Research Protocol
NCT04103593

Title: **Effects of 12-weeks of High-intensity Resistance Aerobic Circuit Exercise Training on Epigenetic Aging and Inflammation in Older HIV-infected Veterans**

Sub-title: *Functional Interval Training for Veterans Exercising through Telehealth (**FIT VET**)*

Principal Investigators (PI)

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Table of Contents

1. BACKGROUND

1.1 Summary and Study Design

The goal of the study is to provide a high-intensity exercise program for older Veterans that can be widely disseminated and attenuates processes underlying aging. The study is a randomized trial of 12-weeks of high-intensity functional circuit exercise that will be broadcasted from the Salem VAMC to older Veterans living with HIV in the Atlanta and Baltimore VAMCs. Our primary objective is to determine the effect of VTEL exercise compared to sedentary controls on cardiorespiratory fitness, anaerobic threshold, and O_2pulse based on gas exchange at peak exercise (Aim 1) in addition to sarcopenia and frailty (Aim 2). We also will investigate the effect of VTEL exercise on DNA methylation as a biomarker of advanced aging and determine correlates with biomarkers of systemic inflammation (Aim 3). The sedentary control group will be offered the 12-week exercise training and follow-up testing in a delayed entry design to ensure equity of the benefits of exercise to all participants.

1.2 Rationale and Significance

Treated HIV infection is a chronic disease with numerous elements of biological aging and age-related conditions that are advanced or accelerated.[1–4] Not surprisingly, adults living with HIV (HIV+ adults) have an increased risk of disability with reduced cardiorespiratory fitness[5] and increased sarcopenia[6]. The VHA is the largest U.S. HIV health provider with 64% of these Veterans 50+ years of age.[7] Effective and feasible exercise rehabilitation programs are needed to prevent widespread disability in these Veterans. Our findings and the work of others support the argument that higher intensity exercise may be needed to reverse the putative mechanisms of aging.[8–11] Our goal is to provide a high-intensity exercise program for older Veterans that can be widely disseminated and attenuates processes underlying aging.

Effective strategies for high-intensity exercise in older adults remain a challenge.[12] Circuit exercise, which involves quick transitions between exercises, is a high-intensity training approach that can increase cardiorespiratory fitness (VO_2peak) and strength in adults 65+ years of age[13,14] but has yet to be tested in HIV+ adults. At the Salem and Baltimore VA Medical Centers (VAMCs), Veterans 65+ years of age participate in a center-based exercise program, Gerofit, that increases endurance and strength.[15–17] We recently converted Gerofit into video telehealth (VTEL) circuit exercise classes that are functional (no stationary equipment), yet improve endurance and strength. The objective of this project is to adapt our center-based high-intensity aerobic (AEX) and resistance training (RT) intervention in older HIV+ Veterans into a VTEL-delivered high-intensity circuit exercise program that has the potential for large-scale implementation into VAMCs nationwide.

With increasing age, epigenetic alterations occur that affect transcription in reproducible patterns of DNA methylation (DNAm).[18,19] DNAm Age is an estimate of epigenetic age based on 353 methylation sites which Horvath derived and validated in diverse populations and multiple tissues.[20] DNAm Age is expressed in units of years that can be compared to chronologic age, thus providing a concrete measure of advanced aging. Recent molecular studies support DNAm Age as a comprehensive biomarker of advanced aging.[21] We and others found that HIV+ adults have increased DNAm Age compared to age-matched adults without HIV.[22,23] In adults without HIV increased DNAm Age is associated with physical inactivity,[24,25] weakness[25] and frailty.[26] Our preliminary data show that DNAm Age correlates with the VACS Index,[27] a measure of frailty in HIV+ adults. However, the impact of exercise training on DNAm Age has yet to be determined in any patient population.

In observational studies of HIV+ adults, systemic inflammation independently predicts cardiovascular events,[28] sarcopenia, and frailty.[29] However, the anti-inflammatory response to exercise varies in HIV+ adults, likely affected by comorbidity and lifestyle factors.[30–33] Methylation profiles in candidate genes have been identified that are associated independently with aging phenotypes (age-related diseases, frailty)[34] and lifestyle factors (smoking, physical activity).[35,36] Many of these candidate

genes encode pro-inflammatory cytokines.[37] Further, randomized exercise trials demonstrate epigenetic changes that downregulate pro-inflammatory cytokines.[38–41] Significant methylation changes in DNA isolated from blood leukocytes can occur after acute exercise[42] and only 8 weeks of training.[43] Yet, the effect of exercise on methylation in HIV+ adults is unknown.

This study leverages our exercise training experience in HIV and VTEL, availability of 3,000 HIV+ Veterans at Atlanta and Baltimore VAMCs, and the VA VTEL infrastructure. Results will impact the fields of exercise rehabilitation, HIV, and aging and can be rapidly implemented using existing resources. Exercise-mediated changes to methylation provide a pre-clinical benchmark that demonstrate anti-aging effects of exercise. Epigenetic driven exercise strategies could play a valuable role in precision medicine.

1.3 Hypothesis and Study Aims

The overarching hypothesis is that VTEL high-intensity functional circuit exercise in older HIV+ Veterans will improve the advanced aging phenotype and attenuate DNAm epigenetic processes underlying aging. We will explore the use of DNAm Age as an innovative biomarker that can predict adaptation to exercise by testing its association with exercise outcomes. Our experimental approach includes a 12-week VTEL exercise intervention in 80 older HIV+ Veterans who are randomized to exercise or standard of care sedentary control groups with the specific aims:

AIM 1. To determine the effect of VTEL exercise compared to control on:

1.1 Cardiorespiratory fitness, anaerobic threshold, and O_2pulse based on gas exchange at peak exercise

1.2 Sarcopenia defined by the European Working Group on Sarcopenia[44]

1.3 Frailty defined by the Fried Phenotype[45] and the Veterans Aging Cohort Study (VACS) Index[27]

AIM 2. To investigate the effect of VTEL exercise on DNAm Age[20] as a biomarker of advanced aging

AIM 3. To determine the effect of VTEL exercise on systemic inflammation by measure of:

3.1 DNA methylation of genes encoding pro-inflammatory cytokines in leukocytes (transcriptional regulation)

3.2 Corresponding systemic levels of pro-inflammatory cytokines IL-6 and IL-18 (protein expression)

2 STUDY STRUCTURE AND GOVERNANCE

2.1 Overview

The Salem VAMC (SVAMC) will function as the coordinating site under direction of the contact PI (Dr. Oursler) for the primary multi-PI Merit award (I01 RX002790, Oursler/Ryan/Marconi). Atlanta (AVAMC) and Baltimore (BVAMC) will function as the participant enrollment sites. SVAMC research staff will lead the exercise intervention which will be provided by VTEL broadcast to designated telehealth rooms located at Atlanta and Baltimore VAMCS which are supervised by local research staff. The study responsibilities and activities of each of the three medical centers is summarized in Table 1 below. Guidance for each research activity is provided in a single study *Manual of Operations* which is based on the Merit Award and written by the PIs (Oursler/Ryan/Marconi). The purpose of the Manual of Operations is to ensure scientific rigor and consistency of study activities across enrollment sites and thus attainment of study scientific aims. The Manual of Operations will refer to a specific *Standard Operating Procedure (SOP)* for each research procedure. The coordinating site will provide the template for each SOP to the enrollment sites. The enrollment sites are responsible for site-specific changes to SOPS, e.g., location and local oversight, and obtaining local regulatory approval (IRB and R&D) for

conduct of all research activities at their site. The coordinating site is responsible for all data management and analysis, and overall scientific integrity of the study.

Table 1. Roles & Responsibilities by Site	Salem	Atlanta	Baltimore
Recruitment, Consent, and Enrollment		X	X
Randomization	X		
Baseline and follow-up testing		X	X
Exercise intervention	X		
Data collection		X	X
Data management and analysis	X		
Blood testing and banking for future testing		X	X

2.2 Communication Plan

2.2.a Roles and Responsibilities

Drs. Oursler, Ryan, and Marconi will provide overall scientific leadership for this study and are ultimately responsible for coordinating investigations and activities, for ensuring integrity of the scientific work, and for monitoring budgetary expenditures. They will share administrative responsibilities for the scientific management of this project and meeting the requirements of all regulatory boards (Institutional Review Boards and Research and Development Committees at Atlanta, Baltimore, and Salem VAMCs). As the contact PI of the Merit Award, Dr. Oursler will be responsible for: a) meeting study benchmarks; b) coordination of data collection and quality assurance; c) interpretation of data and data analysis; and d) preparation of manuscripts, presentations, and progress reports. Dr. Oursler will have a primary role in conduct of the VTEL exercise intervention, which will be broadcast from the Salem VAMC. She will supervise all Salem VAMC study staff. Dr. Marconi will be the site PI at the Atlanta VAMC (i.e., PI on the Atlanta VAMC IRB protocol). He will have the primary role of participant recruitment, retention, completion of planned testing, and data collection at the Atlanta VAMC. He will supervise the study staff at the Atlanta VAMC. He will also facilitate collaboration with Atlanta VAMC co-investigators and oversee laboratory analyses conducted at the Atlanta VAMC, including sample collection, handling, processing, storage and assays. Dr. Ryan will be the site PI at the Baltimore VAMC (i.e., PI on the Baltimore VAMC IRB protocol). Dr. Ryan will have the primary role of participant recruitment, retention, completion of planned testing, and data collection at the Baltimore VAMC. Of note, since Dr. Oursler's move to the Salem VAMC, Dr. Ryan has been functioning in this capacity for their RR&D Merit Award (I01 RX000667, Oursler/Ryan), which includes Baltimore site participants in addition to those enrolled at the Salem VAMC. She will supervise the study staff at the Baltimore VAMC. She will oversee laboratory analyses conducted at the Baltimore VAMC, including sample collection, handling, processing, storage and assay of biomarkers, and shipping of samples to the DNA methylation laboratory in Atlanta.

