Protocol #: 21-3163
Project Title: Enhancing Skeletal Adaptation to Exercise by Attenuating the Acute Disruption of Calcium Homeostasis During Exercise
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I. Hypotheses and Specific Aims:

Weight-bearing exercise is critical for optimal bone health.[1,2] However, our recent work, corroborated by others,[3-14] has raised the possibility that current exercise recommendations for bone health may not be appropriate. Multiple studies have confirmed that moderate-to-vigorous exercise stimulates a robust **acute catabolic response** in bone that persists for several hours after exercise. It is mediated by a transient increase in parathyroid hormone (**PTH**) to minimize the decline in serum calcium (**Ca**) during exercise. However, PTH has paradoxical actions on bone, such that a chronic increase in PTH is *catabolic*[15] but repeated transient increases in PTH are *anabolic*.[16-20] Indeed, two medications used to *treat* osteoporosis, teriparatide and abaloparatide,[21] are analogs of PTH. It is not known whether transient *exercise-induced* increases in PTH ultimately generate an anabolic skeletal response with exercise training, similar to that achieved pharmacologically with teriparatide or abaloparatide.

In this context, the **POC phase** will address the question of whether the exercise-induced increase in PTH **1)** generates **only** an **acute catabolic response** in bone that occurs each time exercise is performed (i.e., no attenuation with training), or **2)** generates **both** the **acute catabolic response** and a **chronic anabolic response** that becomes apparent after multiple exercise sessions. If the evidence from the POC phase supports option 1 (i.e., catabolic only) and not option 2 (i.e., catabolic and anabolic), this will support the need for the **RCT** to evaluate whether Ca supplementation before exercise attenuates the acute PTH response to exercise and, therefore, the catabolic response, and whether this enhances skeletal adaptations to exercise training.

*Specific Aims (**SA**) and Hypotheses (**H**) for the POC Phase:*

SA1: Confirm that repeated bouts of exercise continue to elicit the acute catabolic response observed with a single bout of exercise. This will be evaluated by having participants exercise 4 d/wk for 4 wk and measuring changes in a serum marker of bone resorption (i.e., C-telopeptide of type 1 collagen; **CTX**) during and after the 1st, 8th, and 16th sessions.

H1: Repeated bouts of exercise will continue to stimulate an acute catabolic response with an increase in bone resorption, as demonstrated by significant increases in serum CTX from before to after exercise in the 1st, 8th, and 16th exercise sessions.

SA2: Determine whether repeated exercise-induced increases in PTH generate an anabolic skeletal response over time, similar to the anabolic response to pharmacologic PTH analogs (teriparatide, abaloparatide). This will be evaluated by measuring the change in a serum marker of bone formation (i.e., increase in serum procollagen type 1 N-terminal propeptide; **P1NP**) from the 1st to the 16th exercise session.

H2: Exercise-induced increases in PTH will generate a chronic anabolic response in bone, as demonstrated by an increase in pre-exercise serum P1NP from the 1st to the 16th exercise session.

If the results of the POC phase **support H1** (significant catabolic responses) and **refute H2** (non-significant anabolic response), we will proceed to the RCT to determine whether Ca supplementation before exercise can attenuate the catabolic skeletal response and enhance skeletal adaptations to training.

Research Methods

A. Outcome Measure(s): Change in CTX from before to after exercise, Change in pre-exercise P1NP from 1st to 16th exercise session.

B. Description of Population to be Enrolled:

Participants. We will enroll female and male Veterans aged 25 to 45 y or 55 to 75 y. Eligible volunteers will be normally active (e.g., recreational cycling or walking) but will not participate in regular moderate-to-vigorous exercise. Women will be premenopausal with regular menstrual cycles or postmenopausal, defined as

absence of menses for at least 12 mo or, in those who underwent a hysterectomy, a serum follicle stimulating hormone (**FSH**) >30 mIU/mL.

Exclusion criteria include: **1)** initiation or change in dose in the past 6 months of medications that affect bone metabolism (e.g., osteoporosis medications, thiazide/loop diuretics, systemic glucocorticoids); **2)** BMD T-score ≤-2.5 at the total hip, femoral neck, or lumbar spine; **3)** impaired renal function, defined as an estimated glomerular filtration rate (**eGFR**) <60 mL/min/1.73m^2;[22] **4)** abnormal alkaline phosphatase; **5)** untreated thyroid dysfunction, defined as an ultrasensitive thyroid stimulating hormone (**TSH**) <0.5 or >5.0 mU/L; **6)** serum Ca <8.5 or >10.3 mg/dL; **7)** serum 25(OH)D <20 ng/mL; **8)** uncontrolled hypertension (resting systolic blood pressure (**BP**) >150 mmHg or diastolic BP >90 mmHg); **9)** type 1 diabetes; **10)** type 2 diabetes if on insulin or sulfonylurea therapy; **11)** hemoglobin A1c >7%; **12)** 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 by a cardiologist; **13)** anemia (hemoglobin <12.1 g/dL for women, <14.3 g/dL for men); **14)** fracture in the past 6 months, and **15)** body mass index >39 kg/m^2. In the event of abnormal eGFR, alkaline phosphatase, TSH, BP, 25(OH)D, or hemoglobin values, volunteers can be reassessed, including after appropriate follow-up evaluation and treatment by their health care provider.

