Protocol #: 21-2580
Project Title: Comparison of Exercise Mode on Disruptions in Calcium Homeostasis
Principal Investigator: Sarah Wherry, PhD
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I. Hypotheses and Specific Aims:

This study will fill a knowledge gap regarding how endurance exercise mode may differentially impact the bone biomarker response to exercise in older Veterans. Endurance exercise is a first-line strategy to prevent or manage cardiometabolic diseases but can lead to bone loss under certain conditions. The data generated will be used to develop a future clinical trial investigating exercise and lifestyle strategies that minimize both the risk of cardiometabolic diseases and osteoporosis.

SA1: Determine the effect of *exercise mode* on the serum ionized calcium (**iCa**; secondary), parathyroid hormone (**PTH**; secondary), c-telopeptide of type I collagen (**CTX**; **primary**), and procollagen of type-1 n-terminal propeptide (**P1NP**; secondary) responses to exercise in older adults. 30 older adults (15 women, 15 men, aged 60+ years) will each perform two, 60-minute exercise bouts: 1) high-intensity (70-80% heart rate maximum (**HRmax**)) cycling exercise (weight-supported); and 2) high-intensity (70-80% HRmax) treadmill exercise (weight bearing).

- **H1a:** Cycling exercise (weight supported) will result in a greater increase in bone resorption compared to treadmill exercise (weight bearing).
- **H1b:** Treadmill exercise (weight bearing) will result in a greater increase in P1NP at the 24-hour time point compared to cycling exercise (weight supported).

SA2 (exploratory): Determine if men and women have a differential bone biomarker response to same exercise. This aim will be achieved by comparing the iCa, PTH, CTX, and P1NP response from each exercise bout between sexes. This will generate preliminary data to determine if exercise needs to be tailored by sex.

II. Background and Significance:

Overview. The proposed aims expand the research conducted during our previous experiments on the disruption of calcium (**Ca**) homeostasis during exercise. This research evolved from the observation that some athletes, such as cyclists, tend to have low bone mineral density (**BMD**) when compared with normally active adults.[1] A logical conclusion may be that BMD is low in cyclists because they perform weight-supported versus weight-bearing exercise, because it is the latter that has more favorable loading effects on bone.[2] However, over a year of training and competition, road cyclists had significant *decreases* in BMD.[3,4] Thus, it is not that cyclists fail to achieve the skeletal benefits of weight-bearing exercise, but rather that there are unfavorable skeletal effects of competitive cycling. Further, the loss of bone mass in athletes is not limited to weight-supported activities. College basketball players had a 6% decrease in total body bone mineral content (**BMC**) over 1 year.[5] Intriguingly, when the basketball players took supplemental Ca the following year, including during practice sessions, BMC was increased.

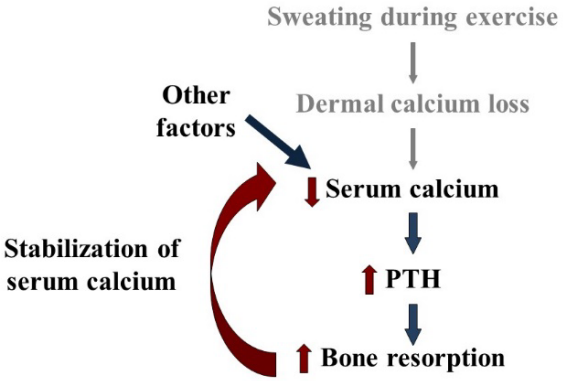

Figure 1. Working model for the disruption of calcium homeostasis during exercise.

These observations led to our **overarching hypothesis** that **activation of bone resorption during exercise counteracts, in part, the skeletal benefits of exercise on bone**. Research on skeletal adaptations to exercise has focused largely on the *mechanical loading characteristics* of exercise, to define the factors that

most effectively stimulate bone formation (i.e., anabolic factors).[6] Potential *metabolic responses* to exercise that influence the extent to which exercise stimulates bone resorption (i.e., catabolic factors) have received less attention. Our research has focused on the disruption of Ca homeostasis during exercise as the trigger for a metabolic cascade that activates bone resorption.[3,7-11] The working model in our previous research (Fig 1) hypothesized that sweating during exercise leads to dermal Ca loss and a decline in iCa. This triggers an increase in parathyroid hormone (**PTH**), which stimulates bone resorption to mobilize Ca from bone and stabilize serum Ca. From this research, we have generated unequivocal evidence that 1) vigorous exercise causes a decline in serum ionized Ca (**iCa**) concentration;[9,11-13] 2) the decline in serum iCa during exercise is the major determinant of the subsequent increases in PTH and bone resorption (as reflected by c-telopeptide of type I collagen; **CTX**);[9] 3) the increases in PTH and CTX are robust and occur after only 15 minutes of exercise;[9,12,13] and 4) despite the fact that serum PTH returns to the pre-exercise level by 1 h after exercise, bone resorption remains activated at least 4 hours.[9] We also generated evidence that, in contrast to our hypothesis, dermal Ca loss through sweating *is not* the major cause of the decrease in serum iCa or increases in PTH and CTX during exercise.[12,13] Other factors regulate this response. Possible factors include changes in metabolic acidosis[14,15] or serum phosphate.[16]

