Study protocol and statistical plan:

Study protocol: Effects of Exercise Training Intensity on Fitness and Insulin Sensitivity in African Americans

Clinical Trials: NCT02892331

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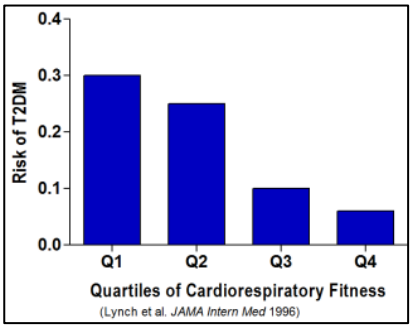
Specific Aims: The American Diabetes Association (ADA) has recognized that the racial health disparities in type 2 diabetes (T2D) represent a major public health concern. African Americans have a substantially greater risk for T2D due to the increased prevalence of risk factors (e.g. low cardiorespiratory fitness [CRF], insulin resistance, and obesity], and are less likely to meet physical activity recommendations compared to their Caucasian counterparts. However, few efficacy-based exercise trials have focused on health outcomes specifically in African Americans. The scientific document from which the Federal Physical Activity Guidelines were derived emphasized **multiple times** that this paucity of data greatly limited the committee's ability to make accurate conclusions regarding the effects of physical activity on health outcomes in African Americans. Thus, randomized controlled trials specifically in African Americans are urgently needed to compare the health benefits of different exercise programs, and identify those which maximize the improvement in T2D risk factors.



Substantial epidemiological evidence exists suggesting that **CRF is an independent risk factor for T2D**. The PI and others have published data demonstrating that CRF is lower in African Americans compared to Caucasians, which likely contributes to the health disparities in T2D. A potential etiology for the racial differences in CRF is that African Americans tend to have a higher percentage of type-II skeletal muscle fibers compared to Caucasians, which are less oxidative and insulin resistant compared to type-I fibers. This disparity is exacerbated in obesity, and racial differences in fiber-type distribution have been suggested to contribute to the lower levels of insulin sensitivity observed in African Americans compared to Caucasians. The PI has published data showing an attenuated response in CRF following **moderate intensity** aerobic training in African Americans compared to Caucasians, which did not resolve by increasing the duration of exercise. **Due to racial differences in fiber-type distribution (promotes low CRF and insulin resistance) and the lower response in CRF in African Americans to moderate intensity exercise training, the evaluation of high intensity aerobic training is warranted**. Studies performed in predominately Caucasian samples suggest that high intensity continuous aerobic training (~75% VO_2 max) promotes a greater increase in CRF and measures of insulin action compared to moderate (~50% VO_2 max) intensity (even when matched for exercise volume). Thus, high intensity aerobic training has the potential to more **readily** resolve the low CRF levels and insulin resistance that predispose African Americans to higher risk for T2D. Further, type-II muscle fibers (more prevalent in African Americans) are recruited to a greater extent (thus greater potential for adaptation) with high intensity exercise compared to moderate. Lastly, due to the higher energy expenditure rate with high intensity exercise, physical activity recommendations can be **achieved in less overall time**.

The **H**igh **I**ntensity exercise to **P**romote **A**ccelerated improvements in **C**ardior**E**spiratory fitness **(HI-PACE)** study will evaluate the effect of exercise intensity on CRF and insulin sensitivity in obese (BMI: 30-45) African Americans (40-65 yrs.) with at least 1 additional T2D risk factor. Participants (n=60) will be recruited in collaboration with the ECU Center for Health Disparities, and subsequently randomized to moderate intensity (MOD-INT, n=20) or high intensity (HIGH-INT, n=20) aerobic exercise training, or to a control group (CON, n=20) for 24 weeks. Supervised exercise training will be performed at a heart rate associated with ~50% and ~75% of VO_2 max in the MOD-INT and the HIGH-INT groups, respectively at the same exercise volume of 600 MET-minutes per week (consistent with public health recommendations). The primary outcome is the change in CRF, which will be assessed at baseline, mid-intervention, and follow-up. Insulin sensitivity will be measured via an intravenous glucose tolerance test at baseline and follow-up. Other secondary measures include mitochondrial oxidative capacity using infrared and measurements on muscle biopsies (PGC-1α and other indices of mitochondrial content), the expression of a protein involved with insulin action (GLUT-4 expression) in skeletal muscle as well as systemic inflammation, adiposity, quality of life and exercise enjoyment measures.

