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Project Title: PTH And Calcium Responses to Exercise in Older Adults Experiment 2 (PACE Sr. 2)
Principal Investigators: Sarah Wherry, PhD & Wendy Kohrt, PhD
Version Date: 05/13/2024

1. Specific aims and hypotheses

Physical activities that load the skeleton through ground- and joint-reaction forces are essential to the maintenance of bone health. Although exercise training is typically thought to have favorable skeletal effects, there is evidence that it can sometimes lead to a *decrease* in bone mineral density (BMD). For example, we found a decline in hip BMD of -1.5% in male road cyclists over a year of training and competition,[1] a rate of loss similar to that of postmenopausal women. The decrease was not explained by changes in body weight or composition. Although cycling may not be an optimal bone-building activity because it is weight-supported, rather than weight-bearing, it remains unclear why it would cause an accelerated loss of BMD. An exercise associated loss of bone mass has also been observed in response to weight-bearing exercise. Male college basketball players were found to have a decrease in bone mass over a year of training and competition.[2] Interestingly, the loss was reversed the following year when additional calcium (Ca) supplementation was provided during practices. *The mechanisms by which exercise training sometimes results in a loss of BMD, even when loading forces are seemingly high (e.g., basketball), are not known*, but could include such possibilities as low energy availability, hypogonadism, metabolic acidosis, excess cortisol exposure, or disrupted Ca metabolism. **The global aim of the proposed research is to investigate a novel and plausible mechanism for exercise-related bone loss. We postulate that the disruption of Ca homeostasis during acute exercise is a trigger for the activation of bone resorption.** The working model (Fig 1) portends that excessive dermal Ca loss (i.e., sweating) causes a decline in serum ionized Ca (iCa; the unbound fraction) and triggers an acute increase in parathyroid hormone (PTH).[3] PTH can defend serum Ca through multiple pathways, including reduced urinary Ca excretion, increased intestinal Ca absorption, and increased bone resorption to mobilize skeletal Ca. If an increase in bone resorption occurs repeatedly over multiple exercise sessions (i.e., exercise training), and is not accompanied by appropriate loading forces to stimulate bone formation, this could lead to a decrease in BMD over time (or diminish the increase in BMD).

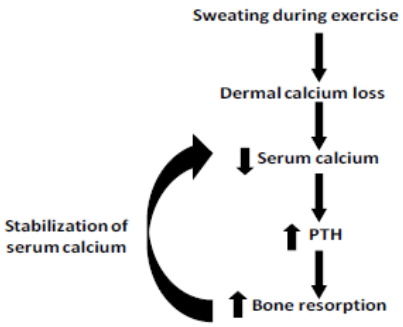

Figure 1: conceptual model by which dermal Ca loss during exercise triggers a decline in serum Ca and compensatory increases in parathyroid hormone (PTH) and bone resorption which stabilizes serum calcium.

1.1 Overarching Hypothesis

The **overarching hypothesis** for this line of research is that **exercise that causes a decline in serum Ca as a result of dermal Ca loss stimulates bone resorption via an increase in PTH**. The working model for this line of research assumes that an exercise bout can activate both bone formation and resorption through distinct mechanisms. Whereas the **loading characteristics** of an activity are the primary determinant of how effectively bone formation is stimulated,[2] the **metabolic characteristics** of an activity may determine the extent to which bone resorption is stimulated. Under this paradigm, the net effect of exercise training on BMD would reflect the cumulative effects of how individual exercise bouts disrupt the balance between bone resorption and formation. Although many studies have assessed changes in markers of bone resorption and formation from the beginning to the end of an exercise training intervention lasting weeks to months, there is a paucity of knowledge of the acute effects of exercise on bone metabolism in humans. We will target this knowledge gap through experiments to exploit the working model by which we postulate exercise activates bone resorption (Fig 1). If the proposed experiment supports the global hypothesis, the long-term goal will be to determine whether the acute activation of bone resorption in response to exercise compromises the expected increases in BMD in response to exercise training.

