Internet]. 1996;156:1307–14. Available from: http://dx.doi.org/10.1001/archinte.1996.00440110073010

12. Wei M. Relationship Between Low Cardiorespiratory Fitness and Mortality in Normal-Weight, Overweight, and Obese Men. JAMA [Internet]. 1999;282:1547. Available from: http://dx.doi.org/10.1001/jama.282.16.1547

13. Swift DL, Staiano AE, Johannsen NM, Lavie CJ, Earnest CP, Katzmarzyk PT, et al. Low Cardiorespiratory Fitness in African Americans: A Health Disparity Risk Factor? Sport Med [Internet]. Springer International Publishing; 2013;43:1301–13. Available from: http://dx.doi.org/10.1007/s40279-013-0092-3

14. Wang C-Y, Haskell WL, Farrell SW, LaMonte MJ, Blair SN, Curtin LR, et al. Cardiorespiratory Fitness Levels Among US Adults 20–49 Years of Age: Findings From the 1999-2004 National Health and Nutrition Examination Survey. Am J Epidemiol [Internet]. 2010;171:426–35. Available from: http://aje.oxfordjournals.org/content/171/4/426.abstract
15. Ceaser TG, Fitzhugh EC, Thompson DL, Bassett DRJ. Association of Physical Activity, Fitness, and Race: NHANES 1999–2004. Med Sci Sports Exerc [Internet]. 2013;45:286–93. Available from: http://journals.lww.com/acsm-msse/Fulltext/2013/02000/Association_of_Physical_Activity,_Fitness,_and.10.aspx

16. Duncan GE, Li SM, Zhou XH. Cardiovascular fitness among U.S. adults: NHANES 1999-2000 and 2001-2002. Med Sci Sports Exerc. 2005/08/25. 2005;37:1324–8.

17. Kokkinos P, Myers J, Kokkinos JP, Pittaras A, Narayan P, Manolis A, et al. Exercise Capacity and Mortality in Black and White Men. Circulation [Internet]. 2008;117:614–22. Available from: http://circ.ahajournals.org/content/117/5/614.abstract

18. Kokkinos P, Myers J, Nylen E, Panagiotakos DB, Manolis A, Pittaras A, et al. Exercise Capacity and All-Cause Mortality in African American and Caucasian Men With Type 2 Diabetes. Diabetes Care [Internet]. 2009;32:623–8. Available from: http://care.diabetesjournals.org/content/32/4/623.abstract

19. Ribisl PM, Lang W, Jaramillo SA, Jakicic JM, Stewart KJ, Bahnson J, et al. Exercise Capacity and Cardiovascular/Metabolic Characteristics of Overweight and Obese Individuals With Type 2 Diabetes. Diabetes Care [Internet]. 2007;30:2679–84. Available from: http://care.diabetesjournals.org/content/30/10/2679.abstract

20. Sidney S, Haskell WL, Crow R, Sternfeld B, Oberman A, Armstrong MA, et al. Symptom-limited graded treadmill exercise testing in young adults in the CARDIA study. Med Sci Sports Exerc [Internet]. 1992;24:176–83. Available from: http://journals.lww.com/acsm-msse/Fulltext/1992/02000/Symptom_limited_graded_treadmill_exercise_testing.4.aspx

21. Zeno SA, Kim-Dorner SJ, Deuster PA, Davis JL, Remaley AT, Poth M. Cardiovascular fitness and risk factors of healthy African Americans and Caucasians. J Natl Med Assoc [Internet]. 2010/02/18. 2010;102:28–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20158133
22. Hunter GR, Chandler-Laney PC, Brock DW, Lara-Castro C, Fernandez JR, Gower BA. Fat Distribution, Aerobic Fitness, Blood Lipids, and Insulin Sensitivity in African-American and European-American Women. Obesity [Internet]. Blackwell Publishing Ltd; 2010;18:274–81. Available from: http://dx.doi.org/10.1038/oby.2009.229