Specific Aims: We seek to provide evidence in obese African American that:

- High intensity aerobic training will lead to greater increase in CRF compared with moderate intensity aerobic exercise
- High intensity aerobic exercise training will lead to greater improvements in insulin sensitivity compared with moderate intensity aerobic exercise

In accordance with the intent of this mechanism, the HI-PACE study will provide **clinically relevant** (CRF/insulin sensitivity) and **mechanistic** data (skeletal muscle), allow us to power outcome variables, and provide recruitment, retention and exercise adherence rates in African Americans for a future R01 proposal.

1. Background and Significance: The American Diabetes Association (ADA) has identified the racial health disparities in type 2 diabetes (T2D) as a major public health concern. African Americans have a disproportionally greater prevalence of T2D (men: 23.4 vs. 15.9 per 1,000 person years; women: 25.1 vs.10.4 per 1,000 person years) compared to their Caucasian counterparts [1]. A recent consensus statement from the ADA [2] recognized the importance of physical activity in the prevention of T2D as low physical activity levels are associated with greater T2D incidence. The majority of African Americans adults in the United States (61.2%) do not meet public health recommendations for physical activity, a rate which is considerably higher than Caucasian (48.9%) adults. Despite the established racial disparities in T2D risk, African Americans are substantially under-represented in exercise research as few randomized controlled trials (RCTs) have a large enough sample size to adequately perform sub-group analyses, and few RCTs have been conducted specifically in African American populations. The scientific report [3] from which the Federal Physical Activity Recommendations were derived emphasized that this **lack of data greatly limited** the committee's ability to make accurate conclusions on the effects of physical activity on health outcomes in African Americans**.**

Low Fitness and T2D Risk in African Americans*:* Low cardiorespiratory fitness (CRF) is an **independent risk factor for T2D incidence** [4-7]. Wei et al. [8] observed a 3.7 fold increased risk of T2D in men with low CRF compared to those with high CRF levels. Sui et al. [4] observed a 3-fold higher incidence rate of T2D in women (25 per 10,000) with low CRF compared to women with high CRF (8.3 per 10,000). Importantly, a **dose-response** was observed between CRF level (quantified in maximal metabolic equivalents [METs]) and T2D incidence (Figure 1). The PI has published a first authored review paper [9] detailing that CRF levels are lower in African Americans compared to Caucasians in a host of studies including the National Health and Nutrition Examination Survey [10-12], other epidemiological studies [13-16], and clinical trials [17-21] . Out of the 24 studies reviewed, 21 showed significantly lower CRF in African Americans ranging from 0.2 to 2.9 METs. Furthermore, a greater proportion of African Americans had categorically defined low CRF compared with Caucasian adults [12,17,22,23], which is associated with the greatest risk of T2D [4-6]. Thus, **lower levels of CRF in African Americans compared to Caucasians may contribute to the health disparities in T2D**.

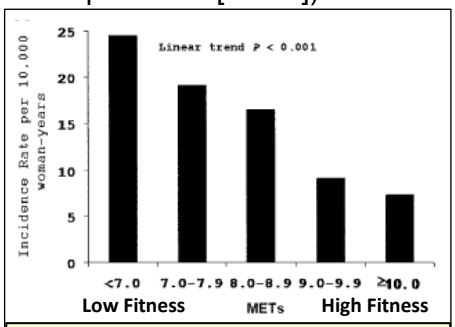


Figure 1. Fitness and T2DM incidence rates. Sui et al. *Diabetes Care* 2008

A contributor to the racial disparities in T2D mediated by CRF is the lower oxidative characteristics (and subsequent insulin sensitivity) of the skeletal muscle in African Americans compared to Caucasians, which has been noted in several reviews [9,24,25] (including 1 published by the PI[26]). African Americans tend to have a greater proportion of type-II muscle fibers compared to Caucasians, which are less oxidative (lower mitochondrial density/enzymes), less vascularized, have a lower proportion of GLUT-4 transporters, and are more insulin resistant compared to type-I fibers [25,27,28]. Evidence from Tanner et al. [27] showed that racial disparities in the distribution (greater type-II compared to type-I fibers) are especially apparent in African American obese adults. Additionally, Sirikul et al.[29] observed racial differences in oxidative capacity contributed to the lower levels of insulin sensitivity observed in obese African American compared to Caucasians. A major adaptation to aerobic training is the shift of both type-I and type-II to become more oxidative (e.g. increased function of the mitochondria [size, density, enzymes] and insulin sensitive (increased GLUT-4 expression) [30,31]. However, the PI has published data suggesting that African Americans have an attenuated response in CRF levels compared with Caucasians (despite similar adherence rates [~90%]) after 6 months of **moderate intensity aerobic training** (50% of VO_2 max), and CRF did not increase by **providing additional moderate intensity exercise** (Figure 2) [21]. Thus, a higher intensity of aerobic exercise may be necessary to maximize the CRF response in African Americans.