2.2.b Investigators

To ensure close communication, Drs. Oursler, Ryan, and Marconi will have standing scheduled conference calls which may also include each site's research coordinator. During the initial study implementation period (first 3-6 months), calls will occur weekly and then as needed. Monthly teleconferences will occur using the VTEL research rooms throughout the 4- year study period and will include all PI's and key staff. The purpose of the monthly VTEL meetings will be to monitor study benchmarks and for ongoing problem solving. The research team will review any important revisions to procedures and ensure that protocol fidelity is maintained across all sites. Drs. Oursler, Ryan, and

Marconi will have at least one in-person meeting annually to discuss plans for data analysis, presentations, publications, and future grant applications. Collaborators will be included. Support for this travel is included in the budget. Dr. Oursler already routinely travels to Baltimore to meet with Dr. Ryan and other collaborators. Drs. Oursler and Marconi will continue to participate in monthly VACS-related conference calls, including the steering committee and the Physical Function Working Group calls. In the unlikely event that conflict would arise related to the current proposal, Drs. Oursler, Ryan, and Marconi have several mutual resources and colleagues in the VACS and Gerofit networks. These individuals would help resolve any disputes related to this project.

2.2.c Staff Coordination and Training

Informal regular calls, emails and interactive Skype calls (shared computer screen) will occur among the research staff in addition to the formal monthly research team meetings. The program coordinator will be available daily for the research coordinators and will also facilitate communication with the exercise physiologist and data manager. As is currently routine for Dr. Oursler's research team, the program coordinator will meet regularly with the data manager to discuss QA/QC issues. Dr. Oursler will meet weekly with the exercise physiologist to ensure intervention fidelity and discuss any safety issues. Dr. Oursler and the program coordinator meet daily. Similarly, Drs. Marconi and Ryan have established research teams at each of their sites with coordinators located within the unit who can meet with them daily as needed.

Coordination of study activities across the three sites will be ensured by using experienced research personnel with well-defined roles, as summarized in Table 2 below. All the research staff involved in the research will also fulfill the VA and NIH guidelines for ethical conduct of research and good clinical practice. Investigators and study staff will receive ongoing training in research ethics and good clinical practice to fulfill all requirements of the VA and local policy. A site visit will be performed by Dr. Oursler and the program coordinator during start-up, and as needed during the project, to provide training on the manual of operations and standard operation procedures. These project-related documents as well as the case report forms (CRFs) will be located on the study's network research folder and available at all time to all research staff. The program coordinator and data manager will provide ongoing education to the enrollment sites as part of their routine duties at Salem.

Table 2. Research Staff for Project

Title/role	Location and effort	Description
Research coordinator	Atlanta VAMC (100%)	Responsible for the daily management of the study at the enrollment site which includes: 1) recruitment and screening of participants; 2) providing informed consent; 3) scheduling all testing and training visits; 4) completion of case report forms; 5) management of participant reimbursements; 6) maintenance of recruitment and retention statistics; 7) reporting and correspondence to regulatory and oversight committees; 8) maintenance of the regulatory study binder and participant research folders; 9) performing quality assurance of all study activities in coordination with the data manager.
Research coordinator	Baltimore VAMC (100%)	
Program coordinator	Salem VAMC (50%)	Responsible for overall oversight of the project which includes: 1) monitoring numbers for recruitment, enrollment, and completion; 2)

		maintenance of current Manual of Operations; 3) providing template SOPs and CRFs to enrollment sites; 4) coordinating calls and meetings; 5) conducting audits of CRFs and protocol deviations to assess and correct inconsistencies between sites and coordinators; 6) reporting and correspondence to Salem VAMC regulatory committees; 7) maintenance of the Salem protocol regulatory study binder
Exercise physiologist	Salem VAMC (50%)	1) conduct VTEL exercise per protocol; 2) instruct and supervise participants during all exercise sessions, including maintenance of exercise training logs and HR data; 3) coordinate VTEL classes with staff at AVAMC and BVAMC
Data manager	Salem VAMC (25%)	1) oversee data collection and entry, including data QA/QC; 2) design and maintain databases located on Salem VAMC server and VINCI (REDCAP); 3) conduct data analysis and generate tables and figures

2.3 Data Handling and Analysis

The project contact PI (Oursler) is responsible for the accuracy, completeness, and timeliness of all data management and analysis. At the enrollment sites, the Atlanta site PI (Marconi) and Baltimore site PI (Ryan) are responsible for their site's data collection using the SOPs and CRFs provided by the Salem coordinating site. Data will be access and stored on two VA computer servers: 1) the Salem VAMC network server ("p drive"), and 2) the national VHA server located in Austin, Texas called VA Informatics and Computing Infrastructure (VINCI). All of the data collected throughout the study will be stored in a secure and access-controlled database. The database will be housed and maintained by Salem VAMC. The Atlanta and Baltimore VAMCs will contribute to the database.

2.3.a Data Management

Paper forms for use as data collection will be derived from the case report forms (CRFs) to record and maintain data for each participant enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Data reported in the CRF derived from data collection forms or source documents should be consistent or any discrepancies should be explained. Data from paper CRFs will be entered into VINCI using VA REDCap (detailed below).

Source data that is generated directly as an electronic file or print out will be stored in a shared VA network folder with access only to authorized research staff. The shared folder will be located on a Salem VAMC network folder (P:\GeriatricResearchCollaboration) that is backed up, has limited access, and is protected by the VA firewall. Individuals will be coded with study IDs in the research database. A separate file with PHI, PII, and the study key code will be kept in a different location in a password protected file. Paper copies with PHI (e.g., ICF, HIPAA) are stored in a locked drawer separately. Paper case report forms will be coded with the study ID. Any publications or presentations will not use PHI. Research records will be retained in accordance with the VA records control schedule. Access to research study data will be removed for study personnel when they are no longer part of the research team. Results will be presented and published in aggregate format unless separate permission is obtained by the Veteran. Publications will be reported per VHA policy. PHI will not be shared with any

non-VA entity. For any Information Security or Privacy incidents, suspected or actual, the ACOS for Research, Privacy Officer and Information Security Officer will be notified within 1 hour of discovery.

2.3.b Data Entry

Only authorized VA network users are permitted to use VA REDCap. For purposes of data entry, research personnel will log into the network-based application from a VA computer and create a username and profile. Once they are approved to use the application by REDCap, the study's data manager will grant individual access to the project's folder within REDCap. Research personnel will enter data into the REDCap study folder using the case report forms which will not include PII or PHI. REDCap (Research Electronic Data Capture) is a software toolset and workflow methodology for electronic collection and management of research data. REDCap projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process. REDCap was developed specifically around HIPAA-Security guidelines. In addition to VAMCs, REDCap currently supports 240+ academic/non-profit consortium partners on six continents and over 26,000 research end-users (www.project-redcap.org). Specific for VA research, VA REDCap servers (where data are housed) reside on VA Informatics and Computing Infrastructure (VINCI) which is physically located at the VA Austin Information Technology Center (AITC) in Austin, Texas. Thus, VA REDCap is accessible through the *VA intranet only.*

2.3.c Data Storage and Access

All data storage will be compliant with the guidelines set forth by Veterans Health Administration (VHA) Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and all other applicable VA and VHA policies and regulations. Study data will be kept in accordance with the Department of Veterans Affairs Record Control Schedule 10-1 (RCS 10-1). Data will be stored on and accessed from study-specific folders located on two different VA computer servers: 1) the Salem VAMC network server ("p drive"), and 2) the national VHA server located in Austin, Texas called VA Informatics and Computing Infrastructure (VINCI).

The following information was provided by VINCI Central at *vaww.vinci.med.va.gov*. The VA Informatics and Computing Infrastructure (VINCI) is a major informatics initiative of the Department of Veterans Affairs (VA) that provides a secure, central analytic platform for performing research and supporting clinical operations activities. It is a partnership between the VA Office of Information Technology (OI&T) and the Veterans Health Administration Office of Research and Development (VHA ORD). VINCI includes a cluster of servers for securely hosting suites of databases integrated from select national VA data sources (including CDW). VINCI servers for data, software applications, and virtual sessions are physically located at the VA Austin Information Technology Center (AITC), 1615 Woodward St., Austin, TX 78772-0001. This secure enclave with 105 high-performance servers and 1.5 petabytes of high-speed data storage has multiple layers of security and disaster recovery to prevent data loss. To ensure the protection of Veteran data, VINCI maintains compliance with the guidelines set forth by Veterans Health Administration (VHA) Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and all other applicable VA and VHA policies and regulations. In addition, VINCI has undergone all security certification activities in support of obtaining an Authorization to Operate (ATO). Access to VINCI resources are approved in accordance with the requirements of National Data Systems (NDS), VHA Handbook 1200.12, Use of Data and Data Repositories in VHA Research, and all other applicable VA and VHA policies and regulations. All data transferred from VINCI may be audited for compliance. Only study team personnel explicitly authorized by the PI will have access to project data. VINCI data managers and VA OI&T personnel not under the purview of the study principal investigator control the servers, network, processors, firewall and software in the VINCI environment, including access rights granted to study personnel. The specific server where the data are stored within the VINCI environment will be chosen by VINCI personnel. The server name and location within the Austin Information Technology

Center may be changed at any time at the discretion of VINCI personnel. All software used to access sensitive patient data, whether provided by VINCI or developed by the study team, will run in virtual desktop sessions on VINCI servers within the Austin Information Technology Center.