23. Hunter GR, Weinsier RL, Darnell BE, Zuckerman PA, Goran Ml. Racial differences in energy expenditure and aerobic fitness in premenopausal women. Am J Clin Nutr [Internet]. 2000;71:500–6. Available from: http://www.ajcn.org/content/71/2/500.abstract

24. Hunter GR, Weinsier RL, Mccarthy JP, Enette Larson-Meyer D, Newcomer BR. Hemoglobin, muscle oxidative capacity, and VO2 max in African-American and Caucasian women. Med Sci Sports Exerc. 2001;33:1739–43.

25. Swift DL, Johannsen NN, Lavie CJ, Earnest CP, Johnson WD, Blair SN, et al. Racial Differences in the Response of Cardiorespiratory Fitness to Aerobic Exercise Training in Caucasian and African American Postmenopausal Women. J Appl Physiol. 2013/03/09. 2013;114:1375–82.

26. Swift DL, Johannsen NM, Earnest CP, Newton RL, McGee JE, Church TS. Cardiorespiratory Fitness and Exercise Training in African Americans. Prog Cardiovasc Dis [Internet]. Elsevier Inc.; 2017;60:96–102. Available from: http://dx.doi.org/10.1016/j.pcad.2017.06.001

27. Sawada SS, Lee I-M, Muto T, Matuszaki K, Blair SN. Cardiorespiratory Fitness and the Incidence of Type 2 Diabetes: Prospective study of Japanese men. Diabetes Care [Internet]. 2003;26:2918–22. Available from: http://care.diabetesjournals.org/content/26/10/2918.abstract

28. Ashor AW, Lara J, Siervo M, Celis-morales C, Mathers JC. Effects of Exercise Modalities on Arterial Stiffness and Wave Reflection: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. PLoS One. 2014;9:1-15.
29. Seo J-B, Chung W-Y, Kim S-H, Kim M-A, Zo J-H. Immediate impact of exercise on arterial stiffness in humans. World J Cardiovasc Dis [Internet]. 2013;3:40-5. Available from: http://www.scirp.org/journal/PaperDownload.aspx?DOI=10.4236/wjcd.2013.31009

30. Kang S-J, Kim E-H, Ko K-J. Effects of aerobic exercise on the resting heart rate, physical fitness, and arterial stiffness of female patients with metabolic syndrome. Phys Ther Sci. 2016;28:1764-8.

31. Ceaser T, Hunter G. Black and White Race Differences in Aerobic Capacity, Muscle Fiber Type, and Their Influence on Metabolic Processes. Sport Med [Internet]. Springer International Publishing; 2015;45:615-23. Available from: http://dx.doi.org/10.1007/s40279-015-0318-7

32. Suminski RR, Mattern CO, Devor ST. Influence of Racial Origin and Skeletal Muscle Properties on Disease Prevalence and Physical Performance. Sport Med [Internet]. ADIS International Limited; 2002;32:667-73. Available from: http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=7241032&site=ehost-live

33. Staiano AE, Harrington DM, Johannsen NM, Newton RL, Sarzynski MA, Swift DL, et al. Uncovering physiological mechanisms for health disparities in type 2 diabetes. Ethn Dis [Internet]. 2015;25:31-7. Available from: http://europepmc.org/abstract/MED/25812249

34. Tanner CJ, Barakat HA, Dohm GL, Pories WJ, MacDonald KG, Cunningham PRG, et al. Muscle fiber type is associated with obesity and weight loss. Am J Physiol - Endocrinol Metab [Internet]. 2002;282:E1191-6. Available from: http://ajpendo.physiology.org/content/282/6/E1191.abstract

35. Ama PF, Simoneau JA, Boulay MR, Serresse O, Theriault G, Bouchard C. Skeletal muscle characteristics in sedentary black and Caucasian males. J Appl Physiol [Internet]. 1986;61:1758-61. Available from: http://jap.physiology.org/content/61/5/1758.abstract
36. Wang Y, Simar D, Fiatarone Singh MA. Adaptations to exercise training within skeletal muscle in adults with type 2 diabetes or impaired glucose tolerance: a systematic review. Diabetes Metab Res Rev. 2009/01/15. 2009;25:13–40.