1.2 Specific Aims (SA) and Hypotheses (H)

SA2: Determine whether preventing the decline in serum iCa during exercise via intravenous Caadministration (i.e., iCa clamp) prevents an increase in serum PTH and c-telopeptide (CTX). EXP2 will manipulate serum Ca level during exercise to determine whether this is the trigger for downstream steps in Figure 1. This will be achieved by having participants perform two identical exercise bouts under conditions of saline (Fig 2, left) vs Ca infusion (Fig 2, right).

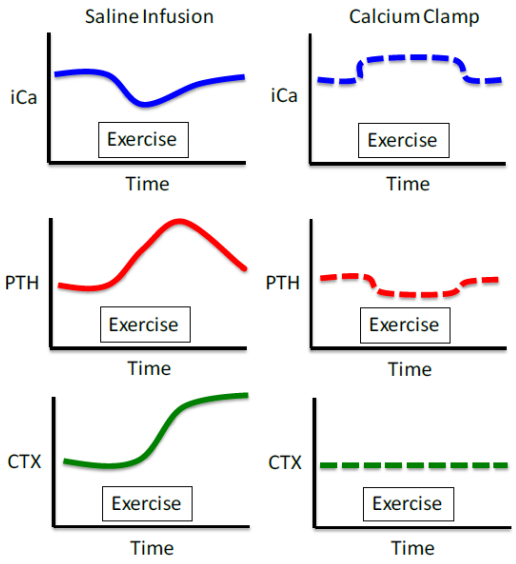

Figure 2. Changes in serum iCa, PTH, and CTX during exercise (left panels) and postulated responses to intravenous Ca infusion to prevent a decline in serum iCa (right panels).

H2: When compared with saline infusion, intravenous Ca infusion during exercise to prevent a decline in serum iCa will attenuate the increases in serum PTH (primary) and CTX (secondary).

Interpretation: Our expectation is that preventing a decline in serum iCa during exercise will prevent the increase in PTH. The goal of the iCa clamp will be to increase serum iCa by ~0.10 mg/dL above the pre-exercise level and maintain this during exercise; this should generate a decrease in PTH (Fig 2, right). If preventing the decline in serum iCa during exercise is not fully effective in preventing the increases in serum PTH and CTX, this will suggest that other mechanisms, such as increased metabolic acidosis,[4] contribute to the stimulation of PTH during exercise. Future experiments would challenge this possibility.

2. Significance of this research

Exercise is recommended for both the prevention and treatment of osteoporosis. Although there have been no randomized controlled trials to document the anti-fracture efficacy of exercise, animal studies demonstrate that even small increases in bone mass and density (4-6%) in response to mechanical loading generate very large improvements in bone strength (60-90%).[5] This is in contrast to pharmacologic therapies with proven anti-fracture efficacy, which generate increases in bone strength (10-20%) in animals that are only proportional to the increases in bone mass.[6,7] Such findings suggest that exercise is an important strategy for building and maintaining bone strength. Accordingly, identifying novel factors that either compromise or enhance the skeletal adaptations to exercise is of high priority.

It is our hypothesis that the beneficial effects of exercise on BMD in humans may be attenuated by **metabolic responses** to exercise, and specifically the disruption of Ca homeostasis under conditions of high dermal Ca loss. Importantly, despite the vast literature on the effects of exercise on bone in rodents, it is unlikely the proposed mechanism has ever been considered because of the lack of a sweating response in rodents. However, as will be discussed further in the Research Strategy section, there is compelling preliminary evidence from our lab and from others to support the proposed mechanism as a modifier of bone metabolism in humans.