Effects of mode of exercise. The majority of our research in this area[3,7-9,11] involved moderate- or high-intensity cycling exercise in young adults (mostly men) who were accustomed to vigorous exercise. The PTH and CTX responses to exercise have been well characterized in *athletic young men during vigorous cycling exercise*. We have published two studies of *normally active older adults during brisk treadmill walking* and found that they, too, had a decrease in serum iCa and increases in PTH and CTX in response to exercise.[10] However, we found that the iCa, PTH, and CTX responses to exercise were similar, in terms of pattern of change, in older adults as young adults, but that the absolute magnitude of the decrease in iCa and increases in PTH and CTX were approximately 2-fold higher in young adults than in older adults. There are many potential explanations for the difference in magnitude between young and older adults, but one potential explanation is that the mode of exercise was different between experiments (treadmill vs cycling). Between-lab studies suggest that cycling induces higher PTH and CTX responses than treadmill exercise (Fig 2). To the best of our knowledge, this has never been studied using a within-subject approach and the potential reasons for a mode-specific disruption of Ca homeostasis, such as differences in the mechanical and metabolic characteristics of the exercise performed, are not clear.

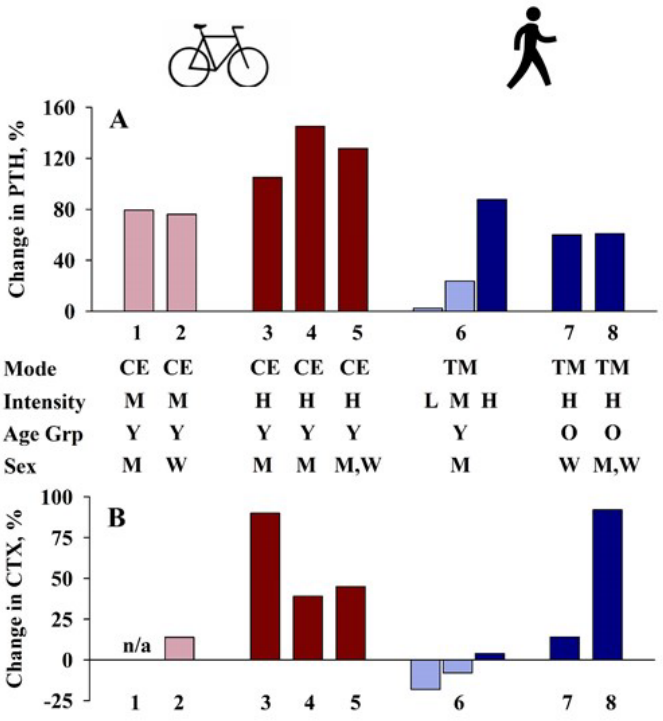

Figure 2. Exercise-induced changes in serum PTH (A) and CTX (B) in multiple cohorts of young (Y) and older (O) women (W) and men (M). Participants were studied during cycle ergometer (CE) or treadmill exercise TE) at low (L), moderate (M), or high intensity (H). From left to right, results are from the following studies: 1 – Barry 2007; 2 – Haakonssen 2015; 3 – Kohrt 2018; 4 – Barry 2011; 5 – Kohrt 2019; 6 – Scott 2011; 7 – Shea 2015; 8 – Wherry 2019.

Without knowing how mode of exercise influences disruptions in calcium homeostasis during exercise, it has been challenging to design interventions to maintain bone health while also improving cardiovascular health and reducing the risk for cardiometabolic diseases. While all forms of aerobic, endurance exercise has a demonstrated benefit for reducing diabetes or cardiovascular disease,[17-20] our labs and other have generated preliminary data suggesting that some forms of endurance exercise may be better for bone health.[4,21-24] As mentioned previously, it is not clear if these are real differences or a reflection of other contributing factors such as age, sex, or lab-to-lab differences. Given the large burden of cardiometabolic disease in the Veteran population,[25-28] recommending health lifestyle behaviors, such as regular exercise, could provide a substantial benefit to Veteran health and function.

However, to also reduce the burden of osteoporotic fracture, we need to ensure that intervention strategies benefit cardiometabolic health without jeopardizing musculoskeletal health.