37. Zorzano A, Palacin M, Guma A. Mechanisms regulating GLUT4 glucose transporter expression and glucose transport in skeletal muscle. Acta Physiol Scand. 2005/01/19. 2005;183:43–58.

38. Swain DP, Franklin BA. Comparison of Cardioprotective Benefits of Vigorous Versus Moderate Intensity Aerobic Exercise. Am J Cardiol [Internet]. 2006;97:141–7. Available from: http://www.sciencedirect.com/science/article/pii/S0002914905016991

39. Roberts CK, Little JP, Thyfault JP. Modification of insulin sensitivity and glycemic control by activity and exercise. Med Sci Sports Exerc. 2013;45:1868–77.

40. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap) – A metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform [Internet]. 2009;42:377–81. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2700030/

41. Swift DL, Dover SE, Nevels TR, Solar CA, Brophy PM, Hall TR, et al. The intervention composed of aerobic training and non-exercise physical activity (I-CAN) study: Rationale, design and methods. Contemp Clin Trials [Internet]. Elsevier Inc.; 2015;45:435–42. Available from: http://www.sciencedirect.com/science/article/pii/S1551714415301166

42. Myers CA, Johnson WD, Earnest CP, Rood JC, Tudor-Locke C, Johannsen NM, et al. Examination of mechanisms (E-MECHANIC) of exercise-induced weight compensation: Study protocol for a randomized controlled trial. Trials. 2014/06/08. 2014;15:1–12.

43. Swift DL, Johannsen NM, Tudor-Locke C, Earnest CP, Johnson WD, Blair SN, et al. Exercise Training and Habitual Physical Activity: A Randomized Controlled Trial. Am J Prev Med [Internet]. 2012;43:629–35. Available from:
44. Kozey-Keadle S, Libertine A, Lyden K, Staudenmayer J, Freedson PS. Validation of Wearable Monitors for Assessing Sedentary Behavior. Med Sci Sport Exerc. 2011;43:1561-7.

45. Bergman RN, Finegood DT, Ader M. Assessment of insulin sensitivity in vivo. Endocr Rev. 1985;6:45-86.

46. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. J Hypertens. 2012;30:445-8.

47. Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, et al. Recommendations for Improving and Standardizing Vascular Research on Arterial Stiffness: A Scientific Statement from the American Heart Association. Hypertension [Internet]. 2015;66:698-722. Available from: https://sslgate.uni-luebeck.de/pmc/articles/PMC4587661/, DanaInfo=www.ncbi.nlm.nih.gov+

48. David M, Malti O, AlGhatrif M, Wright J, Canepa M, Strait JB. Pulse Wave Velocity Testing in the Baltimore Longitudinal Study of Aging. J Vis Exp. 2014;84:1-6.

49. Shanely RA, Zwetsloot KA, Triplett NT, Meaney MP, Farris GE, Nieman DC. Human Skeletal Muscle Biopsy Procedures Using the Modified Bergström Technique. J Vis Exp [Internet]. 2014;91:1-8. Available from: http://www.jove.com/video/51812/human-skeletal-muscle-biopsy-procedures-using-modified-bergstrom

50. Wen X, Wu J, Chang JS. Effect of exercise intensity on isoform-specific expressions of NT-PGC-1 alpha mRNA in mouse skeletal muscle. 2014;1-11.