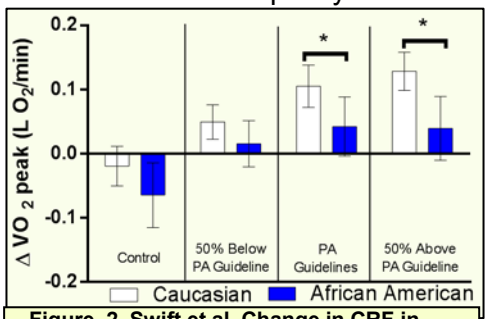


Figure 2. Swift et al. Change in CRF in response to moderate intensity training in African American and Caucasian women *Journal of Applied Physiology* 2013

Rationale for High Intensity Exercise Training: In this application, the term "exercise training" refers specifically to continuous **aerobic** exercise training (e.g. not interval). Several studies from predominately Caucasian study populations have shown enhanced improvements in CRF or insulin action with high intensity exercise training compared to moderate even when matched for energy expenditure. Very recently, Ross et al.[32] observed that CRF and 2-hour glucose (from an oral glucose tolerance test) was improved to a greater

extent following 6 months of high intensity (75% of VO_2 max) exercise training compared to moderate (50% of VO_2 max) in a Canadian (99% Caucasian) sample. O'Donovan et al. [33] (100% Caucasian) evaluated the effect of 24 weeks of moderate (60% of VO_2 max) versus high intensity (80% VO_2 max) aerobic exercise training in dyslipidemic men. The authors observed a significant (intensity*time) interaction, suggesting that CRF was increased to a greater extent with a higher exercise intensity. In regard to insulin sensitivity, DiPietro et al. [34] (race distribution not reported) showed that 9 months of high intensity (80% of VO2 peak) exercise improved insulin sensitivity in older women (measured via a hyperinuslinemic euglycemic clamp) to a greater degree than moderate (65% of VO2 peak). Thus, enhanced effects for measures of CRF [32,33,35-37] and glucose/insulin metabolism [34,38-40] have been demonstrated with high intensity exercise intensity compared to moderate. A key aspect of why high intensity aerobic exercise training may be especially relevant for obese African Americans is that it may represent a more effective strategy to treat both low CRF, insulin resistance **while meeting physical activity guidelines in less overall time** compared to moderate intensity exercise. Additionally, high intensity exercise may represent a better stimulus to address the less oxidative/insulin resistant fiber-type distribution (higher percentage of type-II) in African Americans compared to Caucasians since there is **increased recruitment of type-II fibers with increasing exercise intensity** [41,42]. In contrast, moderate intensity exercise recruits type-I fibers preferentially and a lower percentage of the available type-II fibers [41,42].

No exercise intensity training studies have determined if the enhanced improvements in whole body CRF and insulin sensitivity observed with high intensity exercise compared to moderate are explained by adaptations in the skeletal muscle. It is possible that high intensity exercise may induce a greater increase in peroxisome proliferator-activated receptor-gamma co-activator (PGC-1α) compared to moderate, which is a global activator of gene expression with a primary role in mitochondrial biogenesis. **PGC-1α expression** is **increased with aerobic exercise training, and through its signaling cascade can also induce increases in oxidative enzymes (TCA cycle, oxidative phosphorylation), insulin sensitivity (increased GLUT-4 expression), and fatty acid oxidation** [43]. Due to these effects, increases in PGC-1α expression have been speculated as a mechanism for the improvement in CRF and insulin action with exercise [43]. The expression of PGC-1α is lower in type-II fibers compared to type-I, but expression can be increased specifically within type-II fibers with aerobic exercise training [44]. Additionally, recent data suggest that PGC-1α may increase to a greater extent with acute high intensity aerobic exercise compared to lower intensities in animals [45] and humans [46-48], which may be related to the increased recruitment of skeletal muscle with high exercise intensity [47]. Further study of the effects of exercise intensity on PGC-1α and other proteins in its signaling cascade may provide a rationale for why enhanced effects for CRF/insulin action are observed with high intensity exercise training.