Impact on Veteran health. Regardless of the outcome, understanding the contribution of exercise mode to the activation of bone resorption and/or formation will provide critical information for improving Veteran functional independence and quality of life. Veterans have a high prevalence of cardiometabolic diseases;[26,28] exercise is often used to improve metabolic health. However, there is evidence that Veterans have impaired bone health, and that impairment could be exacerbated by exercise-induced bone resorption. Women Veterans have a *40% greater incidence of hip fracture* compared to their civilian peers, regardless of where they receive their care.[29] If Veterans are encouraged to reduce their risk of other chronic diseases through regular endurance exercise, the repeated activation of bone resorption by exercise could diminish skeletal benefits of exercise or potentially increase the already elevated risk of hip fracture. Exercise is a critical component of healthy aging, and should be encouraged to maintain a healthy lifestyle, **but we need to ensure that these increased metabolic benefits do not have unintended consequences of impaired bone health or increased osteoporotic fracture**. Osteoporotic fracture can be devastating, as one year after hip fracture, mortality is 24% and 38.3% in women and men, respectively.[30] Of those who survive, ~50% are unable to live independently.[31,32]

III. Preliminary Studies/Progress Report:

Preliminary Studies. As mentioned previously, we have conducted experiments in both young and older adults. In one such series of experiments, we infused calcium gluconate intravenously during exercise to prevent the decrease in serum iCa during exercise[9] and then measured how PTH and CTX were influenced. The exercise bout was then repeated under a saline (control) condition and we have recently repeated this experiment in older adults (Wherry, under review); this has provided preliminary data to compare the bone biomarker response to exercise across two studies of the same exercise intensity (~85% HRmax), but utilized different modes of exercise. As can be seen in Figure 3, which reflects the bone biomarker responses to exercise under the saline condition, despite the same overall patterns of change in bone biomarkers, the magnitude of the change is smaller in older compared to younger adults. There are multiple potential explanations for this, including 1) age; 2) exercise intensity; and 3) mode of exercise (younger adults completed vigorous cycling exercise, older adults completed brisk treadmill walking). Age and exercise intensity are likely inter-related, as younger adults can exercise at a higher absolute intensity compared to older adults. In the experiments presented, although the relative intensity was ~85% HRmax for both younger and older adults, the absolute exercise intensity was 9 metabolic equivalents (METs) for the young adults and 4 METs for the older adults. This corresponds to vigorous intensity exercise for the young adults but moderate intensity for the older adults.

The effects of exercise intensity have been previously investigated and could, at least in part, explain the

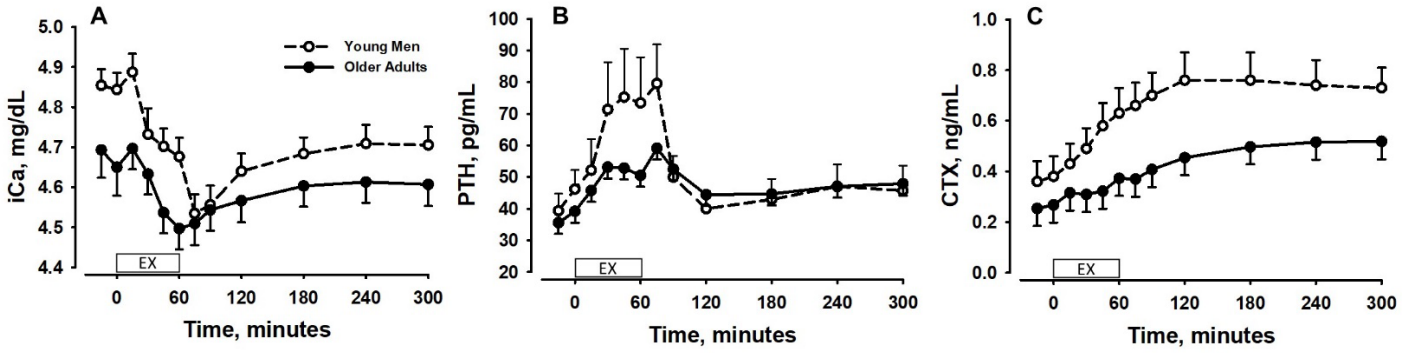

Figure 3. Changes in serum iCa (A), PTH (B), and CTX (C) in response to 1 h of exercise and 4 h of recovery. Dashed line, open symbols are data from young men during vigorous cycling exercise at ~85% HRmax (adapted from Kohrt, 2018). Solid lines, closed symbols are data from older adults during brisk treadmill walking at ~85% HRmax (Wherry, under review).

differences observed.[22] However, our preliminary data and the evidence presented in Figure 2 cannot rule out the possibility that mode of exercise is an important contributing factor. Without knowing how mode of exercise contributes to the difference in bone biomarkers, information is lacking about what types of exercise may be the most beneficial to bone. To be able to appropriately prescribe exercise for health and to maintain or improve quality of life, we must fully understand the positive and potential negative effects of the mode of

exercise on bone turnover. The proposed research is the first step in determining how exercise mode may need to be tailored to Veteran needs, especially older Veterans using exercise to reduce the risk of diabetes, cardiovascular disease, and/or osteoporosis, but may have orthopedic limitations that impair the ability to perform weight-bearing exercise.