The new concept being advanced is that the skeletal response to exercise is determined by both the mechanical loading characteristics and metabolic characteristics of the exercise. Our premise is that exercise can acutely activate both bone formation and bone resorption, and that it is the balance of these processes with repeated exercise bouts that is a determinant of the BMD adaptation to exercise training. Under this paradigm, activities such as basketball, soccer, and cycling could all have similar metabolic characteristics (i.e., could trigger the cascade in Fig 1), but the more favorable loading characteristics of basketball and soccer could explain the higher BMD in such athletes than in cyclists.[8] The proposed disruption of Ca homeostasis during vigorous exercise training could underlie the increased risk of stress fracture during basic training in military recruits.[9] This is supported by the finding that Ca (2000 mg/d) and vitamin D (800 IU/d) supplements taken with morning and evening meals reduced the incidence of stress fractures in Navy recruits.[9] The proposed experiments will develop mechanistic evidence for this contention and could lead to improved recommendations regarding the timing and dose of Ca supplementation to prevent unfavorable bone metabolism responses to exercise. We believe that acute increases in PTH and bone resorption during and after exercise could increase risk for stress fracture and diminish the potential benefits of exercise on BMD. However, we also recognize that transient increases in PTH in response to exercise could have favorable effects on bone metabolism, similar to the benefits of transient increases in PTH with pharmacologic therapy.[10-12] In future intervention trials, it will be important to advance the proposed acute exercise studies to more chronic interventions that challenge our hypotheses.

3. Research Strategy

SA: Determine whether preventing the decline in serum iCa during exercise via intravenous Ca administration (i.e., iCa clamp) prevents an increase in serum PTH and CTX. The experiment will manipulate serum iCa level during exercise to determine whether this is the trigger for downstream steps in Fig 1.

Rationale

The goal is to isolate the decline in serum iCa during exercise as the trigger for the increase in PTH. In healthy adults, Ca-sensing receptors in the parathyroid glands are exquisitely sensitive to serum iCa. Small increases in iCa in response to Ca infusion result in a decline in serum PTH within minutes (Fig 3).[11]

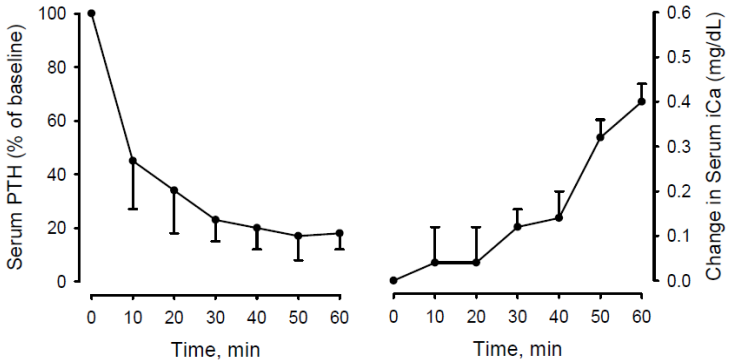

Figure 3. Relative changes in serum PTH (left; mean±sd) in response to a rise in serum iCa (right) during an intravenous Ca gluconate infusion. Adapted from Goodman WG et al. *J Clin Endocrinol Metab* 83:2765 1998

Importantly, changes in total serum Ca levels during exercise, which are more commonly measured than iCa, do not reflect the changes in iCa (Fig 4).[12] Our lab is currently in the process of investigating this protocol in younger adults. However, to the best of our knowledge, this will be the first study to evaluate whether preventing the decline in serum iCa during exercise prevents an increase in PTH and CTX in healthy older adults.

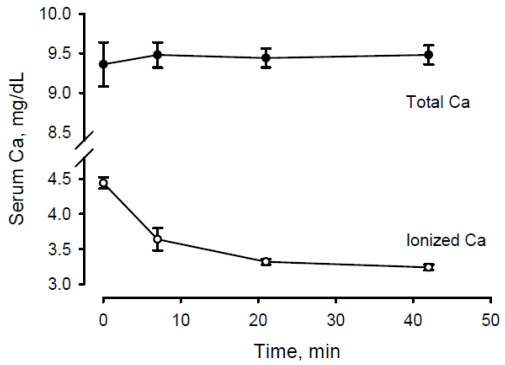

Figure 4. Changes in serum total and ionized Ca in response to running at 70% VO_2max (21 min) and 85% VO_2max (21 min). Adapted from Bouassida A et al. *Eur J Appl Physiol* 88:339 2003

H: When compared with saline infusion, intravenous Ca infusion during exercise to prevent a decline in serum iCa will prevent the increases in serum PTH and CTX.