Rationales for evaluating high intensity exercise training in African Americans are as follows:

1. No prospective RCTs exist **comparing the health benefits** of different exercise training programs in African Americans despite the substantially greater T2D risk compared to Caucasians
2. Data from predominately Caucasian samples suggest that high intensity exercise training results in **greater** improvements in CRF and insulin action compared to moderate [49]. Thus, it may more readily improve the low CRF and insulin resistant phenotype observed in obese African Americans
3. African Americans have a higher percentage of less oxidative/insulin resistant type-II fibers compared to Caucasians. High intensity exercise will recruit type-II fibers to a greater extent than moderate
4. From a practical standpoint, high intensity exercise training has a greater energy expenditure rate. Thus, the recommended amount of physical activity (based on current public health guidelines) can be obtained in **less total time** compared with moderate intensity training
5. The PI has published data suggesting an attenuated improvement in CRF levels in postmenopausal African American women compared with Caucasians after 6 months of **moderate intensity exercise training** (50% of VO_2 max) (Figure 2) [21], which did not resolve with **increased duration** of exercise

2. Innovation: The **H**igh **I**ntensity exercise to **P**romote **A**ccelerated improvements in **C**ardior**E**spiratory fitness study **(HI-PACE)** study will be the first study to evaluate the effects of exercise intensity on CRF and insulin action in African Americans at high risk for T2D. Our proposal addresses **two major needs for future research** directly identified by the Federal Physical Activity Guidelines scientific advisory committee:

- Obtain data on the effects of physical activity on health outcomes in African Americans
- Quantify and clarify the effects of exercise training **intensity** on health outcomes.

We will directly address these research needs by employing **high quality** exercise training methodology:

- Training sessions will occur in a controlled laboratory setting under the direct supervision of study staff
- Periodic measurement of EE rate at the prescribed exercise intensity will occur at steady state exercise

(indirect calorimetry) to adjust the rate predicted from ACSM equations. This has **never** been performed in an exercise intensity study, and will allows us to more accurately match exercise volume in both groups

- Skeletal muscle measures will be evaluated to support conclusions on whole body CRF and insulin action

HI-PACE will be the first study to compare the health benefits of moderate and high intensity exercise training on T2D risk factors specifically in African Americans. The present research will have high public health implications given the substantially greater T2D risk in African Americans compared to Caucasians, and the **under-representation** of African Americans in physical activity research. The results of HI-PACE will allow us to adequately power our main primary and secondary outcomes to inform the design of an R01 application on exercise intensity in African American adults with a larger array of secondary outcomes. Completion of HI-PACE and the subsequent R01 will likely impact future physical activity recommendations, and will help guide health professionals on the type of exercise to recommend to obese African Americans with high T2D risk.

3. Approach: The HI-PACE study will enroll 60 sedentary obese African Americans (BMI: 30-45 kg/m^2, age 40-65 yrs). We will randomize participants to moderate intensity (MOD-INT, n=20), high intensity (HIGH-INT, n=20), or a control group (CON, n=20) for 24 weeks. The primary outcome measure will be the change in CRF following the intervention. Main secondary outcomes include insulin sensitivity measured via an intravenous glucose tolerance test (IVGTT), mitochondrial capacity, skeletal muscle measurements, body fat, c-reactive protein, and psychological outcomes (quality of life/exercise enjoyment).

Inclusion/exclusion criteria: We will recruit obese (30-45 kg/m^2) African Americans (age: 40-65 yrs.) with one additional T2D risk factor. Selection of these risk factors are based on a position stand from the ADA[50], which provided definitions for impaired fasting glucose, and additional risk factors which are associated with increased risk for T2D (Triglycerides (TG) ≥ 150mg/dL, HDL-C < 50 mg/dL or taking cholesterol lowering medications, Blood pressure: systolic ≥ 130 mmHG or diastolic ≥ 85 mmHg or the use of BP medication, Fasting glucose 100-125 mg/dL, waist circumference ≥ 88cm). All participants must be sedentary/low active (step count ≤ 6,500 step/day) and not participating in exercise training (≥ 20 minutes on ≥3 days per week for the last 3 months). We will exclude participants with diagnosed T2D (or blood glucose value>125 mg/dL) or known cardiovascular diseases, excessively high resting blood pressure (systolic >180 mmHg and diastolic >100 mmHg), significant medical conditions, life threatening conditions, pregnancy or plans to become pregnant, conditions that are contraindicated for exercise. In addition, we will exclude individuals who have plans to diet or engage in weight loss, or demonstrate non-compliance during screening visits.