Analysis of the nutrition data collected using the 24-hour dietary recall questionnaire will occur using the a freely-available, web-based tool developed by NIH that called Automated Self-Administered 24-hour Dietary Assessment tool (ASA24). Study staff will access the web-based ASA24 system from a VA computer and enter each participant's dietary recall data using only a study ID number and date of the assessment. No PHI will be stored in ASA24. ASA24 provides data analysis options that will produce a data output in Excel or SAS file format. The data output is downloaded directly onto the VA network drive. IP addresses are not stored or tracked by the ASA24 system. Data entered are secured at the hosting site using industry standard security controls, including firewalls and encryption. All data entered into the ASA24 system at the study staff's computer is encrypted by the internet browser (e.g., Internet Explorer) using Secure Socket Layer (SSL) Technology. SSL allows for the authentication of data transfer to and from the ASA24 servers from VA computers. Only the research staff and the ASA24 operations team can access the data for each study. Access is gained through usernames and strong passwords. Additional information is available by the National Cancer Institute at www.asa24.nci.nhi.gov.

Only the following will have access to full participant records that include PHI and all research data collected: study PIs, Salem VAMC data manger and Salem VAMC program coordinator, representatives of the funding agency, and the Institutional Review Boards, or other regulatory agencies. If data is shared with other researchers, it will be done in such a way that participants will not be identified. Information gathered from this study may be published in abstract or article form in the medical literature. However, no participant will be identified by name in any publication. When study personnel are no longer part of the research team, we will amend the data access request to terminate that person's access to all study data and notify the VA Information Security Officer of such action. No sensitive patient data will be shared with anyone who does not have a VA appointment. All study team personnel with access to sensitive patient data will stay current on required VA information security and privacy policy trainings.

2.3.d Data Security and Confidentiality

Beyond the data security measures of VINCI and REDCap discussed above, additional safeguards will be in place to allow secure and confidential data sharing between the Salem, Atlanta, and Baltimore VAMCs. An experienced data manager will have dedicated effort to ensure all collected data is maintained per VA regulations and will manage the data sharing research project folder which is located on the Salem VAMC network folder behind the VA firewall with limited access. This project folder will allow secure data sharing between the Salem and Atlanta VAMCs; a similar folder system is in place now between the Salem VAMC and Baltimore VAMC for conduct of an ongoing collaborative Merit Award project. The Salem VAMC network is compliant with FISMA (Federal Information Security Management Act) with appropriate security checks and backup systems.

Protected healthcare information will be obtained during the course of this study. The collection of data will be HIPAA compliant under a Full Waiver of Authorization for Salem VAMC, where no participants will be enrolled. Atlanta and Baltimore VAMCs will have a partial HIPAA waiver for screening of the electronic medical record or local clinical database to identify potential participants. HIPAA and written informed consent will be obtained prior to any research procedures.

Written records will be stored in locked research offices at the VA Medical Centers located in Salem, Atlanta, and Baltimore. Some of the results of the research tests performed may be stored in the VA electronic medical record (EMR), per local policy. These may include participant enrollment notes, documentation of the informed consent process, and electronic scanned copies of the consent forms,

and DXA. The sensitive information collected will be maintained in accordance with the security requirements of 38 CFR Section 1.466, or more stringent requirements, the information will not be re-disclosed except back to VA; and the information will not identify any individual patient in any report of the research, or otherwise disclose patient identities.

Several provisions will be made to maintain data confidentiality. Unique identifiers will not be on any study related forms that contain data collected during the study. A unique study ID will be used on all study forms (case report forms (CRFs), test reports, and other study-related information). Only the informed consent form, HIPAA, and contact sheet will have protected health information (PHI) and will be stored in a separate folder from the research data in a different locked filing cabinet and VA computer folder. The list with linkage of study ID to name and SSN will be stored in a specific table, password protected and filed separately from the rest of the study data. Only select members of the research team will have a password and access to this table.

Data entered from paper CRFs into the REDCap project folder by staff at enrollment sites is securely stored on the national VINCI server, as discussed above. However, it should be noted that this data will be identified by study ID and research visit dates only. No PHI will be stored on VINCI. Data that is not collected on VINCI will be stored on the Salem VAMC network shared folder. This source data will be uploaded as an excel or scanned file directly from computers located in the Baltimore and Atlanta VAMCs to the Salem network folder. This data includes: blood sample results, Polar and Garmin heart rate data, and printouts from the treadmill test and DXA scan. The linkage table of PHI to study ID or specimen ID will be maintained in a separate Salem VAMC network shared folder, and only select members of the research team will have the password and access to this table.

Blood samples will be collected and assayed per the specific aims during the study period at laboratories located at the Baltimore and Atlanta VAMCs and Emory University. In addition, blood samples will be stored for future testing (banked) at laboratories located at the Baltimore and Atlanta VAMCs. All blood specimens will be stored with study ID and code numbers as the only identifiers. Results of laboratory assays from the study will be labeled only with study ID and code numbers. Any specimen remaining after testing at the off-site laboratory will be destroyed per written a priori agreement with the laboratory director and PIs per VA regulations. Human research protection of all blood samples regardless of when blood samples assays are performed is under the prevue of the site performing the testing.

Data from Polar heart rate monitors cannot be analyzed in aggregate without extraction of the data from the individual monitors. To do so, data from each individual monitor of participants in the VTEL exercise group will be uploaded to the Polar company commercial server using a VA approved USB interface and access to a confirmed secure non-VA internet server at each enrollment site. Data on the HR monitor has no PHI and is limited to heart rate, year of birth, and study ID number. This information will be temporarily stored on the Polar server. The file with linkage of the study ID number to PHI will always remain behind the VA server at all times and is never released to Polar. Therefore, there is no PHI in the storage, extraction, and transfer of data from the Polar monitors. Heart rate data is essential for exercise research and there is no available VA system that can perform this function.

A Garmin Connect web account will be generated by research staff for the participants in the delayed entry exercise group. The account, including user ID and password, will not include any element of PHI or PII. Individual-level data is limited to heart rate, height, weight, sex and year of birth. For any individual over the age of 85, year of birth will be set at 1930. Data from the Garmin activity tracker device will be uploaded from the wrist tracker to the Garmin Connect web site via the app installed on each participant's personal cell phone. Location tracking on the Garmin app will NOT be enabled on the phone. Data from the Garmin device will be temporarily stored on the Garmin commercial server (https://connect.garmin.com/en-US) (TLS 1.2, AES with 256-bit encryption (High); ECDH_P256 with 256 bit exchange). The file with linkage of the participant Garmin user ID to PHI will always remain behind

the VA server at all times and is never released to Garmin. Therefore, there is no PHI/PII in the storage, extraction, and transfer of data from the Garmin device, phone app, or web site.

The metabolic cart that collects gas exchange data and the DXA scan report are paper source documents. Participants are identified for both procedures by a study ID with no PHI. Scanned copies of these documents will be uploaded by the enrollment site to the Salem network folder.

All data collection and storage procedures will be approved by each respective VAMC Information Security Officer (ISO) and Privacy Officer (PO). Salem VAMC already has procedures and approval in place for current exercise studies.

2.3.e Statistical Plan

Phenotypic Outcomes (AIM 1) and Biomarker DNAm Age (AIM 2): We hypothesize that the VTEL high-intensity circuit exercise intervention will increase cardiorespiratory fitness and decrease sarcopenia and frailty (AIM 1) and decrease DNAm Age (AIM 2). ANOVA models will be used with dependent variables of cardiorespiratory fitness (VO_2peak, 2-minute step test) and DNAm Age (years). Logistic or multinomial models will be used with dependent variables of sarcopenia (present/not present) and frailty (robust/pre-frail/frail). Secondary analyses will be performed that use the individual components of composite outcome as their own dependent variable (e.g., lean mass and strength from sarcopenia). ANCOVA will be used to test for between group difference by time (pre/post exercise). Baseline performance and demographic and clinical characteristics will be included in models to assess and adjust for known potential confounding factors based on our experience with older HIV+ adults. We do not expect significant variability or effect from HIV-related factors (e.g., antiretroviral therapy, viral load) since we will use our previous established eligibility criteria. We do not anticipate needing to adjust for sex or race since each individual will function as their own control. Nutritional analyses of macro and micronutrients will be performed to measure any change during the study, and if present, will be tested for within group and between group effects. A similar approach will be applied to smoking exposure, which will be quantitated at baseline and follow-up testing. Treatment fidelity will be measured as percent of AEX time at target heart and entered in ANOVA models. For future planning, feasibility analyses will include calculation of rates for enrollment, retention, and attendance overall and by HIV group. Aggregate data will be collected on adverse events and reasons for lost to follow-up.