IV. Research Methods

A. Outcome Measure(s): Change in iCa, PTH, CTX, and procollagen of type 1 n-terminal propeptide (P1NP; a marker of bone formation)

B. Description of Population to be Enrolled:

Participants. Healthy older (60+ y) women and men who are accustomed to regular exercise lasting approximately 1 hour in duration will be eligible to participate. All volunteers must be accustomed to performing 60 minutes of vigorous cycling and treadmill exercise.

Exclusion criteria include: **1)** impaired renal function, defined as an eGRF of <60 mL/min/1.73m^2;[33] **2)** hepatobiliary disease, defined as liver function tests (AST, ALT) >1.5 times the upper limit of normal; **3)** thyroid dysfunction, defined as an ultrasensitive thyroid stimulating hormone (**TSH**) <0.5 or >5.0 mU/L; **4)** serum Ca <8.5 or >10.3 mg/dL; **5)** serum 25(OH)D <20 ng/mL; **6)** uncontrolled hypertension, defined as resting systolic blood pressure (**BP**) >150 mmHg or diastolic BP >90 mmHg; **7)** history of type 1 or type 2 diabetes; **8)** cardiovascular disease; defined as subjective or objective indicators of ischemic heart disease (e.g., angina, ST segment depression) or serious arrhythmias at rest or during the graded exercise test (**GXT**); volunteers who have a positive GXT can be re-considered after follow-up evaluation, which must include diagnostic testing (e.g., stress echocardiogram or thallium stress test) with interpretation by a cardiologist; **9)** anemia, defined as a serum hemoglobin <12.1 g/dL for women and <14.3 g/dL for men; **10)** fracture in the past 6 months; **11)** current diagnosis or symptoms of COVID-19**.** In the event of abnormal BP, live function, TSH, 25(OH)D, or hemoglobin values, volunteers can be reassessed, including after appropriate follow-up evaluation and treatment by a primary care provider. Those who have experienced symptoms of COVID-19 or have been formally diagnosed will be allowed to participate once symptoms have resolved and they are approved to return to exercise by their primary care provider. This study will not enroll vulnerable populations. Non-English speakers will also be excluded due to the need to constantly communicate verbally to ensure participant safety during exercise testing.

C. Study Design and Research Methods

Screening tests. Volunteers will provide a medical history and undergo a physical examination by the study clinician. A blood sample will be obtained for screening chemistries (comprehensive metabolic panel, complete blood count, 25(OH)D, TSH). A treadmill GXT will be performed, with monitoring of BP and 12-lead electrocardiogram, as previously described.[10] The maximal HR attained during the test will be used to determine the exercise intensity for the submaximal exercise sessions. Peak oxygen consumption (**VO_2peak**) will be measured by indirect calorimetry using a TruMax 2400 metabolic cart (ParvoMedics, Sandy UT). Dual-energy x-ray absorptiometry (**DXA**) scans of the lumbar spine, proximal femur, and total body will be performed for the measurement of BMD and body composition, as previously described.[8]

Familiarization session. Eligible subjects will undergo a familiarization session to experience the targeted exercise intensity levels and to become accustomed to the exercise equipment. They will exercise for 20 minutes on the treadmill at 70-80% HRmax. After a ~15-minute rest, they will exercise for 20 minutes on the cycle ergometer at 70-80% HRmax. Subjects will provide a rating of perceived exercise (**RPE**)[34] every 5 minutes during exercise. Based on our experience, participants will be able to complete treadmill exercise, but high-intensity cycling may be challenging for some individuals. After 15 minutes of high-intensity cycling, they will be asked if they could complete 60 minutes at this intensity. If it is questionable, the power output will be decreased by 10% for the last 5 minutes of cycling and the question will be repeated. Subjects who do not believe they can complete 60 minutes of high-intensity cycling will not undergo the testing sessions.

Blinding and randomization. Neither the research team nor the participants can be blinded to the mode of exercise. However, samples will be labeled in a manner that ensures the individual analyzing the samples will blinded to the test condition. The order of the testing condition will be randomized and counter-balanced. Once a participant has been verified to meet enrollment criteria and initial sign-off by the study physician and the primary investigator has occurred, participant details (sex, ID number) will be entered into REDCap, which houses the randomization scheme (developed by the GRECC biostatistician). The randomization scheme will include allocation by sex, but not by race/ethnicity or age due to the small sample size.