In recent discussions with colleagues who study osteocytes, it has been noted that the changes we observe with exercise may be due to osteocytic osteolysis (bone resorption by the osteocyte)[13,14], rather than the assumed bone resorption by the osteoclast. Osteocytes are known to contribute to the regulation of bone remodeling in response to various stimuli, such as mechanical loading.[15] However, until the discovery of the osteoclast, osteocytes were also believed to be involved in calcium homeostasis in response to acute fluctuations in serum calcium.[14,16] Recently, research has resurrected the idea that osteocytes may contribute to mineral homeostasis, but the majority of research was done *in vitro,* which may not have direct implications for human physiology.[14] Studying osteocytic osteolysis *in vivo* in humans is challenging because the majority of osteocyte research focuses on osteocyte isolation and culture.[17,18] However, osteoporosis drugs that have direct action on osteoclasts (i.e. bisphosphonates) have the potential to determine the contribution of the osteocyte to calcium homeostasis via studies of exercise in older adults.

Although we are generating the scientific basis to suggest that the decline in serum iCa during exercise is the driver behind increases in both PTH and CTX, our research to date does not provide information regarding which bone cell is responsible for regulating calcium homeostasis and increasing bone resorption during exercise. Given that this research is ongoing in older adults, it is possible to recruit participants who are already taking bisphosphonate medications and conduct the clamp experiments in this group. Because bisphosphonates slow bone loss by increasing osteoclast apoptosis, if those on bisphosphonate medication respond similarly to healthy older adults not taking bone altering medications (i.e., the decrease in iCa during exercise stimulates increases in PTH and CTX), the osteocyte is likely the cell responsible for regulating mineral homeostasis and increasing bone resorption. However, if we do not see similar responses (i.e., a failure of CTX to increase in response to a decline in iCa and increase in PTH), this will suggest that the osteoclast is the cell primarily responsible for calcium homeostasis and bone resorption during exercise. Knowing which cell type is responsible for the large and sustained increases in CTX that we observe during long duration, high intensity exercise can help create targeted exercise and supplementation strategies to preserve bone health with age. Further, if those on bisphosphonates do respond differently, it will provide preliminary evidence to design more personalized exercise interventions for those taking those medications.

Accordingly, we will repeat the calcium clamp (methods detailed below) in older women currently taking a bisphosphonate medication to generate pilot data as part of the larger research project. We will compare the iCa, PTH, and CTX responses between the saline and control conditions. We will also compare these results to those from the ongoing studies in healthy older adults not taking a bisphosphonate.

Approach

Study Overview. Healthy older adult men and women will undergo two 1-hour exercise bouts with concomitant intravenous infusion of saline or CaCl2 (Fig 5). Because of a shortage of CaCl2, Ca gluconate may be used in some experiments. The exercise bouts will be held approximately 1 week apart.

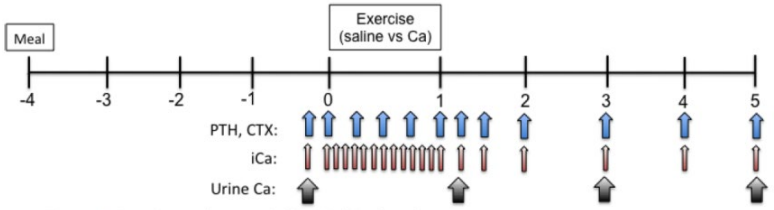

Figure 5. Experimental approach for SA2 (iCa clamp)

Participants will be asked to record their dietary intake the day before the first exercise bout and repeat that intake the day before the second exercise bout. A pre-exercise meal that contains <100 mg Ca will be consumed 4 hours before exercise (same meal on both days; Figure 5). The macronutrient composition of the meal is as follows: CHO/protein/fat of 50%/15%/35%. Meals will contain 575 kcal for men and 335 kcal for women (20% of daily caloric intake).