Primary Outcome- CRF: We have selected CRF as the primary outcome measurement due to the well-established association between CRF levels and T2D [4-7]. We will measure CRF by maximal oxygen consumption from a maximal exertion treadmill test, which is considered the gold standard technique.

Maximal Exercise Test: We will use a modified Balke treadmill (Trackmaster 425, Carefusion, Newton Kansas) protocol to determine CRF, and the appropriate heart rate range for aerobic exercise training. Participants will walk at an initial speed of 2.0 mph with 0% grade for the first 2 minutes after which the treadmill speed will increase to 3.0 mph for the next 2 minutes. Treadmill grade will be increased by 2.5% every 2 minutes until volitional exhaustion. Respiratory gases (VO_2, CO_2) and ventilation will be measured continuously using a True Max 2400 Metabolic Measurement Cart (Parvomedics, Salt Lake City, Utah).

Secondary outcomes: The majority of secondary measures will be evaluated at baseline and follow-up. Exceptions to this are anthropometry, CRF, and weight data which will also be evaluated at mid-intervention (see Figure 3), and exercise enjoyment/affective valence measures which will occur during exercise sessions.

IVGTT: Insulin sensitivity will be assessed at baseline and follow-up (24 hours following the last exercise session in exercise groups). After the collection of fasting blood samples, glucose (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 min. Insulin will be injected at minute 20 of the test at a dose of 0.025 U/kg body mass. 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 [51].

Mitochondrial Function: In vivo skeletal muscle mitochondrial oxidative capacity will be measured non-invasively by near-infrared spectroscopy (NIRS). Submaximal knee extension exercise is used to increase muscle oxygen consumption (mVO_2) and the recovery kinetics of mVO_2 are determined during a series of repeated arterial occlusions. The mVO_2 data are fit to a mono-exponential function to calculate the rate constant, which is directly related to the mitochondrial respiratory capacity. NIRS has been validated against the 'gold standard' of phosphocreatine recovery kinetics measured using magnetic resonance spectroscopy [52].

Skeletal muscle measures: A collaborator on the proposal (Joe Houmard) has extensive experience with the muscle biopsy technique and the study of skeletal muscle metabolism (see letter of support/biosketch). With a percutaneous needle biopsy, approximately 100-200 mg of tissue will be obtained which is more than adequate for the proposed assays; some muscle will be frozen and achieved for future analyses. The rationale for selecting the following proteins (citrate synthase, COX IV, GLUT-4, CPT-1) is that they are downstream of PGC1-α and also represent distinct steps in oxidative metabolism [53]. Citrate synthase activity (10-20 mg of muscle) will be determined with a colorimetric reaction using reagents in a commercial kit (Sigma CS0720, St. Louis, MO) as in a previous study [54]. Citrate synthase is a component of the TCA cycle and is also indicative of mitochondrial density and CRF [55]. Protein content will be determined using western-blot techniques (10-15 mg of muscle total) for COX IV (Cell Signaling Technology, Beverly, MA) which is involved with the electron transport system and indicative of mitochondrial content [54], GLUT-4 protein content (Sigma, St Louis, MO) which is involved with insulin action and increases with exercise training (Cox), CPT1 content (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) which is involved in lipid oxidation and increases with exercise training [56], and PGC1-α (Cell Signaling, Beverly, MA).

Other secondary measures: Body weight will be evaluated a calibrated scale, with the participant fasted and wearing only a hospital gown. Dual Energy X-ray Absorptiometry (DEXA) will be used to measure body composition (fat and lean mass). C-reactive protein (CRP) will be measured using standard analytical techniques. Waist circumference will be evaluated with a gulick tape measure, and recorded at the natural waist. Quality of life (QOL) will be measured by the short form health survey (SF-36)[57]. Exercise enjoyment will be assessed via a 5-item short version of the commonly used Physical Activity Enjoyment Scale (PACE)[58,59] and will be during exercise training sessions. Affective valance (pleasure/displeasure) will be assessed during exercise sessions by the Feeling Scale [60], which is an 11 point scale that assesses how individuals feel at a specific moment in time ranging from -5 (very bad) to +5 (very good) with 0 representing neutral feelings.