Submaximal exercise sessions. Each participant will complete 2 exercise sessions that vary by mode: cycling at 70-80% HRmax and treadmill at 70-80% HRmax. HRmax will come from the GXT at screening. Each session will be 60 minutes in duration (plus a 5-minute warm-up at ~50% of HR max) and sessions will be at least 1 week apart. Sources of variance in the CTX response to exercise (primary outcome) other than mode of exercise may include pre-exercise Ca ingestion and the time of day the test is performed, because CTX has a diurnal rhythm.[35] To minimize variance from these sources, all exercise tests will be performed in the morning after a calcium-controlled meal and participants will be asked to avoid taking Ca supplements and multi-vitamins for 24 h before a test. Participants will be fasted when they return for the 24-hour blood draw.

Participants will arrive between 0900h and 1000h. After vital signs are obtained, an intravenous catheter for blood sampling will be positioned in a forearm vein. Participants will remain rested in a semi-recumbent position for ~15 minutes; a blood sample will be obtained at the end of this interval. After voiding, body weight (in hospital gown) will be measured and, after dressing, participants will move to the cycle ergometer or treadmill and remain seated or standing for 5 minutes before exercise. A second pre-exercise blood sample will be obtained immediately before exercise. This sampling paradigm will enable us to address the contention that positional changes have not been adequately considered in evaluating the changes in plasma volume in response to cycling and running.[36] Participants will be allowed deionized water *ad libitum* during exercise and recovery. Water consumption and urine volumes will be recorded for calculation of plasma volume changes. They will return between 1000h and 1100h the next morning after a 10- to 12-h fast for the 24-h blood sample.

Blood collections (Fig 4) and assays. For each of the exercise tests, two pre-exercise blood samples will be obtained as described in the preceding paragraph. Blood samples (10 mL each) will be obtained after 15, 30, 45, and 60 minutes (exercise), and at 75, 90, 120, and 300 minutes during recovery, and 24 hours after the end of exercise. An additional blood sample 48 hours after exercise will be available as an optional procedure for analysis of iCa, P1NP, and CTX. After exercise, participants will remain in a semi-recumbent position for the duration of recovery. Blood samples will be processed by the CTRC Core Laboratory (partial off-site waiver will be pursued) and stored at -80°C. Samples from all exercise sessions for an individual will be analyzed in batch to minimize batch effects. The exceptions to this will be the parameters measured in real time (hematocrit (**HCT**), iCa, pH) during the study visits by the iSTAT analyzer (Abbot Point of Care, Princeton, NJ). Serum (or plasma) assays will include: **1)** PTH by immulite two-site enzyme immunoassay (Siemens, Erlangen, Germany; CTRC Core Lab), **2)** CTX by chemiluminescence (Immunodiagnostic Systems, Boldon Business Park, UK; CTRC Core Lab), **3)** P1NP by chemiluminescence (Immunodiagnostic Systems; CTRC Core Lab), and **4)** tCa by indirect ion-selective electrode (Beckman Coulter Inc; UCH Clinical Lab). Laboratories where assays will be conducted are CAP and CLIA certified. Laboratory-specific intra- and inter-assay coefficients of variations are generally lower than those determined by the manufacturers. PTH, CTX, iCa, and tCa, concentrations during and after exercise will be adjusted for shifts in plasma volume as previously described.[8] These outcomes cannot be assayed in a VA laboratory due to the lack of the necessary combined analysis system. Performing each assay individually in a wet lab would double the blood required, increasing participant burden and risk.

Feasibility measures. Participants will be asked to rate multiple aspects of their experience, including preference for each mode of exercise, ease of getting to and from the exercise facility, and their likelihood of participating in a center-based exercise program that would require multiple trips to the VA facility several times per week over multiple months. This information will be used to inform future intervention designs.

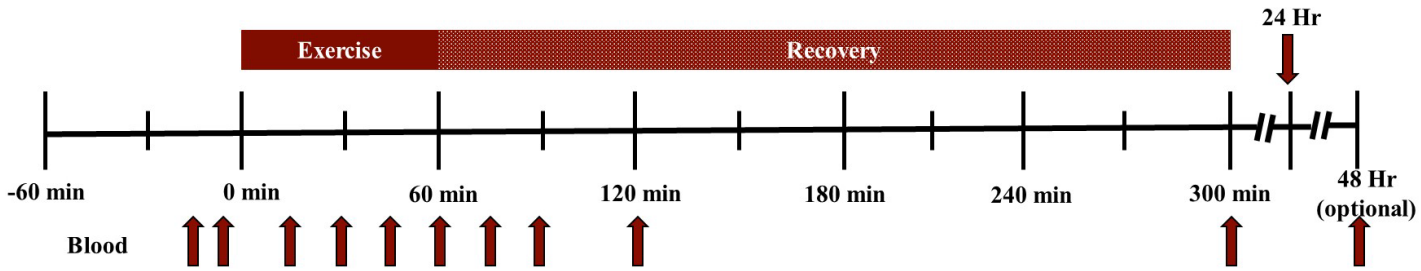

Figure 4. Timing of blood and urine collections before and during 60 minutes of exercise and 4 hours of recovery.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Potential Risks:

Exercise: The risks of this study are those of any exercise testing or exercise program and include musculoskeletal injury, falls during exercise, and symptoms of unknown underlying cardiac disease.

Ionizing radiation: Participants will be exposed to radiation from the dual-energy x-ray absorptiometry (**DXA**) testing that will occur at screening. Like any x-ray imaging modality, the DXA system used in this study exposes the participant to ionizing radiation. The ionizing radiation from the DXA scan of the total body, pelvic region, and lumbar spine is roughly the equivalent dose of 4 days of radiation exposure from the environment.

Venipuncture and IV: There is a small risk of local hematoma, infection, syncope, and thrombosis associated with intravenous blood sampling.

Confidentiality and privacy: The use of questionnaires, interviews, and collection of personal medical information poses a risk to confidentiality and privacy and may cause embarrassment.

Psychological risks are minor and include frustration with exercise program and frequent visits to the research facility.

Sources of Materials: Data from each participant's medical history will be acquired from patient interview and from the patient medical record to assure they are appropriate for an exercise study. Data will be acquired with the patient's permission during the screening process. Once the patient is enrolled in the study, additional information may be acquired from the participant's medical record and recorded in the study database. This includes information such as medical history and medications. Participants will also be interviewed for additional information such as exercise history, history of bone and muscle problems, as well as sedentary behavior. Blood samples will be collected at the time of enrollment for assessment of renal function and electrolytes and additional samples will be stored for future analysis of bone turnover markers and inflammatory mediators.

The material, records, and data obtained through participation in the study will be specifically for research purposes. All collected data will be entered and stored in the Research Electronic Data Capture (**REDCap**) database. This database was designed specifically for clinical research data. It is a web-based secure application and is freely available through the University of Colorado Clinical and Translational Sciences Institute and the VA. REDCap is ideal for this study in that it allows data to be securely entered at each site of data collection. An important REDCap feature is that different staff can be given different levels of data access so that only appointed personnel can enter and adjusted collected data. PHI is not stored in REDCap but on a secure server separate from the research data. The study team will have access to the PHI. This includes participant name, phone number, address, and medical record number. REDCap data and only the PHI necessary for analysis will be linked by study number. Once analysis is

complete, all PHI will be removed. Imaging data will be stored on a VA research group server, which is password-protected and backed up regularly by the VA. Data that is not generated from a core lab (such as questionnaire data) will be double-entered via REDCap based on unique participant identifiers for analysis and later merged with the VA server data.

Therapeutic Risk: There are no therapeutic risks in this study.

Research Risk: This is an exercise intervention, there are no alternatives. Additional procedures include radiologic testing with DXA. There are no alternatives to this testing.

To reduce the risk of the submaximal exercise bout, an initial chart review will occur to acquire history. If there are any concerns the patient's history will be discussed with their physician. To reduce the risk of cardiac events, potential participants with an abnormal ECG, resting blood pressure ≥150/90 or with abnormal exercise testing will be excluded from further participation in the study. To reduce the risk of bony injury during the exercise tests, participants will receive a bone density scan. Those participants who qualify for osteoporosis with a T score of ≤ -2.5 at the lumbar spine or either femoral neck will be excluded from further study participation.

As part of the fitness testing for the exercise prescription and to gauge safety/screen for cardiovascular abnormalities, baseline graded exercise testing will be performed by the research assistant and either a nurse practitioner, a physician assistant, or a physician. The research assistant will administer the test (change treadmill speed and grade, assess ratings of perceived exercise, operate metabolic cart) and the clinician will interpret the ECG, monitor clinical signs and symptoms, and alert research staff of any abnormalities. During the 1-reptition maximum testing, only trained personnel will perform these tests. Both tests will occur in dedicated research space with immediate access to emergency response staff, if needed. Proper chart review by the PI and the study physician will help to protect against any participants with known cardiovascular or musculoskeletal conditions from proceeding to portions of the study that may result in physical harm.