C. Study Design and Research Methods

C.1. Screening procedures

Volunteers will provide a medical history and undergo a physical examination by a study clinician. A blood sample will be obtained for screening chemistries, which will include a comprehensive metabolic panel, complete blood count, 25(OH)D, and TSH (and FSH for women without a uterus). A treadmill graded exercise test (**GXT**) will be performed, with monitoring of BP and 12-lead electrocardiogram and measurement of peak oxygen consumption (**VO_2peak**).

The protocol for the GXT will be as previously described.[5] Briefly, the volunteer will rest for ~15 min before a resting ECG and BP are recorded. If there are no contraindications to proceeding, the participant walks on the treadmill for ~5 min to find the speed (at 0% grade) that generates a HR that is ~70% of the age-predicted HRmax. After a brief rest, the GXT will start at the speed achieved during the warm-up, and treadmill grade will by increased by 2% every 2 min until volitional exhaustion or until the test is stopped by the proctor. The PI has used this protocol for young and older women and men over the past 20 years and found that it results in a test duration of 8 to 12 min (the goal) in most participants. The HRmax attained during the test will be used to determine the exercise intensity for the submaximal exercise sessions. VO_2peak will be measured by indirect calorimetry using a TruMax 2400 metabolic cart (ParvoMedics, Sandy UT). Contraindications to start or continue the GXT will be those recommended in the American College of Sports Medicine *Guidelines for Exercise Testing and Prescription*.[23]

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.[4] The scans will be reviewed by Dr. Swanson, who is an ISCD-certified clinical densitometrist. If there is the possibility that a participant could be pregnant (e.g., premenopausal woman), a urine pregnancy test will be conducted prior to the DXA scan.

Study eligibility will be determined by completing a checklist that includes all eligibility criteria. Two investigators, including one clinician, will review the checklist to confirm whether a volunteer is or is not eligible.

C.2. Exercise intervention

All participants will undergo the same exercise intervention for the POC phase, so there is no randomization. The exercise will be treadmill walking at 70-80% of HRmax for 60 min/d, 4 d/wk, for 4 wk. We will extend the intervention up to 2 weeks (total of 6 weeks) to enable participants to complete 16 exercise sessions. Compliance will be defined as completing at least 75% of the prescribed exercise sessions. The mode and intensity of exercise were selected because high-intensity weight-bearing exercise is recommended for bone health, and walking is the most common weight-bearing activity. Although “high-intensity” exercise for bone health refers to the intensity of bone-loading forces, %HRmax is a good proxy because peak bone-loading forces increase as walking or running speed increases.[24,25]

It might be questioned whether untrained adults, and particularly older adults, can tolerate relatively high-intensity exercise. This exercise prescription was used in the studies of young and older adults described above and resulted in robust PTH and CTX responses (Fig 5, 8) What may not be appreciated is that untrained older adults can exercise at a high relative intensity with less stress or fatigue than young adults. Dr. Kohrt conducted a study that compared plasma lactate, norepinephrine, and epinephrine responses to high-intensity exercise (78% of VO_2max) in untrained young (20 to 35 years) and older (60 to 74 years) women and men. Exercise at the same relative intensity resulted in significantly lower lactate (3.7±0.1 vs 6.5±0.5 mmol/L), norepinephrine (1,444±74 vs 1,983±222 pg/mL) and epinephrine levels (109±10 vs 228±29 pg/mL) in older adults than in young.[26] Subjective ratings of perceived exertion during high-intensity exercise are also lower in older adults than typically observed in young adults.[27] Based on our extensive experience, study participants will be able to complete the exercise intervention

C.3. Bone resorption and formation responses to exercise

To achieve the Aims, markers of bone resorption and formation will be measured before, during, and after the 1st, 8th, and 16th exercise sessions. The exercise stimulus will be the same for all sessions. For each of these sessions, participants will arrive at the CTRC between 0700h and 0800h following an overnight fast. They will be asked to keep a record of what they ate the evening prior to the blood collection visit and will be asked to eat the same meal prior to all blood collection visits. An intravenous catheter for blood sampling will be positioned in a forearm vein. Participants will remain rested in a semi-recumbent position for ~15 min; a blood sample will be obtained at the end of this interval. After voiding, body weight will be measured, and participants will move to the treadmill and remain standing for 5 min 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.[28] After exercise, participants will remain in a semi-recumbent position during a 4-h recovery interval. They will be allowed water *ad libitum* during exercise and recovery. Water consumption and urine volumes will be recorded for calculation of plasma volume changes. A standardized snack will be provided after the 120-min blood draw. Macronutrient composition will be 50% carbohydrate, 35% fat, and 15% protein, and total energy content will be ~500 kcal for men and ~350 kcal for women. Participants will return to the CTRC between 0800h and 0900h the next morning after an overnight fast for the 24-h post-exercise blood sample.