Participants will be instructed to avoid Ca supplements for ~24 hours prior to testing. Blood samples will be obtained at 15-min intervals starting 15 min before exercise until 30 min after exercise, and then at 1 hour intervals starting 1 hour after exercise and ending 4 hours after exercise for the measurement of iCa, PTH and CTX. Samples will also be obtained every 5 min during exercise for the measurement of iCa (on the Ca and saline days), which will be used to adjust the infusion of CaCl2 or Ca gluconate.

Urine samples will be collected before exercise, immediately after exercise, 2 hours after exercise and 4 hours after exercise for the measurement of urine Ca concentration.

Following completion of the testing visit, participants will receive a boxed lunch.

Subjects. Participants will be healthy older adult women and men aged 60-80 y who are accustomed to vigorous walking (i.e., walking for intervals over 1 hour in duration at an intensity that causes sweating). Volunteers will undergo an orientation session and a screening session. The screening session will include a medical history, physical exam, blood chemistries (complete metabolic panel, complete blood count, TSH, 25-hydroxy-vitamin D (25(OH)D), dual-energy x-ray absorptiometry (DXA) scan, and graded exercise test (GXT) on a treadmill for measurement of VO_2peak. Exclusion criteria are described in the section on the Protection of Human Subjects.

Blinding and randomization. Because of the nature of the experiments, neither the research team nor the participants will be blinded because of the need to adjust the rate of CaCl2 or Ca gluconate infusion during the iCa clamp. However, samples (other than iCa) will be processed in a way that ensures the individual analyzing the samples will blinded to the test condition. The order of the test conditions will not be randomized. The iCa clamp will be performed first for all participants, for reasons described below.

Procedures.

GXT. The walking GXT will be done to determine whether the BP/ECG responses to exercise are normal and measure VO_2peak, which will be used to determine the exercise intensity for the two exercise bouts. VO_2peak will be measured by indirect calorimetry using a TruMax2400 metabolic cart (ParvoMedics, Sandy UT). GXT tests will be supervised by either the study physician (Dr. Rebecca Boxer) or the CTRC PA.

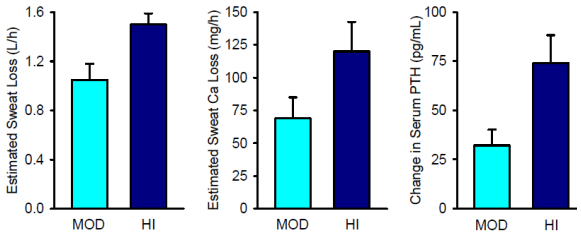

Figure 6. Estimated sweat loss (left) and sweat calcium loss (center) and change in serum PTH in response to 2 hours of moderate-intensity cycling (Barry DW, Kohrt WM Calc Tiss Int 80:359 2007) and 1 hour of high-intensity cycling (Barry DW et al. Med Sci Sports Exerc 43:617 2011). Values are mean ± se.

DXA. Participants will undergo a DXA (Hologic, Bedford, MA) for the total body, left hip (unless metal/injury prevents this; in which case, right hip will be used), and lumbar spine. All three scans take approximately 30 minutes to complete, and the total radiation exposure is the equivalent of one day on Earth. All scans will be conducted by a certified technician who has undergone the appropriate training.

Exercise bout. The exercise bout will be 1 hour of walking at ~80% of walking VO_2peak. 3- to 5-min warm-up at a self-selected intensity will precede the exercise bout and a 3- to 5-min cool-down at a self-selected intensity will follow the exercise bout. This intensity and duration is expected to result in a significant decrease in serum iCa and increase in serum PTH (Fig 6). The first exercise bout will be the iCa clamp. The volume of fluid infused intravenously will be recorded and the same volume will be delivered in the same manner during the subsequent saline infusion. Participants will be allowed to drink water *ad libitum*.

Blood sampling and analysis. Two indwelling intravenous catheters will be positioned approximately 30 minutes before the start of exercise for serial blood sampling. Baseline samples will be obtained 15 minutes before exercise, immediately before the start of exercise and then every 15 minutes during exercise and for 1 hour following exercise. Additional blood samples will be obtained at 5-min intervals during exercise for iCa.