Analyses will include participants who drop out before completion of the 12-week intervention period by way of multiple imputation. This intention to treat approach will investigate factors that may affect compliance and difficulty with exercise training, including soreness and medical comorbidity. If the pattern of missing data is missing completely at random, or simply at random, our multiple imputation should eliminate bias due to loss to follow-up. Because the participants will be recruited from clinic-based VA cohorts, we will be able to obtain clinical data from participants who drop out of the study, but remain in clinical care, to determine if drop-out was related to medical conditions that may influence outcomes. Thus, we will be able to estimate bias introduced by loss to follow-up. If there is sufficient information, we may be able to model the drop-out process and adjust our analyses for loss to follow-up even if the missing data pattern is non-ignorable. If 36 training sessions cannot be completed in 12 weeks, then total training time will be tracked, and incorporated into the data analysis.

DNA Methylation (AIM 3): We will examine the change of methylation levels after 12 weeks for each candidate marker using ANOVA models. To examine epigenetic association with each outcome, we will fit the linear regression model as follows: where Yi is the quantitative outcome measurement, Zi is the vector of covariates including age, race, smoking status and leukocyte proportions, and Ei is the candidate epigenetic marker. ANCOVA will be used to test for between group difference by time (pre/post exercise). The candidate epigenetic markers will involve individual CpG sites previously identified and discussed in detail section D4c. We will extend this linear regression model to account for batch/chip effects and

longitudinal measures using a linear mixed model. We will adjust the significance threshold for multiple testing using Bonferroni correction ($p<0.05$/number of tests).

Sample Size Estimates:

AIM 1. Paired t-test was used to test for a mean change given a range of correlation between pre and post values with assumptions of $\alpha= 0.05$ and 80% power. (Mean (SD) values for the VO_2peak are from our current high-intensity AEX + RT trial in HIV+ Veterans (Table 3). Data for 2-minute step test are from our VTEL Gerofit exercise program (C3). Values for chair stands are results from a 10-week RT in older men.[46] We will have adequate sample size even with attrition as high as 40% and a correlation of within person values as low as 0.3.

Table 3. Sample Size Estimates by Outcome

AIM	Dependent Variable	Pre/ Post Intervention: Pre-Mean (SD)	Pre/ Post Intervention: Post-Mean (SD)	Sample Size: correlation pre/post 0.3	0.5	0.7
1	VO_2peak, L/min	2.17(0.13)	2.46(0.14)	4	3	2
	2-min. step test	67 (18)	86 (17)	16	14	9
	Chair stands[46]	17.8 (5.4)	25.4 (5.6)	8	7	5
2	DNAm Age,yrs[23]	62(10.8)	68.7(10)	29	21	14

AIM 2: Since change in DNAm Age with exercise training is unknown, we used values from our observational study of 19 male Veterans before and after antiretroviral therapy was started.[23] We will have adequate sample size even with attrition of 28% and a correlation of within person values as low as 0.3.

AIM 3: As summarized by Tsai and Bell, epigenetic data generated by EWAS have two additional factors that affect power, stability of CpG sites and their variance within a biological sample.[47] They performed power simulations based on micro-array based datasets (e.g., Illumina 450K) with the assumption that disease risk is affected by a single CpG site. Using Wilcoxon signed-rank test for paired study design (one sample), 80% power and $\alpha <0.05$, they generated sample size estimates based on percent methylation difference (p.1437).[47] Denham reported a 11.6% methylation difference after 4 weeks of AEX in a single CpG site in the EGF gene, which also showed decreased mRNA expression.[48] On the basis of this literature, a sample size of 34 participants is sufficient to find an 11% difference in methylation of a relevant candidate gene with exercise.

2.3.f Data Sharing and Publication

Final data sets underlying all publications resulting from the proposed research will be shared outside the VA. A Limited Dataset (LDS) will be created and shared pursuant to a Data Use Agreement (DUA) appropriately limiting use of the dataset and prohibiting the recipient from identifying or re-identifying (or taking steps to identify or re-identify) any individual whose data are included in the dataset. Data sharing and preservation will enable validation of results and advancing science by broadening the value of research data for intra and inter disciplinary developments ultimately benefiting the scientific community, industry, and the public at large.

Publications from this research will be made available to the public through the National Library of Medicine PubMed Central website within one year after the date of publication (per guidance on the ORD website).

2.3.g Data Safety Monitory Plan and Reporting

VA regulatory requirements include a plan for data and safety monitoring (DSMP) for all studies greater than minimal risk. The DSMP for this study includes a biannual formal review by Drs. Oursler, Marconi, and Ryan of all adverse events and note to files reported by the study coordinators, data manager, and exercise physiologist. Additionally, an independent clinical monitor will act in an advisory

capacity to the PIs to monitor participant safety, evaluate the study progress, and review adverse events and any safety issues. This review will include correspondence and logs maintained of all adverse events, protocol exceptions and deviations, which are reported to the respective IRB committee per local policy. Drs. Oursler, Marconi, and Ryan are readily available to research staff on a daily basis to manage any adverse event and are experienced with the target population of older patients with HIV. Dr. Marconi, an infectious diseases physician at the Atlanta VAMC, is an experienced clinician investigator who will be on site during study activities which occur at the Atlanta VAMC Rehabilitation R&D Center. Dr. Ryan, a Research Scientist at the Baltimore VAMC with a doctorate in Exercise Physiology, is experienced with conduct of exercise testing and training and will be on site during study activities which occur at the Baltimore VA GRECC. The major focus of the DSMP is safety, particularly on expected and unexpected adverse events directly attributable to participation in the research; i.e. muscle strain due to exercise. All protocols will adhere to good clinical practice guidelines. After review of the adverse events, the PIs will review policies and procedures and make recommendations accordingly. If there is a serious unexpected adverse event that brings into question the safety of a procedure or of the protocol, the PIs will temporarily suspend new enrollment into the protocol and work in concert with the VA Research Office and IRB to further evaluate the situation and determine if additional steps are necessary.

On a weekly basis, each site PI is responsible for conducting safety reviews with staff and will be available by phone for urgent issues. When unavailable to staff, a designee is indicated. During exercise training, ongoing monitoring by the research coordinator is conducted prior to each exercise training session that includes vital signs and an established panel of safety questions and is documented on the exercise log CRF. The exercise physiologist will also monitor participant's heart rate and rating of perceived exertion (RPE) during the exercise training. If there is an issue, the participant will not exercise, and the site PI will be contacted and will evaluate the participant. A participant will be discontinued from the study and referred for medical care if any adverse event (AE), inter-current illness, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant. Emergency procedures are in place should a participant need immediate medical attention during exercise training or testing.

Discontinuation Criteria:

- Lost to follow-up: Missing study visits and cannot be contacted
- Requested withdrawal: Participant requests to discontinue participation
- Death: Also an SAE that is reported to IRB
- Excluded: After study enrollment, development of a medical problem that meets exclusion criteria
- Completed: All study procedures have been performed after the exercise intervention

<u>Minimal reporting requirements for the study are as follows:</u>
AE/SAEs: All AEs must be graded for severity and relationship to the research procedure or intervention (see below) by a licensed clinician (i.e., physician, nurse, nurse practitioner, physician's assistant) but the relationship to the study can only be assessed by a clinician investigator familiar with the protocol. All SAEs will be reported to the site's local IRB as per VHA Handbook 1058.01 and local policy.

Severity of Event: All AEs will be assessed by the clinician using the following defined grading system:

- Mild: events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- Severe: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
- Life-threatening: any adverse drug experience that places the patient or participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

*Serious Adverse Event (SAE)*is defined as an AE meeting one of the following conditions, per the International Conference on Harmonization (ICH) definitions:

- Results in death during the period of protocol defined surveillance.
- Is life-threatening (defined as a participant at immediate risk of death at the time of the event).
- Requires inpatient hospitalization or prolongation of existing hospitalization during the period of protocol defined surveillance.
- Results in congenital anomaly or birth defect.
- Results in a persistent or significant disability/incapacity.

Any other important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Relationship to Study Intervention: The clinician investigator's assessment of the relationship of an AE to study interventions is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their possible relationship to study intervention assessed using the following terms: associated or not associated. To help assess, the following guidelines are use:

- Associated – The event is temporally related to the participation of the study procedure and no other etiology explains the event.
- Not Associated – The event is temporally independent of study procedure and/or the event appears to be explained by another etiology.

New Abnormal Clinical Findings from Research Testing: Any medical condition that is present at screening will be considered a baseline condition and will not be reported as an AE. If the severity of any pre-existing medical condition increases to a new level of diagnosis during the study period, then it will be recorded as an AE.

Unanticipated and Unexpected Events: The terms "unanticipated" and "unexpected" refer to an adverse event or problem that is new or greater than previously known in terms of nature, severity, or frequency, given the procedures described in protocol-related documents and the characteristics of the study population and affects rights, safety or welfare of participants.
Serious adverse events and serious problems which are UN-anticipated (regardless of relatedness to the research) will be reported to the IRB Chair or designee within 5 business days.