51. Battaglia GM, Zheng D, Hickner RC, Houmard JA. Effect of exercise training on metabolic flexibility in response to a high-fat diet in obese individuals [Internet]. 2012. Available from: http://ajpendo.physiology.org/ajpendo/303/12/E1440.full.pdf
52. Ryan TE, Southern WM, Reynolds MA, McCully KK. A cross-validation of near-infrared spectroscopy measurements of skeletal muscle oxidative capacity with phosphorus magnetic resonance spectroscopy. J Appl Physiol [Internet]. 2013;115:1757–66. Available from: http://jap.physiology.org/cgi/doi/10.1152/japplphysiol.00835.2013

53. Ryan TE, Brophy P, Lin C-T, Hickner RC, Neufer PD. Assessment of in vivo skeletal muscle mitochondrial respiratory capacity in humans by near-infrared spectroscopy: a comparison with in situ measurements. J Physiol [Internet]. 2014;592:3231–41. Available from: http://doi.wiley.com/10.1113/jphysiol.2014.274456

54. Block G, Thompson FE, Hartman AM, Larkin FA, Guire KE. Comparison of two dietary questionnaires validated against multiple dietary records collected during a 1-year period. J Am Diet Assoc. 1992;92:686-93.

55. Ware, Jr. JE, Sherbourne CD, Ware Jr. JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care [Internet]. 1992/06/11. Lippincott Williams & Wilkins; 1992;30:473–83. Available from: http://www.jstor.org/stable/3765916

56. Kendzierski D, DeCarlo KJ. Physical Activity Enjoyment Scale: Two Validation Studies. J Sport Exerc Psychol. 1991;13:50–64.

57. Hardy Jack CR. Not What, But How One Feels: The Measurement of Affect During Exercise. 1989;11:304-17.

58. Raedeke TD. The Relationship Between Enjoyment and Affective Responses to Exercise. J Appl Sport Psychol [Internet]. Routledge; 2007;19:105–15. Available from: http://dx.doi.org/10.1080/10413200601113638

59. McArthur LH, Raedeke TD. Race and sex differences in college student physical activity correlates. Am J Heal Behav. 2008/10/11. 2009;33:80–90.

60. Azizan A, Justine M, Kuan CS. Effects of a behavioral program on exercise adherence
and exercise self-efficacy in community-dwelling older persons. Curr Gerontol Geriatr Res. 2013;2013:1–9.

61. American College of Sports Medicine. ACSM’s Guidelines for Exercise Testing and Prescription. 8th ed. Whaley M, editor. Baltimore: Lippincott Williams& Williams; 2010.

62. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exerc. 1982/01/01. 1982;14:377–81.

63. Donnelly JE, Honas JJ, Smith BK, Mayo MS, Gibson CA, Sullivan DK, et al. Aerobic exercise alone results in clinically significant weight loss for men and women: Midwest Exercise Trial-2. Obesity [Internet]. John Wiley & Sons, Inc.; 2013;21:E219–28. Available from: http://dx.doi.org/10.1002/oby.20145

64. Donnelly JE, Hill JO, Jacobsen DJ, Potteiger J, Sullivan DK, Johnson SL, et al. Effects of a 16-month randomized controlled exercise trial on body weight and composition in young, overweight men and women: The midwest exercise trial. Arch Intern Med [Internet]. 2003;163:1343–50. Available from: http://dx.doi.org/10.1001/archinte.163.11.1343

65. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: R Foundation for Statistical Computing; 2018. Available from: http://www.r-project.org/

Tables

Table 1

Major inclusion and exclusion criteria.
**Inclusion criteria**

| Age | 35-65 years |
| --- | --- |
| Sex | Men and women |
| Overweight/obese BMI | 25.0-45.0 kg/m² |
| Physically inactive | Sedentary/low active, not participating in regular aerobic or resistance exercise <20 minutes, ≤2 days/week for last 3 months |
| African American | Self-identify as African American |
| Informed consent | Willingness and capability to provide written consent and to understand the exclusion criteria |