All exercise testing visits will be one-on one supervised and occur in a private setting. Study present at these visits will include a nurse or nurse practitioner, the study physician, the study research assistant, and Dr. Wherry. All staff members are trained in CPR and proper exercise technique. While exercising, participants will be monitored throughout their exercise protocol for any concerning musculoskeletal or cardiopulmonary symptoms. Participants who experience any concerning symptoms will be asked to stop exercise and will be immediately evaluated.

All members of our research group are trained to manage urgent and emergent situations in the exercise laboratory. Our group conducts quarterly mock codes and will have ad-hoc mock codes when new staff are hired for exercise supervision. These mock codes cover topics related to exercise-related injuries or emergencies, such as: 1) shortness of breath, low oxygen saturation, abnormally high/low injury; 2) falls, strains, or other musculoskeletal injuries; 3) nausea, vertigo, lightheadedness, loss of consciousness; 4) general feelings of fatigue. The exercise gym is located within a University of Colorado Hospital building with access to clinical staff and emergency care in the event of an emergency. We have a multi-step documenting method to record any adverse events that may be related to the exercise protocol that occur during supervised exercise. This documentation includes: 1) an on-site record of emergency contact information; 2) paper copies of any vital signs or signs/symptoms recorded by gym staff before emergency personnel arrive; 3) carbon copies of all recorded vitals to accompany participants to the emergency room (if needed), a copy for the research gym, and a copy for the study physician on the protocol.

To reduce risks associated with blood sampling, only trained personnel perform the procedures using sterile techniques. This is the same for reducing the risks of ionizing radiation. Scans will only be performed by trained technicians, which includes Dr. Wherry. All participants will be scanned using pre-determined protocols from the manufacturer.

To reduce concerns regarding confidentiality sharing of personal information during interview and questionnaires, subjects will be interviewed in a private room. The identity of each study subject will be protected by assigning a study specific identification number. Study specific identification numbers will be used for all participant data. The key which ties the study subject to their PHI will be kept separate from

the database on a secure server at the VA. All data sheets will be stored in a double lock system with access granted only to those directly affiliated with the research protocol. Data for publication will be presented only in aggregate form preventing identification of individual participants.

Data and Safety Monitoring

Data collection at study visits will take the form of subjective measures (forms) and will be identified with a participant ID number. Data will be collected and stored with the participant ID code only. The research coordinator will keep the code that links the participant ID with the identity of the participant in a secure location and will store it separately from the data on the secure server at the VA. Data will be stored on data forms and entered into the REDCap database which is a secure web-based database made available to researchers through the VA. No data or subject information will be stored on computer hard drives. No PHI is permitted in the REDCap database. Needed PHI will be kept separate and securely form research data. Data management will be provided through the GRECC and the data manager will construct database for recruitment and participant procedures. Data will be quality controlled using random double data entry to identify discrepancies. Data will be audited for missing data on a monthly basis and missing data acquired if possible. Data regarding participant adherence to the intervention will be reported at the monthly investigator meeting and strategies to improve adherence recommended.

Unanticipated Problems Involving Risks to Subjects or Others: Members of the VA ECHCS research community are required to ensure that unanticipated problems involving risks to subjects or others in research are reported promptly to the appropriate IRB in accordance with the time period established by each VA ECHCS IRB of record.

Serious Unanticipated Problems Involving Risks to Subjects or Others: Within 5 business days of becoming aware of any serious unanticipated problem involving risks to subjects or others in VA research, members of the VA ECHCS research community are required to ensure that the problem has been reported in writing to the appropriate IRB of record for the study, including incidents related to research information protection.

Incidents related to research information protection which include: unauthorized access to VA sensitive information (including unauthorized use, disclosure, transmission, removal, theft, or loss) related to research, including but not limited to, protected health information, individually-identifiable private information and confidential information protected by HIPAA, or by Federal records requirements; any research-related incident reportable to the Office of Information and Technology (OI&T) Network Security Operation Center (NSOC) that impacts, inhibits, or compromises network security, members of the VA research community are required to ensure that the situation has been reported to the ACOS/R&D, the facility ISO, and the facility PO within 1 hour of becoming aware of the incident The ACOS/R&D must immediately notify the VA ECHCS Director, the RDC, and any relevant research review committee, upon discovering, receiving, or otherwise becoming aware of a credible report of a research information protection incident and must ensure that the facility ISO and facility PO have been notified. These immediate reporting requirements are in addition to the regular reporting requirements to the IRBs, as stated in the first paragraph in this section.

Local Unanticipated SAEs: Within 5 business days of becoming aware of any local (i.e., occurring in the VA ECHCS) unanticipated SAE in VA research, members of the VA ECHCS research community are required to ensure that the SAE has been reported in writing to the appropriate IRB. NOTE: This requirement is in addition to other applicable reporting requirements (e.g., reporting to the sponsor under FDA requirements). The unfounded classification of an SAE as “anticipated” constitutes serious noncompliance.