4. Recruitment: Participants will be recruited primarily through the ECU Center of Health Disparities (CHD) (directed by Hope Landrine, Ph.D., letter of support included). The Community Core of the CHD specializes in recruiting African Americans for research and clinical trials. The Core produces expertly-designed, culturally-tailored recruitment flyers, DVDs, CDs etc., and distributes these through community partners. Using these strategies, the Core has recruited more than **400 African American adults** per year for research. For the present proposal, the Core will design recruitment flyers tailored for African Americans; distribute these in six local community settings frequented by them; distribute these to their partners; advertise the project on the Center's African-American community website, *Minority Health Matters*; and maintain a phone number to answer potential-participants' questions. We will also utilize standard techniques (advertisement, ECU email server, flyering).

5. Pre-screening: After phone-screen, individuals will be invited to an **orientation session**, where we will provide information about the study design, procedures, and risks/benefits. Individuals interested in participation will be consented, and scheduled for run-in visits.

Run-in visits: The purpose of the run-in visit is to ensure participants are able to make the time commitment to come to ECU approximately three times per week. In our past training studies, this run-in period has helped to obtain excellent training adherence by allowing potential participants to fully evaluate their ability to commit to study time demands. To simulate participation in HI-PACE, run-ins sessions will be scheduled 3 sessions per week over the course of one week, and duration will be similar to training sessions (~30-60 minutes). Study staff will verify sedentary status, and screen for other inclusion/exclusion criteria (e.g. medical history questionnaire/study surveys, BMI, waist circumference, blood pressure assessment, etc.). As a safeguard against low exercise adherence, participants will complete an exercise calendar (identification of expected exercise days/times, and potential back-up times). Participants will also complete a questionnaire on time commitments, child/elder care, distance from the exercise facility, motivations for exercise, family support.

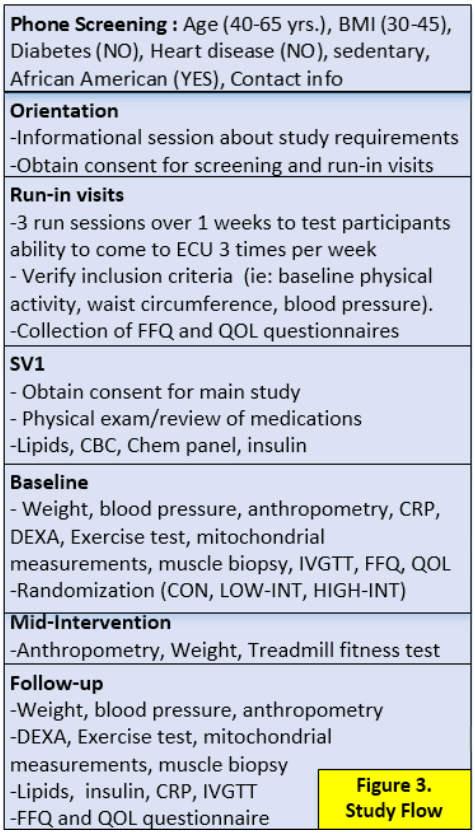


Figure 3. Study Flow

Screening visit 1 (SV1): At SV1, fasting blood draws will be performed to evaluate hepatic, renal, hematological, endocrine, lipids, blood chemistries, and metabolic function for inclusion/exclusion purposes. Pre-menopausal women will take a pregnancy test.

Assessment visits: We will obtain primary (CRF) and secondary outcome measures (IVGTT, DEXA, QOL, mitochondrial measures) at baseline and follow-up (24 weeks) (Figure 3). At mid-intervention (week 12), CRF, body weight and anthropometry will be evaluated. At follow-up (week 24), we will re-evaluate all outcomes measures. Randomization (stratified by sex) will occur at the completion of baseline assessments. Additionally, a blood sample (~10 mL) will be obtained at baseline and follow-up and archived at -80°C for future analysis.