Blood and urine collections will be the same for each of the three exercise sessions. Two pre-exercise blood samples will be obtained as described in the preceding paragraph. Blood samples (~5 mL each) will also be obtained after 15, 30, 45 and 60 min of exercise, during the 4-h recovery as indicated in Fig 20, and 24 h after exercise. Urine samples will be obtained shortly after the completion of the exercise session and after 4 h of recovery; other voids during the recovery period will be pooled with the final sample. Urine will then be collected through the 24-h post-exercise visit. Urine and blood samples will be processed by the CTRC Core Laboratory and stored at -80°C. Samples from all 3 exercise sessions for an individual will be analyzed in batch to minimize between-batch variance. 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). Urine samples will be assayed for total Ca (**tCa**) at the University of Colorado Hospital (**UCH**) Clinical Laboratory by indirect ion-selective electrode (Beckman Coulter Inc, Brea, CA, USA). Urine Ca concentration will be multiplied by void volume to calculate Ca loss for each time interval. Serum (or plasma) assays will include: **1)** PTH by chemiluminescent immunoassay (Beckman Coulter Inc; 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), **4)** tCa by indirect ion-selective electrode (Beckman Coulter Inc; UCH Clinical Lab), **5)** phosphorus (**PO4**) by colorimetry (UCH Clinical Lab), and **6)** hemoglobin (**Hgb**) by automated cell counter (UCH Clinical Lab). PTH, CTX, iCa, tCa, and PO4 concentrations during and after exercise will be adjusted for shifts in plasma volume as previously described.[4]

Participation in the study is considered completed when one of the following criteria is met:

- Participant completes the final study procedure
- Participant elects to withdraw from the study

- Study clinician decides to withdraw the participant because of a health or safety concern

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

Potential Risks:

Exercise: The potential serious risks of exercise testing and training include the development of serious arrhythmias, cardiac arrest, myocardial infarction, asthma, and death; these are rare. More common but less serious risks include musculoskeletal (muscle, ligament, tendon, joint) discomfort or injury.

Fasting: Participants will be asked to fast prior to the screening blood draw and before each of the blood collection visits. Participant risks include feeling faint or lightheaded or loss of consciousness. The risk of feeling faint is common but not serious. The risk of loss of consciousness is rare.

Ionizing radiation for the POC: Participants will be exposed to radiation from the dual-energy x-ray absorptiometry (**DXA**) testing that will occur at screening. The ionizing radiation from the DXA scan of the total body, pelvic region, and lumbar spine for the POC study is roughly the equivalent dose of 4 days of radiation exposure from the environment.

Ionizing radiation for the RCT: Participants in the RCT will be exposed to ionizing radiation for the DXA and HR-pQCT scans at baseline and follow-up. The ionizing radiation from the DXA scan of the total body, pelvic region, and lumbar spine for the POC study is roughly the equivalent dose of 4 days of radiation exposure from the environment. The radiation exposure for the HR-pQCT is similar to the DXA. The total effective radiation exposure over 36 weeks for the RCT is <40 mrems, which is ~8% of the average annual exposure in the U.S.

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

Randomization: Participants will be assigned to either study condition by chance, and each condition may differently influence how the skeleton adapts to exercise. They also may have more side effects, such as GI upset, with one study condition versus the other condition.

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.

Ca citrate and vitamin D3 for the RCT: Side effects of Ca intake include weakness, confusion, fatigue, headache, and bone pain; these are rare. More common side effects of Ca intake are constipation and nausea. Rare side effects of excess D3 intake include hypercalcemia, hypercalciuria, and skin rash.

Sources of Materials: Data from the medical history will be acquired from participant interview and from the medical record to ensure eligibility for an exercise study. Permission to access information will be obtained during the screening process. Once the volunteer is enrolled in the study, additional information may be obtained from the 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 and history of bone and muscle problems. Blood samples will be collected at the time of enrollment for assessment of eligibility criteria and additional samples will be stored for analysis of bone turnover markers and factors association with the disruption of Ca homeostasis.

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 VA and the University of Colorado Clinical and Translational Sciences Institute. 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, birthdate, social security number (for participant payment) 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 are not generated or received

electronically will be manually double-entered in REDCap based on unique participant identifiers for analysis and later merged into the VA server.