All adverse events (AE’s) occurring during the study will be collected, documented, and reported to the PI. Every month the investigators will review AE forms for the month. The study investigators will follow all AE’s to the point of a satisfactory resolution. All AEs will be reported to the VA and COMIRB in accordance with institutional policies. Serious adverse events (SAEs), whether or not related to the study protocol, will be reported (within 24 hours) to the local IRB. Any recommended changes to the protocol as a result of monthly monitor meetings will be submitted and approved by the IRB prior to implementation. A summary of the SAEs that occurred during the previous year will be included in the annual progress report. The

principal investigator will be responsible for safety monitoring and review de-identified data on a quarterly basis and as necessary.

E. Potential Scientific Problems:

Recruitment may be a challenge due to the limited number of older women Veterans and the physical activity requirements of potential participants. However, the PI is involved with a clinical exercise program that includes women Veterans in the appropriate age range, and they will be provided the option to volunteer for the proposed research. Additionally, our research group has performed targeted recruiting through our local Community-Based Outpatient Clinics (CBOCs), the Corporate Data Warehouse (CDW), and VA Information and Computing Infrastructure (VINCI) and we have been successful in recruiting similar participants for other VA research studies.

F. Data Analysis Plan:

Sample size and statistical analysis plan. The primary outcome is the change in CTX from pre-exercise to the end of exercise. The change in CTX is hypothesized to be positive for both conditions. With 30 subjects, we will have at least 80% power to detect a change of 0.12 ng/mL or greater between conditions. The study is not powered to detect subgroup differences on the effects of sex. However, exploratory analyses will be conducted to be able to power future studies and to generate hypotheses. To achieve 15 completers per sex, 18 men and 18 women will be enrolled based on an expected drop-out rate of ~20%.

The effects of treadmill versus cycling exercise on the change in CTX will be evaluated using linear contrasts in a repeated measures maximum likelihood model with all available data. We have utilized this approach in similar experiments,[9,37,38] and it is conceptually identical to repeated measures analysis of variance but avoids the deletion of cases with missing assessments. Estimates are unbiased under the assumption that missing data are missing at random. Linear contrasts will be used to estimate within-condition changes and between-condition differences in changes over the 60-minute exercise bouts and the recovery periods separately. Primary comparisons will include up to the 24-hour blood draw. Secondary analyses will be conducted utilizing the 48-hour timepoint, but results will be interpreted with caution due to the potential for low sample size. This analysis will be repeated for secondary outcomes (change in iCa, tCa, PTH). The distributions of the outcome measures will be checked. If there are severe departures from a normal distribution, transformations (e.g., natural logarithm) will be applied as appropriate to achieve an endpoint that is approximately normally distributed. To minimize missing data, all participants will be informed of the importance of complete follow-up and encouraged to complete the study per the protocol. Although statistical methods can be used to adjust for missing data, these methods rely on the untestable assumption that data are either **1)** missing completely at random, for which no adjustment is needed, or **2)** missing at random, for which the effect of the “missingness” can be removed through statistical modeling. We will instead focus on preventing missed exercise tests and samples. Due to the mechanistic nature of the studies and the use of the results to guide decisions about a future randomized controlled trial, no adjustments for multiple comparisons will be performed. All analyses will be conducted using SAS software version 9.4 M4 (SAS Institute Inc, Cary, NC, USA) and a p-value ≤ 0.05 defining statistical significance. For the exploratory analyses in SA2, we will use the same statistical approach as SA1 while including sex in the maximum likelihood model. Results will be interpreted with caution due to the low sample size, and will be used to generate hypotheses for future research.

G. Summarize Knowledge to be Gained:

The results from this pilot study will provide the foundation for further study to discern the most effective types of exercises to improve bone strength and ultimately reduce the risk of fracture. The current exercise recommendations for the prevention and treatment of osteoporosis have evolved from preclinical research (i.e., animal models) on the loading characteristics of an activity that are important for the activation of bone formation (i.e., dynamic, high magnitude bone-loading forces, high strain rate). The current application is focused on how the mode of exercise can influence an acute bone biomarker response, which could impact bone strength and fracture risk long-term. In the short-term, this study will be informative to

determine *if* mode of exercise is an important determining factor in the bone biomarker response to exercise, which will aid in the design of future trials. Longer-term, if a difference in mode of exercise is found, this research will be used to determine what discrepancy exists between modes of exercise, and how different exercise programs can be designed to maximize benefit to bone. This is especially relevant to older Veterans, as service-related orthopedic limitations do not always allow for all forms of exercise to be completed safely, but this research could lead to strategies to enhance the benefits of exercise on osteoporosis prevention for both Veterans and the general public.

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