Protocol deviation (PD): A PD is any noncompliance with the clinical trial protocol or Good Clinical Practice (GCP). The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. It is the responsibility of all sites to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to each site's

corresponding IRB. All deviations from the protocol must be addressed in study participant data collection forms. A completed copy of the Protocol Deviation (PD) Form must be maintained in the Regulatory File, as well as in the participant's data collection forms folder/binder. Protocol deviations must be sent to the local IRB per their guidelines. All research personnel are responsible for knowing and adhering to their IRB requirements.

3 STUDY METHODS

3.1 Study Design

This study is a multi-site exercise intervention for Veterans receiving care at the Atlanta and Baltimore VAMCs that will randomize approximately 80 participants (40 per site) at these VAMCS, herein referred as enrollment sites. The intervention is 12-weeks of high-intensity functional circuit exercise performed 3 times weekly in a supervised group setting. Exercise training will be led by an exercise physiologist at the Salem VAMC (coordinating site) and broadcast to the enrollment sites. HIV-infected Veterans 50 years of age and older will be enrolled at the Atlanta and Baltimore VAMCs. Eligible participants will be randomized to exercise training (N=40) or control (N=40) groups. All participants will undergo baseline testing and follow up testing after 12 weeks at their respective enrollment site. Participants in the Control Group will be instructed to maintain their baseline sedentary lifestyle (as defined in eligibility criteria) during the 12-week intervention period. After follow-up testing is complete, participants in the control group will have the option of completing 12-week exercise training (delayed entry exercise) which will be a combined home and center-based exercise program. Pre-post exercise testing will be conducted in these participants to gauge individual progress and provide individual feedback that is a benefit of study participation, but these results will not be part of the randomized trial analyses.

3.2 Coordinating Center at the Salem VAMC (Research Activities Conducted at SVAMC)

3.2.a Exercise Intervention: VTEL Exercise Training

Exercise Training Description

The exercise physiologist located at the SVAMC will be responsible for conduct of all exercise training under the supervision of the PI. Exercise training will be delivered by VTEL broadcast from the Salem VAMC to participants at the Atlanta VAMC and Baltimore VAMC. In the event that the Salem EP is not available to deliver the training, the exercise training can be delivered by another experienced EP through a telehealth platform. Research staff at the Atlanta and Baltimore VAMCs will be in the room with the participant to help with heart rate monitors and portable exercise equipment, and in the event of an emergency. Rooms will be equipped with steps, hand and ankle weights, dumbbells, chairs, and bands. No stationary exercise equipment will be used in either aerobic (AEX) or resistance training (RT). The intervention consists of high-intensity circuit exercise training performed 3 times weekly for 12 weeks in a group setting. Circuit training is an exercise modality consisting of a series of exercises at different stations.

Functional Circuit Training Details*:* The group exercise training sessions will be performed 3 times weekly and last approximately an hour, including warm up (8-10 min.), functional circuit training (48 min.), and cool-down/stretching (8-10 min.). Our clinical program, Tele-Gerofit, supports the feasibility of this design in a small area where each station is not defined necessarily by an area but by available equipment.[49] Each exercise circuit will consist of one AEX station and three RT stations with minimal rest between stations. The AEX will be performed for three minutes. Each RT station will include 1-3 sets of a strength exercise that is completed within 3 minutes (min). The total exercise time per circuit is 12 minutes (3 min. AEX + 9 min. RT (3 x 3 min.)). Heart rate is monitored during the entire circuit with targets set for AEX and RT that is analogous to high-intensity interval training. Details are provided under exercise intensity. Specific exercises to be used for AEX and RT for each circuit are summarized in Table 4. Progression of intensity is performed by increasing the size of the steps (AEX) and number of repetitions and weight of dumbbells or hand/ankle weights (RT). In the first week, only 1-2 RT exercise set will be performed as the participant becomes acclimated to the exercises for a total of 24-36 minutes (6-9 mins/circuit). Then participants will advance to three sets per RT exercise for a total of 48 minutes of high-intensity circuit training.

Exercise Intensity: To ensure that *AEX intensity* is met, participants will wear Polar HR monitors (Polar, Lake Success NY) with alarm limits set by the exercise physiologist and programmed into the watch by enrollment site staff. The AEX protocol will follow exercise interventions protocols that have been successful in older HIV+ Veterans. Participants will initially train at 50% heart rate reserve (HRR), increasing target HR by 5-10% weekly until 70-80% HRR is reached. Based on our work to date, three minutes of AEX per circuit is feasible. *Resistance training (RT)* progression will also be based on our prior experience with center-based progressive high-intensity RT in HIV+ adults. This protocol involves initial weight lifting of 50-60% 1-RM in two sets of 8 repetitions with progression to 12 repetitions. Then training weight is increased by an amount (~10%) that causes the muscle group to fatigue/fail after eight repetitions. However, functional RT exercises utilize multiple muscle groups, unlike traditional RT. Therefore, we will use the number of repetitions for specific functional RT exercises as the target, based on thresholds set by Rikli and Jones. The use of hand and ankle weights and multiple muscle engagement will help provide higher intensity RT. Strength exercises will be performed until muscle fatigue at three stations of the circuit with each strength exercise performed quickly.

Table 4. Circuit Exercises by Modality

Circuit	AEX (Target HR)	RT (8-12 reps)
1	Step Ups	Bicep curls
		Chest Press
		Lateral Shoulder Raise
2	Shadow Boxing	Romanian Dead Lift
		Side Bends
		Calf Raises
3	Step Ups	Squat
		Reverse Wood Chop
		Bent Over Row
4	Lateral Side Steps	Lateral Band Walk
		Triceps Extension
		Wide Pull

AEX, aerobic exercise; RT, resistance training

Treatment Fidelity: The exercise physiologist will maintain a training log for each exercise session for each participant. The log includes the type of each RT exercise and number of sets, the type and duration of each AEX exercise, and any special participant feedback or concern. The heart rate will be measured and recorded continuously using a chest-strap heart rate monitor (Polar Electro Inc., Lake Success, NY) and downloaded by the enrollment site staff at their site. The exercise physiologist will compile and review weekly HR data on the Salem VAMC shared folder to document AEX duration and intensity. The exercise physiologist will meet weekly with Dr. Oursler to ensure interim training targets are met for each participant. In addition,

progress and any issues will be discussed on the monthly VTEL conferences with Drs. Oursler (Salem), Ryan (Baltimore), and Marconi (Atlanta).

Adherence to Exercise Training Sessions: The intervention is designed as 12-weeks of exercise training performed 3 times weekly on Monday, Wednesday, and Friday for a total of 36 sessions. However, due to federal holidays, 36 sessions may not be feasible within 12 weeks and so time allowance will be made for 36 consecutive sessions to be performed with 100% adherence. Given that occasional missed exercise sessions will occur (usually due to travel in our experience), up to 3 additional exercise sessions will be offered to allow participants to reach 36 exercise training sessions. This provision minimizes the risk of muscle strain and exercise intolerance by allowing sufficient time to reestablish training progression and is a standard approach in progressive exercise training trials.

3.2.b Randomization

After baseline testing is complete, eligible participants will be randomized 1:1 to VTEL exercise or standard of care control groups. Randomization will occur in blocks of 2-10 participants at each site. An independent statistician will provide an allocation list for each site that will be managed by an individual without access to study data or contact with participants. The size of the block may vary by site and COVID safety practices. Block randomization is routinely used in smaller trials to ensure equal distribution between groups. In this application, it also serves to adjust the cohort size of each exercise group from a minimum of one participant to a maximum of five participants.

3.2.c Delayed Entry Exercise for Control Group

Since the start of the study, participants randomized to the standard of care control group have had the option of entering an exercise training program after completion of 12-week follow-up testing, extending their participation time. This delayed entry exercise program, beyond the RCT design, has been provided so that the potential benefits of exercise are available to all participants despite initial randomization allocation. In the interest of ensuring that all participants may receive exercise toward the end of the award, the delayed entry exercise program will be changed from an identical one in the VTEL intervention arm to a hybrid home and center-based program. Since this may affect willingness to participate, the change in protocol will be implemented at the initial screening and recruitment of a cohort, not active participants.

The new delayed entry exercise program leverages advances in commercial physical activity trackers which now accurately measure wrist-based heart rate and are validated in older adults.[50]Delayed entry exercise will consist of 12-weeks of home-based exercise that is an individualized exercise prescription based on maximum heart rate, like the VTEL exercise group. However, a Garmin wrist device (Garmin Vivosmart 5, Garmin International, Inc. Olathe, KS, USA) will be used rather than a Polar heart rate band for heart rate monitoring. A Garmin Connect account will be generated by study staff which will include a “dummy” email that is not accessible to the participant and has no PHI/PII. The participant’s heart rate data from treadmill testing will be entered into each Garmin Connect account to define moderate intensity and vigorous intensity based on the same heart rate zones as used by the Polar monitors. Participants will be asked to wear the Garmin device continuously during waking hours, placing it on the charger each night at bedtime. Wear-time adherence will be assessed by a participant Garmin paper log which will include non-wear time and technical issues. This Garmin-based exercise program will include phone feedback at least weekly and in-person support group sessions at least monthly. These sessions will be conducted by an exercise physiologist who will adjust and progress the participant’s exercise prescription based on individualized weekly targets and personal tolerance.