Exercise intervention: All exercise sessions will be supervised by study staff at the FITT Center (open: 12.5 hrs. Mon-Fri [2.5 hrs.: Sat.], and Heart Institute exercise facilities (open: 6 hrs. Mon-Thurs [4 hrs. Fri.]). All exercise will be performed on a treadmill to maintain close control over kcal expenditure. Participants in the MOD-INT group will exercise at the heart rate associated with 45-55% VO_2 max, and participants in the HIGH-INT group will exercise at a heart rate associated with 70-80% VO_2 max (heart rate ranges will be updated at mid-intervention CRF assessment). The exercise volume for both groups will be 600 MET-minutes per week, which is consistent with current public health guidelines (500-1,000 MET-minutes)[3]. Since all participants will be sedentary at baseline, we will gradually increase the volume of training. All participants will exercise at 300 MET-minutes during week 1. The volume of exercise will increase 50 MET-minutes per week until they reach the maximum exercise volume of 600 MET-minutes (week 7). Using standard American College of Sports Medicine (ACSM) equations, we will calculate the amount of MET-minutes exercised based on treadmill speed/grade and the participants' weight [61]. Laptop carts with custom-made spreadsheets will be used to track MET-minutes, exercise training data, and aggregation of weekly values (mean RPE, speed, grade, caloric expenditure, etc). Exercise heart rates will be monitored **continuously** using Zephyr Bioharness 3 monitors (Annapolis, MD) to confirm exercise intensity. Adherence to exercise volume will be calculated as MET-minutes exercised divided by required MET-minutes. Exercise intensity adherence will be quantified as time within the required heart rate range divided by total exercise time.

Exercise Intensity	METs	Session time
75% VO_2 peak	4.9	~41 min.
50% VO_2 peak	3.3	~61 min.
Estimated exercise time (3 times per week) of a woman with VO_2 peak of 23 $ml \cdot kg^{-1} \cdot min^{-1}$ at a volume of 600 MET-minutes per week		

Exercise economy: To address potential variability in economy during training at a given exercise workload, we will also **directly** measure energy expenditure (EE) rate by indirect calorimetry at the prescribed exercise intensity at baseline, every two weeks during the first month of the study, and then monthly until the conclusion of the intervention. The rate of EE determined by indirect calorimetry will be divided by the EE estimated by ACSM equations (actual EE rate/predicted EE rate) to develop a correction factor. This correction factor will be used to adjust the EE calculated from ACSM equations within our exercise training spreadsheet to more accurately implement the EE which **corresponds with 600 MET-minutes per week at both intensities.**

Psych. exercise session data: During exercise training sessions, affective valence (Feeling scale) will be assessed at exercise sessions at rest, every 10 minutes during exercise, and at 5 minutes post-exercise. The PACE scale (exercise enjoyment) will be measured at 5 minutes after the completion of the exercise session.

Promotion of high exercise adherence: The HI-PACE study has several protocols to promote high exercise adherence during the intervention: 1) Participants will complete an exercise calendar during screening (described above), and the first exercise session of the each week during intervention (e.g. planned exercise days, planned back-up days). 2) Bi-weekly progress reports (e.g. adherence rate, miles walked, heart rate compliance) will be provided to participants to increase intrinsic motivation for attainment of exercise goals. 3) We will actively monitor exercise adherence on a weekly basis in study meetings. Participants with adherence levels <75% or display major warning signs for low adherence (e.g. frequent rescheduling, missed sessions, low morale) will be assigned a "case manager" from the staff to create a plan to intervene and improve exercise levels and problem solve the causes of poor adherence. 4) Participants missing an exercise session will be contacted by the exercise intervention coordinator immediately and rescheduled for a make-up session.

6. **Dietary assessment and non-exercise physical activity:** Participants in all groups will be asked not to alter dietary habits or non-exercise physical activity during the study. Non-exercise physical activity will be tracked using the Fitbit Flex (Fitbit Inc., San Francisco, CA) in all randomization groups. The Fitbit Flex will be worn in blind-mode (steps will not be represented on the device display), and the device will be removed during exercise training sessions for participants in the exercise training groups. Control participants will upload their data using Fitbit software at home. Staff will use a database program (Fitabase®, Small Steps Labs) to centralize all non-exercise physical activity data from the Fitbits inclusive of steps, moderate to vigorous

physical activity, and charge status of all active devices in the study. We will utilize food frequency questionnaires (FFQ) [62] to measure estimated daily intake (kilocalories, macronutrients, and micronutrients).