**Exclusion criteria**

| Diabetes | Diagnosed type 1 or type 2 diabetes, or fasting glucose ≥126 mg/dL |
| --- | --- |
| Cardiovascular disease or disorders | Diagnosed congestive heart failure, serious arrhythmias, peripheral vascular disease with intermittent claudication, previous stroke or myocardial infarction |
| Resting blood pressure | Excessively high resting systolic (>180 mmHg) or diastolic (>100 mmHg blood pressure). Participants taking blood pressure medications at time of recruitment are permitted to enroll in the HI-PACE study |
| Blood lipids | Total cholesterol ≥240 mg/dL, low-density lipoprotein cholesterol ≥160 mg/dL, or triglycerides ≥300 mg/dL |
| Other exclusionary medical conditions | Chronic or reoccurring neuromuscular, respiratory, gastrointestinal, neurological, HIV, or psychiatric conditions. Musculoskeletal conditions affecting exercise. Current treatment for mental illness or hospitalization from mental illness within previous 5 years. Autoimmune or collagen vascular diseases. Other medical conditions considered life-threatening or that can be provoked from exercise training |
| Other exclusion criteria | Pregnancy or plans to become pregnant. Currently or plans to engage in weight loss or dieting program. Addition of medication and/or dosage unstable in past 3 months. Previous bariatric surgery or current weight loss medications. Plans to leave the Pitt County area for more than 2 weeks during the next 6 months. Non-compliance in wearing pedometer or demonstration of high risk for non-compliance/drop-out during screening |

**Table 2**

**Detailed summary of data collection at study visits.**
Screening visit and Informed consent

- Informational session about study requirements
- Obtain informed consent
- Verify inclusion criteria (i.e. BMI, blood pressure)
- Physical exam/ review of medications
- Non-exercise physical activity via activPAL
- Exercise calendar and Barriers screening forms
- Complete metabolic panel, lipids, insulin, C-reactive protein, blood chemistries

Baseline

- PWV, muscle biopsy, IVGTT, NIRS
- SF-36 and FFQ
- Body weight, blood pressure, anthropometry, DEXA, maximal exercise test

Randomization

- CON, MOD-INT, or HIGH-INT group

Mid-intervention (12 weeks)

- Waist circumference
- Body weight
- Maximal exercise test

Follow-up (24 weeks)

- Non-exercise physical activity via activPAL
- PWV, muscle biopsy, IVGTT, NIRS
- SF-36 and FFQ
- Complete metabolic panel, lipids, insulin, C-reactive protein, blood chemistries
- Body weight, blood pressure, anthropometry, DEXA, maximal exercise test

Figures
Figure 1

Flow chart of study visits in the HI-PACE study.

| STUDY PERIOD | Timepoint          | Enrollment | Randomization | Post-randomization | Close-out |
|--------------|--------------------|------------|---------------|--------------------|-----------|
| TIMEPOINT    | -t₁                | 0          | t₁            | t₂                 | t₃        |
|  | **ENROLLMENT:**    |            |               |                    |           |
|  | Eligibility screen| X          |               |                    |           |
| Study schedule of enrollment, intervention, and assessments. Alx, augmentation index; CON, non-exercise control group; DEXA, dual-energy x-ray absorptiometry; FFQ, food frequency questionnaire; HIGH-INT, high-intensity exercise group; IVGTT, intravenous glucose tolerance test; MOD-INT, moderate-intensity exercise |  |  |  |  |
group; NIRS, near infrared spectroscopy; PA, physical activity; PWV, pulse wave velocity; SF-36, short-form health survey; t1, baseline; t2, mid-intervention (week 12); t3, follow-up (week 24).

Figure 3

Ramping protocol of required MET-minutes in both the MOD-INT and HIGH-INT groups in the HI-PACE study.

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

Appendices.docx
SPIRIT_Fillable-checklist-15-Aug-2013.doc
Report7.rtf
Report6.rtf