Research coordinators at the Atlanta and Baltimore sites will conduct an initial orientation visit for each participant to setup the Garmin device and link it with the Garmin Connect app on their personal cell phone. One device will be given to each participant as an incentive for participation in the delayed entry exercise group. Coordinators will help install the Garmin Connect app on the participant's personal phone to ensure location tracking is off and no PII (e.g., personal email) is used. Education with written material will be provided on specific exercise (e.g., walking, jumping jacks) and activities (e.g., mowing lawn) which do not require specialized equipment and are classified as either moderate intensity or vigorous intensity.[51] For the first 4 weeks participants only moderate intensity exercise/activity will be provided. Target duration will begin with 15 minutes of daily moderate intensity activity and will be progressed 10-15% weekly or as tolerated by the participant with a 1-month goal of 150 minutes of moderate physical activity per week ACSM Physical activity Guidelines for Americans.[8] After 4 weeks, participants will be instructed to increase intensity to include vigorous exercise/physical activity with the 12-week goal of at least 75 minutes of vigorous intensity activity per week. The participant will be instructed to synch the Garmin wrist device with the phone app prior to the weekly call with the exercise physiologist, who will then review minutes in each intensity level on the Garmin Connect web site. This weekly call will last 30-60 minutes and will be used to assess tolerance, provide motivational feedback based on behavioral change techniques and make adjustments to progression of exercise in duration and intensity. This combination of activity trackers and feedback sessions has been effective in sedentary older adults.[52–55] A monthly in-person group session will be held at each site which includes participants in the delayed entry control group from the same enrollment cohort. An exercise physiologist will lead a group discussion on challenges and incentives of exercising for the purpose of encouraging peer-support, which has been observed in the VTEL sessions. These sessions will occur after weeks 4, 8, and 12 of the exercise training period. At the 12-week in-person session participants will meet with the local site research coordinator who will uninstall the Garmin Connect app on the participant's phone and reset the Garmin device. Participants will be provided instructions on how to establish a personal Garmin Connect account which cannot be accessed by research staff for use after the end of the study.

3.3 Enrollment Sites: Atlanta VAMC (AVAMC) and Baltimore VAMC (BVAMC)

Overview: Details on the research activity conducted at the enrollment site are provided here in the Salem VAMC protocol for the purpose of demonstrating the project's scientific rigor (robust and unbiased experimental design and methodology) and likelihood of achieving scientific aims. As the coordinating site, the Salem VAMC PI and research team is responsible for creating the manual of operations and standard operating procedures that will be implemented by enrollment sites. This will ensure consistency of tests and procedures among all the sites. All research activity conducted at the enrollment sites is under the FWA of each site's respective IRB. Salem VAMC research team members who have access to PHI will be included as approved collaborators in the IRB protocol at each enrollment site.

3.3.a Participant Enrollment: Recruitment, Informed Consent, and Eligibility

<u>Pool of Potential Participants</u>

Together these sites have established a well-defined pool of HIV+ Veterans in the Veterans Aging Cohort Study (VACS) by recruiting from their Infectious Disease (ID) Clinics.

Atlanta VAMC: Potential participants will be recruited from the 1,900 HIV+ Veterans at the Atlanta VAMC ID Clinic, where approximately 1,000 patients are 50+ years of age and 93% of whom are receiving antiretroviral therapy.

Baltimore VAMC: Potential participants will be recruited from the 950 HIV+ Veterans at the Baltimore VAMC ID Clinic, where approximately 700 patients are 50+ years of age and 85% of whom are receiving antiretroviral therapy.

Recruitment and Screening

Veterans receiving care at the Atlanta and Baltimore VAMCs will be recruited using techniques proven effective in our previous studies, including flyers and brochures. In addition, databases linked to the VA electronic medical record will be used to identify potential participants based on HIV status, age and current medication list. Local clinical registries (i.e. HAVACS and HIV Clinical Case Registry (CCR)), appointment systems (i.e. VISTA) and national data warehouses (i.e. VINCI/CDW) can be used for this purpose. Patients who are identified as potentially eligible will be mailed an introductory letter informing them that a research study is taking place and they might be eligible. The letter will inform them that someone from the study team will be calling them to tell them more about the study. The letter will provide phone numbers and/or a return post-card that allows them to opt-out of further contact. Additionally, providers can request permission to give a patient's name to study staff, or provide study information to patients in order for the patient to contact the study staff directly. Providers will be invited to refer patients they feel might be eligible for the study.

Informed Consent

Written informed consent will be obtained from each participant in a private setting at the enrollment sites prior to any research activity that is not part of the partial HIPAA waiver. Staff will explain the purpose, risks, and benefits of the study during the consenting process. Participants will be given the opportunity to read the consent form or to have it read to them and to ask questions before signing. All sections of the consent will be reviewed with any questions answered. A timeframe will be given to complete the consent and provide an opportunity to ask questions and validate the participant's understanding of the information included in the informed consent document.

Copies of signed consent forms will be kept in a locked filing cabinet within a locked office in the study's research offices at the Atlanta or Baltimore VA Medical Centers. Per VA guidelines, a list of participants who sign an informed consent form will be maintained. HIPAA form will be reviewed and signed at the time of the informed consent. HIPAA forms will be stored with the informed consent form.

Enrollment and Completion Targets

Feasibility of the project is supported by investigators with a track record of collaboration and the existing infrastructure including experience staff, well-defined population, and available exercise, VTEL, and lab equipment. Based on prior exercise trials we have conducted in this population, we expect a 33% eligibility failure rate and a 30% attrition rate. Due to the COVID hold in 2019-2000, first study year, no participant activity occurred and a 1-year cost-extension has been awarded which will allow for completion of 40 participants per site to complete the exercise trial within the project period (Table 5).

Table 5	**Y 2**				**Y 3**				**Y 4**				**Y 5**				**total**
Quarter	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	
Completers	2	6	6	6	6	6	6	6	6	6	6	6	6	4	2		80
AVAMC	*1*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*2*	*1*		*40*
BVAMC	*1*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*2*	*1*		*40*

Eligibility

HIV+ adult men and women of all races and ethnicities who are 50 years of age and older will be eligible. Eligibility criteria are based on our exercise research in older HIV+ adults and American College of Sports Medicine guidelines and are designed to minimize attrition, maximize safety, and limit confounding

factors. Of note, smoking is not an exclusion criterion since approximately 70% of the otherwise eligible HIV+ Veterans are smokers, and if excluded would decrease the study's generalizability.
Inclusion Criteria: Participants will be on antiretroviral therapy with HIV viral suppression because HIV viremia affects DNA methylation and DNAm age. Only sedentary adults will be eligible (not engaging in structured activity for exercise (≥1/week).
Exclusion Criteria: Specific eligibility criteria are listed in Table 6 below and include objective cut-off limits designed to maximize safety and minimize attrition but not prohibitively limit enrollment and maintain generalizability. Eligibility will be determined by information collected by a clinician study investigator during the eligibility visit which occurs after informed consent is obtained. The eligibility visit includes history and physical exam (H&P), review of the electronic medical record, and resting ECG. The following eligibility checklist was used for IRB submission at the enrollment sites:

Table 6. Eligibility Checklist
**based on date of H&P*

		Inclusion Criteria
Yes	No	
		Veteran living with HIV and under care at VAMC
		50 years of age and older
		Stable antiretroviral therapy (ART) (same ARV medications within 3 months)
		At least one HIV-1 PCR < 20 c/ml (viral load) within prior 6 months
		Exclusion Criteria
Yes	No	
		History of AIDS defining illnesses (within 6 months; CDC Criteria)
		Myocardial infarction (within 3 months)
		Exertional or unstable angina (current chest pain that limits activity)
		Severe congestive heart failure (EF < 20% in last year or NYHA Classification III or IV)
		Uncontrolled hypertension (SBP >180 &/or DBP > 110 mm Hg)
		Therapy with beta blockers or non-dihydropyridine calcium channel blocker (within 1 month)
		Screening EKG with ischemia, complex arrhythmia, or high-grade block (per Minnesota Code)
		Poorly controlled DM within prior 1 month (FBS>180 mg/dl, RBS > 299 mg/dl, or HbA1C > 10)
		Receiving treatment for cancer except skin cancer (within 3 months)
		Peripheral vascular disease with claudication
		Severe arthritis limiting ambulation
		Neurologic disease limiting ambulation (requiring assist device)
		End stage liver disease (decompensated liver disease)
		Chronic renal failure (requiring dialysis)
		Severe pulmonary disease (home O2, admission for dyspnea or pneumonia within 1 month)
		Use of systemic steroids (testosterone or glucocorticoids) or growth hormone (within 6 months)
		Dementia (based on Evaluation to Consent)
		Signs or symptoms of any medical comorbidity that would preclude exercise testing or training
		Exercise on routine basis (structured resistance or aerobic exercise > 1 time per week)
		Past medical history of COPD or emphysema AND mMRC score =4
		January 2020

Withdrawal
Participants are free to withdraw from participating in the study at any time upon their request. Participants who elect to withdraw from the study will not perform additional research portions of the

study (i.e., research blood draws). Data that has been already collected prior to withdrawal will be used in analysis, unless the participants request otherwise in writing.