7. Statistical Considerations: Initial CRF will be compared for the three groups using side-by-side boxplots. Side-by-side boxplots will also be made for males and separately for females as we expect the max O2 to be greater for males. Numerical summaries for the boxplots along with mean and standard deviation will be recorded. Outliers and other observations requiring additional scrutiny will be identified. These steps will be repeated for the measurements taken post intervention and for the differences. The groups will be compared in terms age and other covariates using graphs (boxplots for numeric variables and segmented bar plots for categorical data) and numeric summaries. Assuming the descriptive analyses reveal no serious violations of the assumptions, ANOVA will be used to analyze the differences in CRF. In addition to using contrasts for group comparisons, confidence intervals for the difference in means will be reported. Both one-way (factor Group) and two-way (Group and Sex) ANOVA will be used. The analyses described for CRF would also be applied to insulin sensitivity, and other secondary outcomes. Data will be analyzed on an intention to treat basis. We expect attrition rate to be approximately 10-15%. Using -.112, .124, and .200 L/min as the means for the three groups based on previous data [35], a common standard deviation of .250 L/min, and significance level .05, the power ranges from .85 for 15 participants per group to .96 for 20 participants per group.

8. Experience of PI: The PI has 33 publications on exercise training and T2D/CVD risk factors, and is a former NIDDK T-32 postdoctoral fellow (Pennington Biomedical, Mentor: Timothy Church, M.D.). He has worked on a completed trial on exercise intensity and visceral fat [63]. His publications include 3 papers on exercise intensity [35,64,65], 5 papers on racial health disparities [9,21,66-68], and 4 papers dealing with cardiorespiratory fitness as a risk factor for disease [21,65,69] (3 specifically on CRF in adults with T2D [70-72]), and a recently published review paper on racial disparities in T2D [26]. He has been awarded an NIH loan repayment award from NIMHD, and was recently accepted to the NHLBI sponsored PRIDE summer research program for health disparities research.

9. Potential Pitfalls: Recruitment of the necessary sample of African Americans is a potential concern. However, the CHD has documented success with recruitment of African Americans. The PI and the CHD will closely monitor recruitment trends. If the recruitment of participants is below the expected rate, we will develop new recruitment plan to meet the necessary study sample. As the PI is a second year faculty member, another potential pitfall is that the study team has not worked extensively together on a clinical trial. As noted in the study flow diagram (below), during start-up, extensive study planning will take place inclusive of a manual of procedures (MOP), standard operating procedures (SOP), and simulations of data collection of all study visits/databases prior to the start of recruitment. Additionally, Drs. Houmard and Dar are co-investigators on the PI's ongoing AHA funded exercise trial at ECU, which currently has **92% exercise adherence (6 month exercise intervention),** which suggests the PI is capable of implementing an exercise intervention at his new institution. While the PI will assume complete responsibility of leading this trial, Dr. Houmard (expertise: large exercise trials/insulin sensitivity, muscle biopsies) will serve as faculty mentor, and the research team will engage in regular study meetings to closely monitor the progress of important indicators of study success (e.g. recruitment rates, adherence to exercise levels and intensity, etc.). If the results of HI-PACE are not supportive of what we have hypothesized on CRF, the secondary measures (insulin sensitivity, skeletal muscle measures, body composition) and archived blood/muscle samples are potential sources to support an R01 application.

10. Conclusion: 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 African Americans. HI-PACE will be the first study to compare two exercise training programs on T2D risk factors in obese African Americans, and has the potential to influence future physical activity recommendations. We will also obtain valuable psychological parameters of quality of life and enjoyment of exercise, which will help determine whether high intensity aerobic exercise is a feasible strategy over a six month period to improve health outcomes in African Americans. In accordance with the goals of this funding mechanism, the specific direction of the R01 will be dependent on the results of the present proposal, but we hope to do a larger exercise intensity study in African Americans with adequately powered primary and secondary variables. We hope that the pilot data from the present proposal will provide evidence showing the health benefits of high intensity exercise in African Americans and recruitment/exercise adherence data, which will be necessary to write a competitive R01 application in a vulnerable population.

HI-PACE Timeline	Year 1				Year 2				Year 3			
	1	2	3	4	1	2	3	4	1	2	3	4
IRB approval, MOP/SOP development, staff training	■											
Recruitment and screening		■	■	■	■	■	■	■	■	■		
Baseline testing			■	■	■	■	■	■				
Follow-up testing					■	■	■	■	■	■		
Data management/cleaning			■	■	■	■	■	■	■	■	■	■
Data analysis											■	■

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