Intact PTH will be measured using an Immulite two-site EIA (DPC). CTX and will be measured by ELISA (Nordic Bioscience). Ionized Ca will be measured using an iSTAT point-of-care whole blood analyzer (Abbott Point of Care, Inc; Princeton, NJ). The iSTAT analyzer will also measure hemoglobin and hematocrit, to allow adjustments for hemoconcentration), and pH, a potential modulator of PTH secretion.[4,19]

Despite the wealth of information generated from preclinical (i.e., animal) studies of the effects of acute exercise or loading on bone metabolism, there have been relatively few studies that measured changes in bone metabolism in response to acute exercise in humans, as reviewed in 2010[20] and since.[20-23] Recent studies evaluated the effects of training status[23] and exercise intensity[20] on the metabolic response of bone to acute exercise, but to the best of our knowledge no studies have specifically focused on dermal Ca loss as the determinant of the metabolic response. By obtaining blood samples every 15 minutes during and after exercise, we should be able to capture the dynamic regulation of PTH by serum iCa because small changes in iCa trigger an increase in PTH within minutes.[24] Obtaining samples up to 4 hours after exercise should be sufficient time to observe a peak in CTX.[25]

Urine sampling and analysis. Urine samples will be collected immediately before and after exercise and 2 and 4 hours after exercise. Urinary Ca concentrations will be measured with an ion-sensitive electrode.

Data management. Ms. Anusha Guntupalli, who is the data manager for the PI's research group, will create and maintain the database for all of the experiments in a secure centralized SQL server that meets the institutional requirements for security and regulatory compliance. Whenever possible, data will be uploaded electronically from the point of collection (e.g., core lab chemistries) to the database. When key outcomes data must be entered manually, they will be double-entered to provide a means of doing quality assurance checks. The user interface for manual entry will be Research Electronic Data Capture (REDCap), which is a secure, web-based application developed at Vanderbilt University for building and managing online surveys and databases. REDcap is available to investigators at UCD for use at no charge. Data entered via REDcap will eventually be merged electronically into the central SQL server to facilitate data analysis. Data verification and integrity for the database are imposed at the field, table, and relationship levels within the SQL backend. We have minimized data input error by eliminating

manual input of most clinical data by using electronic upload. All data are automatically backed up to off-site servers on a nightly schedule. Voucher copies of the database and supporting documents are copied to permanent media and stored in a secure location on a semi-annual basis.

Sample size estimate and power calculation. The primary outcome for SA2 is the change in serum PTH from the beginning to the end of exercise. The expectation is that PTH will increase under the saline condition by 74±63 pg/mL, as observed in previous studies using a similar exercise intervention (Fig 4).[11] Based on our hypothesis, we expect that serum PTH will be unchanged (or decrease) during the iCa clamp condition. Thus, the expected difference in the PTH response under the two experimental conditions is 74±63 pg/mL. Assuming the within-subject correlation between exercise bouts is 0.5, a paired *t* test at the 0.05 level with a sample size of 10 will provide 91% power to detect this magnitude of difference between conditions. For a sample size of 8 (i.e., if some of the iCa clamps are not successful), the power is 81%. In order to distinguish any potential sex differences, we will enroll both men and women (12 men and 12 women to allow for attrition). To generate pilot data, we will enroll 6 women taking a bisphosphonate medication for comparison with the women who are enrolled in the study and not taking a bisphosphonate. Because the purpose of this is to generate pilot data, sample size is based on what is feasible given available funds.

Statistical analysis plan. The primary statistical test will be a paired *t* test comparing the change in serum PTH from the beginning to end of exercise in the saline condition with the change in the iCa clamp condition. Secondary analyses will include similar comparisons of the changes in serum iCa and CTX. For the primary and secondary outcomes, we will also estimate the changes in slope over time and the integrated responses using data obtained before, during, and after exercise. Depending on the appearance of changes over time, we will use linear or nonlinear mixed models.[6] Regression analyses will be used to explore the associations of change in serum iCa with the changes in serum PTH and CTX in a manner that informs the working model depicted in Figure 1 (i.e.,upstream events as mediators of downstream events).[10] Potential confounding variables, such as training history or conditioning, will be collected to allow for adjustment of the model, if necessary.