3.3.b Testing Procedures at Enrollment Sites

Summary of the research procedures to be performed at each timepoint are summarized in Table 7 below. All testing will be conducted in participants located at Atlanta and Baltimore VAMCs within 5 weeks of the eligibility visit (baseline) or end of a 12-week intervention phase. For delayed entry controls, follow-up testing will occur at ~12-weeks and ~ 24-weeks.

Table 7. Summary of study procedures and outcome measures

Procedure	Outcome	Measures
Treadmill Test	Exercise Capacity	Aerobic capacity (VO_2peak)
		O_2 pulse
		Anaerobic Threshold (AT)
DXA	Body Composition	Body fat
		Appendicular skeletal mass index (ASMI):
		Bone density
Functional performance	Strength and endurance	Chair stand
		Arm curve
		2-minute step test
Frailty		Fried Frailty Phenotype
		VACS Index
Blood Sample	Biomarker of Aging	DNAm Age[20]
	DNA Methylation (DNAm) of Candidate Genes	Inflammation-related genes: *NFKB2*[56]; *ASC*[57]; 5 CpG sites in genes related to CRP[37,58] Exercise-related genes: *GHRH* and *FGF1*[43]; Epidermal growth factor (*EGF*) [48]
	Biomarkers of Inflammation	e.g., IL-6, IL-18, high-sensitivity CRP[38–41,43]
Survey	Health-related QoL	WHOQOL-HIV [59]
	Social Support	Exercise Social Provisions Scale [60]
	Exercise Barriers	Barriers-Specific Efficacy Questionnaire [61]
	System Usability	System Usability Scale (SUS) [62]
Garmin Tracker*	Physical Activity	Step counts
		MVPA minutes
		Sedentary time
*Delayed exercise group only; MVPA, moderate vigorous physical activity		

Treadmill Testing**:** Participants will be asked to exercise to voluntary exhaustion during a graded treadmill test using a modified Bruce protocol.[63] After a resting 12-lead electrocardiogram is completed and reviewed by the study physician, participants will be asked to exercise to voluntary exhaustion during a treadmill test using a modified Bruce protocol. A final report on the test results will be made by a

cardiologist. If abnormal findings are seen on the test, the participant will be referred to their primary care physician. The visit for the exercise treadmill test will take about 45-60 minutes and will take place in the Baltimore and Atlanta VAMCs.

Oxygen (O_2) consumption, CO_2 production, and minute ventilation will be measured breath-by-breath using a metabolic cart and the following data will be collected:
VO_2peak. Rationale: O_2 consumption at peak exercise effort, VO_2peak, is used as the standard measure of aerobic capacity in sedentary adults since the majority are not able to reach maximal aerobic capacity defined as a plateau in O_2 consumption.[64] Method: VO_2peak will be the average of the final 30 second values of O_2 consumption at peak exercise.
O_2 Pulse. Rationale: Oxygen pulse is a measure of the efficiency of O_2 delivered or consumed. Method: O_2 pulse will be calculated as the milliliters of O_2 consumed per heartbeat (VO_2/HR).
Ventilator Threshold. Rationale: The onset of anaerobic metabolism or anaerobic threshold (AT) is an estimate of submaximal exercise capacity and can be measured in several ways. We will use ventilatory threshold (VT) as a well-described clinical estimate of AT that has a high test-retest reliability in adults with chronic disease.[65] Method: VT will be derived from two different plots including using V-slope method and ventilatory equivalents. O_2 consumption at VT and the percentage of VTO_2/VO_2peak will be determined.

Dual-energy X-ray absorptiometry (DXA)**:** Participants will undergo a DXA after an overnight fast. Total and regional lean tissue mass and bone density will be measured. Change in muscle mass and adiposity will be calculated and tested for between group differences.
Appendicular skeletal mass index (ASMI): ASMI will be calculated as lean appendicular mass/height^2. Established cut-offs for ASMI measured by dual-energy X-ray absorptiometry (DXA) will be used to define low muscle mass.[66,67] ASMI predicts clinical outcomes in diverse patient populations.[68,69]
Adiposity: total, truncal, and appendicular fat mass and percent body fat

Functional Performance Testing Battery: Function tests will include standard measures of functional performance. Body circumferences (waist, hip), weight, and height will be measured using the protocol established in the Third National Health and Nutrition Examination Survey. We have performed this set of physical function tests on over a hundred HIV-infected adults and expect it to take 20-30 minutes to complete. Function testing will take place in the Baltimore and Atlanta VAMCs with VTEL supervision by the Salem VAMC exercise physiologist to ensure testing accuracy.

Lower extremity strength by timed chair stands: Rationale: The 30-second chair stand correlates with 1-RM leg-press performance in older men (r = .78)[70] and predicts mobility disability[71] and mortality.[15] The test-retest reliability is 0.89.[71] Method: The number of times is counted within 30 seconds that a participant can rise to a full stand from a seated position without arm assistance.
Upper extremity strength by timed arm curl: Rationale: The 30-second arm curl test correlates with 1-RM biceps strength (r= 0.62)[71] and improves with RT.[46] Method: The number of times a hand weight can be curled through a full range of motion in 30 seconds is measured. The test-retest reliability is 0.80.[72]
Two-minute step test (2-MST): Rationale: Cardiopulmonary exercise testing will not readily be available when the VTEL exercise program is translated to clinical care. The 2-MST correlates with VO_2peak in older patients ($r=0.33$; $p<0.01$)[73] and is advocated as a surrogate measure of aerobic capacity in space limited settings[72] Method: The test will be performed according to the Rikli and Jones protocol in which the number of times the right knee reaches a pre-determined height is counted in 2 minutes.[71] The test-retest reliability is 0.88.[72] As a secondary analysis, we will correlate the 2-MST with VO_2peak, not previously reported in HIV+ adults.

Frailty:
Frailty phenotype: The frailty phenotype by Fried et al. will be used to measure frailty phenotype.[74] Rationale: The Fried frailty phenotype has been widely studied in HIV+ adults.[75] Method: The five domains of the phenotype (slowness, weakness, low activity, weight loss, and exhaustion) will be measured and scored as previously described.[74]
VACS Index: Based on our research in the Veterans Aging Cohort Study (VACS)[76] and recent epigenetics literature,[77] the VACS Index will be calculated[27] and used as a secondary measure of frailty. The VACS Index will be calculated based on clinically available lab values, such as kidney and renal function, age, and CD4 cell count and HIV-1 RNA level (viral load).[27]

Blood Sampling: Blood collection, storage, and testing will be performed in the Baltimore and Atlanta VAMC laboratories. The samples will be collected across multiple draws. One of the blood draws will be conducted after an overnight fast. Staff will attempt to phlebotomize the patient during the approved fasting DXA visit to minimize discomfort and inconvenience to the patient. Blood samples will be collected in appropriate tubes using established protocols per the planned assays. Plasma will be immediately separated by centrifugation at 4°C, aliquoted, then frozen at -80°C and stored until further processing and analysis. Pre/post intervention samples from the same participant will be analyzed in the same assay.
Inflammatory Biomarkers: Testing of inflammatory biomarkers will occur at each respective enrollment site using the same kits since procedures are very standardized. We appreciate that change in plasma levels of cytokines can vary greatly between and within individuals. Our analysis plan takes this point into consideration by including change in cytokines as an independent variable in models of the primary and secondary outcomes. Changes in plasma levels will be calculated pre/post intervention and will be tested for association with changes in outcomes as well as between group differences.
DNA Methylation Assay: Samples will be tested for DNA methylation in the laboratory of Dr. Yan Sun in Atlanta using established methods as previously described. [23,58,78] Samples from Baltimore participants will be shipped directly to Dr. Sun's lab. In sum, Dr. Sun will conduct a comprehensive interrogation of epigenome-wide CpG sites using the Illumina Infinium Methylation EPIC (850K) BeadChip. The 850K chip covers 99% of the NCBI Reference Sequence (RefSeq) genes, with sites covering the promoter, untranslated regions, and gene body, >95% of CpG islands, enhancer regions from FANTOM5 and ENCODE, as well as differentially methylated sites across tissue types. The EZ DNA Methylation Kit (Zymo Research, Orange CA) will be used to bisulfite-convert 0.5 μg of gDNA per sample. Bisulfite-converted DNA samples will be whole-genome amplified, enzymatically fragmented, and purified. Samples will be hybridized to the array. The array will then be fluorescently stained, scanned, and assessed for fluorescence intensities to quantify the methylation levels of each DNAm site.
Blood Banking: Blood sampling will include additional volume for future testing related to cardiometabolic disease and aging. These samples will be stored at the Atlanta and Baltimore VAMCs.

Clinical Data: Medical history and clinical laboratory values will be collected at the eligibility visit by a research clinician. Data will include comorbid conditions, medication use, CD4 cell count and HIV-1 PCR level, and other clinical labs included in the VACS Index. Both the participant and review of medical records will be used as source of the data.