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

Research Risks: The exercise testing and training for the POC and RCT are required to address the Aims and hypotheses and there are no alternative approaches. Additional procedures include radiologic testing with DXA and HR-pQCT and there are no alternative testing approaches.

Risks of exercise testing and training are minimized by the following strategies:

Endpoints for maximal exercise tests. In asymptomatic individuals who do not develop cardiovascular abnormalities, the endpoint for the maximal exercise tests will be volitional fatigue. The criteria that will be used to stop the exercise test before volitional fatigue include the development of: a) ST-segment depression of more than 0.2 mV that is either horizontal, downsloping, or slowly upsloping (less than 1 mV/sec) and lasts for 0.08 sec, or ST-segment elevation greater than 0.1 mV; b) chest pain or discomfort; c) serious arrhythmias, including multifocal PVCs, ventricular tachycardia, frequent (>10/min) PVCs or couplets, or sustained atrial tachyarrhythmias; d) A-V block or other conduction defects; e) a fall of systolic blood pressure of 10 mmHg or greater from the peak level with increasing exercise intensity; f) diastolic blood pressure above 110 mmHg or systolic above 220 mmHg; g) dizziness; h) ataxic gait; and i) pallor or cyanosis.

Submaximal exercise testing and training. The intensity for the submaximal exercise tests and training sessions will be individualized, based on performance during the maximal exercise test, such that the relative intensity is similar for all participants. Participants will be allowed (and encouraged) to drink water *ad libitum*.

Training of personnel. Study personnel assisting with the administration of screening tests will have BLS certification. A clinician will be available in the facility if not directly proctoring the screening tests. A clinician will interpret ECG and BP responses for screening tests.

Screening of volunteers. To minimize risks of exercise testing in subjects, testing will be conducted only after the volunteer is examined by a clinician and after a resting ECG is obtained and evaluated. During exercise testing, the ECG is monitored constantly, and BP is measured frequently. The AHA-recommended emergency equipment and supplies will be available, including: automated electronic defibrillator; portable oxygen tank, nasal cannula, ventimask, non-rebreathing mask, and appropriate tubing to connect to the oxygen tank; oral airways; bag-valve-mask hand respirator; syringes and needles; IV tubing, solutions, and stand; and adhesive tape.

The risks of blood sampling are minimized by having trained personnel perform the procedures using sterile techniques. For participants with a history of syncope during blood sampling, procedures will be performed in the supine position.

The risk of fasting is minimized by closely monitoring participants during screening and exercise. If participants report feeling unwell, the test will be stopped and food will be offered.

The risks associated with radiation exposure during the DXA and HR-pQCT procedures are minimized by having trained technicians conduct the scans, thereby reducing the likelihood of needing repeat assessments. Anyone who could be pregnant will be required to take a urine pregnancy test prior to any scans.

The risks associated with loss of privacy and confidentiality will be minimized by using coded identification numbers in place of names, conducting interviews and collecting personal information in a private setting, and by following regulations for the secure management of research data. Confidentiality of medical records will be strictly maintained by established procedures. For all research data records, participants will be identified by coded identification numbers that are unique to the study and unrelated to any identifying numbers (e.g., social security, medical record, birthday). Only the research staff will have access to these codes. All hard copies of data will be stored in a locked cabinet in a locked office. For Veteran data stored at the VA, procedures designed to maintain confidentiality will include formal training sessions for all study personnel in the importance of confidentiality and procedures to be followed, and formal mechanisms for limiting access to all information that can link data to individual participants. The PI, the VA RDC, and the VA Privacy Officer will work together to ensure integrity and adherence to participant confidentiality.

Data and Safety Monitoring

Data and safety monitoring will be provided by the VA Clinical Sciences Research & Development (CSRD) Data Monitoring Committee (**DMC**). The DMC is an independent multidisciplinary group, whose members have the requisite knowledge pertinent to the subject areas to be reviewed. Membership details are available on the CSRD website. The DMC will provide ongoing independent evaluation of the study focused on safety and feasibility, including participant accrual and retention, adverse events, and data analyses. Meetings will be held two times per year, at which time recommendations will be made to the Director of CSRD for endorsement. These recommendations will range from approval to continue (unconditionally or with conditions to be addressed) to probation or possibly termination if there are problems with enrollment or safety concerns.

*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 include: unauthorized access to VA sensitive information (including unauthorized use, disclosure, transmission, removal, theft, or loss) related to research, including but not limited to, PHI, 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 report such incidents 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 IRB.

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). The unfounded classification of an SAE as “anticipated” constitutes serious noncompliance.

All non-serious adverse events (AEs) 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 AEs 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) will be reported (within 5 days) 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 AEs that occurred during the previous year will be included in the annual progress report. The PI will be responsible for safety monitoring and review de-identified data on a quarterly basis and as necessary.

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