For the comparison between the bisphosphonate and non-bisphosphonate users, the primary test will be a paired *t* test comparing the change in serum CTX from the beginning to end of exercise in the saline condition with the change in the iCa clamp condition. Secondary analyses will include similar comparisons of the changes in serum iCa and PTH. Independent *t* test analyses will be conducted on the calcium and saline conditions between groups (no bisphosphonate vs. bisphosphonate) to determine if the responses to the calcium clamp and/or saline infusions are different. Given the small sample size, negative findings will be interpreted with caution. We will be looking for trends and differences between groups to determine in further investigation of osteocytic osteolysis in humans is warranted.

Data interpretation and limitations. The expectation is that preventing a decline in iCa will prevent an increase in PTH and the activation of bone resorption. If PTH increases during the iCa clamp, this could indicate that: **1)** Some factor other than serum iCa mediated the increase in PTH. Because metabolic acidosis is known to stimulate PTH,[4,19] we will measure pH

to evaluate this possibility; or **2)** The iCa clamp was not successful in preventing a decline in iCa. Serum iCa concentrations will be measured in real-time during the clamp, so there will be immediate knowledge of whether the clamp was successful. Clamps in which the iCa concentration decreases below the baseline level will not be stopped because of the information that can potentially be gained regarding fluctuations of iCa during exercise and the PTH response. However, secondary statistical analyses will be performed using only data from successful clamps. If we do not have 8 subjects with successful clamps (needed to achieve >80% power), we will enroll additional volunteers; the likelihood that this will occur is low.

For the comparison of the bisphosphonate and non-bisphosphonate users, it is possible that calcium homeostasis during exercise is regulated by both the osteoclast and osteocyte. If this is the case, we may see an intermediate CTX response that would indicate diminished osteoclast activity and normal osteocyte activity; this data would be difficult to interpret. However, the goal of this study is to determine if there is a preliminary signal for osteocytic osteolysis. Even if the results are not what we expect, it will provide some indication on if osteocytic osteolysis is a contributing factor to the bone resorption observed during exercise.

4. Protection of human subjects

4.1. Eligibility criteria

Volunteers who are eligible and willing to participate will undergo the procedures described in the Protocol. They will be healthy adults aged 60-80 y. There will be no discrimination by race or ethnicity for enrollment in the trial. Based on the 2010 U.S. Census, the ethnic composition of the Greater Denver Metropolitan area is 21.8% of Hispanic or Latino origin and the racial composition is 0.1% Native Hawaiian or Pacific Islander, 1.0% American Indian or Alaskan Native, 3.8% Asian, 5.2% Black or African American, 78.6% White, 3.5% multi-racial, and 7.8% other. Therefore, we plan that ~22% of our participants will be of Hispanic or Latino ethnicity and ~21% will be non-white or multi-racial.

Inclusion and exclusion criteria. Healthy adults aged 60-80 y will be enrolled in the experiments. To ensure that participants are familiar with the type of exercise to be performed, volunteers will be recreationally active (i.e., engage in moderate- or vigorous-intensity physical activities at least 2 days per week based on self-report) and accustomed to walking bouts of 1 hour duration (based on self-report). Volunteers will be excluded from participation for the following:

- use of medications in the past 6 months known to affect bone metabolism (e.g., bisphosphonates, thiazide diuretics, oral glucocorticoids)
- BMD t score < -2.5 at the total hip or lumbar spine
- known disease or condition associated with intestinal malabsorption
- moderate or severe renal impairment defined as an estimated glomerular filtration rate of <60 mL/min/1.73m^2 based on the Modification of Diet in Renal Disease (MDRD) equation
- chronic hepatobiliary disease, defined as liver function tests (AST, ALT) >1.5 times the upper limit of normal; if such values are obtained on initial screening and thought to be transient in nature, repeated testing will be allowed