Smoking: Smoking history is part of the baseline medical assessment and will be repeated as part of follow-up testing due to its effect on DNA methylation. Smoking exposure will be quantitated as current, prior, or never use, as well as pack-year use, which has been used to measure effects on DNA methylation.[79]

Alcohol and Illicit Drug Use: Alcohol and illicit drug use will be assessed due to their potential effect on adherence. The AUDIT-C will be used to measure alcohol use. Illicit drug use will be quantitated as current, prior, or never use by drug class.

Diet and Nutrition Assessment: All participants will be interviewed three separate times during baseline and follow-up periods to allow for comprehensive and unbiased assessment of diet. Interviews will be performed at unannounced times by a Baltimore or Atlanta VAMC research team member who has been trained in the interview method for a 24-hour dietary recall questionnaire. All participants will be counseled in the principles of a weight stable American Heart Association diet[80] prior to baseline testing to minimize dietary influences on the metabolic tests. Members of the research team will monitor diets and body weight throughout the study to ensure stability and counsel participants as necessary to maintain diet intake and weight. This will minimize confounding effects of weight loss and dietary heterogeneity.

Surveys: A total of 4 surveys will be completed by participants on paper during the study to cover the domains of health-related quality of life (QoL), social support from group exercise, barriers to exercise, and system usability (Table 7). QoL will be considered a secondary outcome and will be assessed with other outcomes before and after the intervention period. The remaining surveys will be used for program evaluation and will be collected after 4 weeks of exercise training.

VTEL Feasibility**:** Rates will be calculated for enrollment, attendance, and retention. Participant satisfaction with VTEL system will be assessed with the System Usability Scale (SUS) . The relationship of social support and self-reported exercise barriers will be tested with attendance and retention rates. The same four surveys will be collected from participants in the delayed exercise home-based pilot with the SUS adapted for the Garmin device.

Garmin Wrist Device: The Garmin Vivosmart v5 will be used to collected from each participant the following data: minutes of moderate intensity and vigorous intensity exercise/activity, number of exercise bouts, step-counts, and sedentary time. No GPS (location) data will be collected. Data from the wrist device will be uploaded to the Garmin Connect website via the Garmin Connect phone app. Research staff will access the website to extract daily and weekly mean values and record them on a case report form before entering into the study database. A report for each participant will be generated for the weekly feedback session.

3.3.c Risks and Benefits of Procedures at Enrollment Sites

There are potential risks associated with some of the procedures included in this study. However, the procedures have been planned by the investigators to minimize the danger of any major complication. All medical procedures will be supervised by qualified medical personnel who will carefully monitor the participant. If new medical problems are discovered or encountered during this protocol, participants will be referred to their physicians for further evaluation and management. Risks associated with specific procedures are discussed below:

1. Exercise testing and training. The acute risk for a cardiac event during exercise testing and training is small. The AHA consensus statement on exercise standards estimates that the acute risk of sudden cardiac arrest during exercise training in adults with known cardiac disease is approximately 1 event per 60,000 hours of aerobic exercise. The primary risk is muscle soreness and injury, which will be minimized by close supervision and appropriate warm-ups.
2. Blood sampling: There is a minimal risk of bruising and infection from blood drawing.

3. Radiation exposure: For the DXA, the total amount of exposure is 20 mR (whole body = 1 mR, lumbar spine = 7 mR, hip = 7 mR, forearm = 5 mR).
4. Tests of functional performance: These tests involve a variety of timed walks and getting up from a chair. There is a small risk that adults will fall, get chest pain, become short of breath or become dizzy during these tests. Adults will be instructed to stop the test if there are any untoward symptoms such as chest pain. We have performed >100 tests of functional performance without complication.
5. Nutrition: There are minimal risks associated with the administration of the 24-hour diet recall. It is possible that some participants may feel uncomfortable discussing their diet.
6. Surveys: There are minimal risks associated with participants completing the surveys.

3.3.d Measures to Minimize Risk

The exclusion criteria have been developed for older HIV+ adults to minimize risk of adverse events. Exercise training will take place in rooms located within the Atlanta and Baltimore VAMCs near clinical areas and with specific safety procedures developed for VTEL exercise. A research staff member at the enrollment site will measure blood pressure before every exercise session and will remain in the room during the exercise class.
1. Exercise testing and training: Exercise testing will only be conducted after the participant is examined by a physician and the resting ECG and vital signs are obtained. A clinician (MD/NP) and exercise physiologist are present in the room with a defibrillator, emergency medications and resuscitation equipment during all testing. Exercise testing will be performed as per the guidelines of the American College of Sports Medicine. The ECG is continuously monitored. Specifically, a full 12-lead ECG, BP, and breathing are monitored during every minute of the test by personnel who are trained in cardiopulmonary resuscitation, exercise testing and emergency treatment of cardiac arrhythmias. The test will be stopped if electrocardiographic, heart rate, or BP abnormalities occur or if the participant has any symptoms of cardiovascular decompensation (e.g., chest pain, extreme dyspnea, dizziness or faintness, fatigue, pallor or cyanosis, cardiac arrhythmias including atrial fibrillation or flutter, frequent PVC's, second or third degree AV block or other intraventricular conduction defects, significant ST segment elevation or depression (> 2mm), SBP > 240 mmHg, DBP > 120 mmHg, or a decrease in SBP from the previous stage > 20 mmHg). Emergency medications are available should the participant need treatment. ECG, BP, and breathing are monitored for at least 6 minutes post exercise, or until the participant's heart rate and BP have returned to their pretest values. Disqualified participants will be referred to their physician for further evaluation. The clinician (MD/NP) and exercise physiologist are responsible for safety monitoring.
2. Blood sampling: Risk associated with blood sampling is minimized by use of aseptic technique and an experience and certified phlebotomist. The research coordinator is responsible for safety monitoring of this procedure.
3. Radiation exposure: Exposure is minimal for the DXA scans. participants will be instructed, verbally and in writing in the informed consent, to notify research staff of any additional radiologic tests outside of the study. We do not expect women over 50 years of age to be childbearing, but menopause will be confirmed by history and a serum pregnancy test performed, if needed. The PI is responsible for safety monitoring.
4. Tests of functional performance: These tests involve a variety of timed walks, getting up from a chair, and tests of balance. No tests will be performed before the participant is examined by a physician. An exercise technician who is trained in CPR will be administering these tests. A crash cart and emergency medications are available in the area where these tests are performed. The exercise physiologist is responsible for safety monitoring.
5. Nutrition: The 24-hour recall allows participants to discuss their diet with research staff in a private setting and in a non-judgmental manner. The research coordinator is responsible for safety monitoring.

6. Surveys: Confidentiality will be protected by using only study ID number on the surveys and having participants complete them in a private setting.

7. Garmin wrist device: This brand and style of commercial physical activity device was chosen based on prior work with older veterans which showed it was preferred compared to other commercially available devices based on ease of use and comfort. [81] The risk of minimal wrist discomfort will be minimized by appropriate sizing of the device band and the option for participants to remove the device at bedtime.

Confidentiality of data: Confidentiality of data is of concern, particularly when information includes HIV status. The investigative team has taken extensive steps to ensure the confidentiality of the data as discussed above in Data Management section. All members of the research team are responsible for minimizing loss of confidentiality of participant records, and the PI is ultimately responsible for all aspects of study conduct.

3.3.e Benefits

The participants may not benefit from taking part in the study. However, participants will receive a thorough assessment of their functional performance and risk factors for cardiovascular disease and bone disorders, which are common in HIV-infected adults but not routinely considered by their providers. They will be told of abnormal findings and with their written permission, results will be forwarded to their health care providers for appropriate medical follow-up. In prior studies, these types of assessment have detected previously undiagnosed problems (e.g., osteoporosis). Participants enrolled in the intervention study, and delay entry controls will benefit from a training intervention, which should improve their level of functioning and potentially decrease their risk for future cardiac events. Some participants may embrace the new behavior of exercise after exercise training during the study and incorporate the activity into their routine. Any of these changes in the future will give them an opportunity to improve their quality of their life. Comparing the two exercise interventions, the VTEL exercise may have greater, more immediate increase in aerobic capacity but the delayed entry control group may have more durable benefits for all outcomes. We believe that the benefits associated with this study will exceed the risks, thereby resulting in a low risk: benefit ratio for any participant.

3.3.f Costs/Compensation

Compensation will be allocated directly at each site, per their local policies and procedures. Each site has the same target completion rate and annual participation reimbursement budget, but will be allowed to adjust reimbursement per study procedure as deemed appropriate per local standards. Participant name, address, social security number, and amount of payment will be submitted to the Internal Revenue Service for tax reporting purposes for any financial compensation they receive per calendar year as a result of participation in this research study. The Salem VAMC team will not have access to this information. All participants are reimbursed for baseline and follow-up testing. For performance of VTEL exercise training, reimbursement is provided per exercise session for travel cost and a completion bonus at end of 36 sessions (VTEL intervention group). The delayed exercise control group will complete follow-up testing at 24-weeks in addition to 12-weeks, and therefore total study reimbursement will be greater. During the delayed exercise period, the control group will be reimbursed for the weekly phone calls and monthly in-person group visits.

Use of research samples may lead to inventions or discoveries that could become commercially valuable. In the event of scientific discoveries made using participant samples, study participants will not receive compensation or profits from any inventions, discoveries, or products.

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