- thyroid dysfunction, defined as an ultrasensitive TSH <0.5 or >5.0 mU/L; volunteers with abnormal TSH values will be re-considered for participation in the study after follow-up evaluation by the PCP with initiation or adjustment of thyroid hormone replacement
- serum calcium <8.5 or >10.3 mg/dL
- serum 25(OH)D <20 ng/mL; volunteers with abnormal serum 25(OH)D values may be re-considered for participation in the study if serum 25(OH)D is >20 ng/mL after vitamin D supplementation
- uncontrolled hypertension defined as resting systolic BP >150 mmHg or diastolic BP>90 mmHg; participants who do not meet these criteria at first screening will be re-evaluated, including after follow-up evaluation by the PCP with initiation or adjustment of anti-hypertensive medications
- history of type 1 or type 2 diabetes
- cardiovascular disease; 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) without follow-up evaluation will be cause for exclusion; follow-up evaluation must include diagnostic testing (e.g., stress echocardiogram or thallium stress test) with interpretation by a cardiologist
- diagnosis or history of asthma

The addition 6 women enrolled for the bisphosphonate comparison group will meet all the above criteria with two exceptions:

- must be taking a bisphosphonate for a minimum of one year

must have a t-score ≥ -3.0 at the total hip or lumbar since osteoporosis is often the diagnostic criteria necessary to be prescribed a bisphosphonate

4.2. Potential risks

Exercise: The potential risks of exercise include development of ventricular arrhythmia, myocardial infarction, cardiac arrest, asthma, and death, as well as the less serious problems of injury to tendons, ligaments, joints, and muscles.

Venipuncture: 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.

4.3. Adequacy of protection against risks
a. Recruitment and informed consent

Volunteer recruitment. Recruitment of volunteers for the study will rely on strategies that have proven successful in attracting volunteers to intervention trials the PI and CO-Is have conducted. Our intention is that the study cohort will be comprised of men and women with a race/ethnic distribution similar to that of the Denver metropolitan area. The recruitment strategies will be conducted by the research coordinator and the research assistant and will include:

- radio advertisements – utilization of radio stations that target a demographic (age, race, ethnicity) consistent with the study population
- newspaper advertisements – the major publication in the Denver Metropolitan has a Health and Fitness section that runs once per week; advertisements in these sections have targeted appropriate individuals for our previous studies
- suburban newspaper advertisements – there are many suburban journals in the Metro area that are issued on a weekly or monthly basis; we specifically advertise in those that target neighborhoods in relative proximity to our exercise training facility and those that target persons of under-represented minorities
- university advertisements – advertisements will be placed on a quarterly basis in the University of Colorado Denver (UCD) publications that target faculty/staff and alumni
- e-mail bulletins – system-wide (UCD, University of Colorado Hospital, The Childrens Hospital) email advertisements will be distributed quarterlyelectronic networks – electronic networks, such as Craigs List, have been a productive source of study volunteers
- study pamphlets – study pamphlets describing the study will be printed for a variety of purposes (for study participants to give to family and friends who may be interested in participating; to place in the UCD Primary Care Clinics and other relevant clinics; to place in community recreation centers; to hand out when the investigators give community lectures)
- RECRUIT- this offers a variety of recruitment (RECRUIT) tools which are intended to assist research teams in their recruitment efforts by providing a mechanism for teams to connect with UCHealth patients using My Health Connection. We will specifically use Epic Recruitment to recruit potential participants. Patients have the opportunity through My Health Connection to click the "I'm Interested" button and be on a list in Epic where interventionist can further determine eligibility for the study and reach out to them through secure messaging.

Participants will receive volunteer remuneration, although this will not be advertised as a study benefit. The amount of remuneration in the study will be $100 for the completion of the exercise test.

Informed consent process. The first interaction with volunteers is typically by telephone or email, initiated by individuals who have seen or heard an advertisement and call to learn more about the study. A brief screening interview is conducted, either by phone or by email (form containing the questions in the phone script). Callers who meet general study criteria and are interested in learning more about the study are scheduled for an orientation session. The consenting process is part of the orientation session, which typically takes ~60 min. Volunteers are either sent a copy of the consent form in advance or are given one at the start of the orientation; time is allowed for review of the consent form. Volunteers then meet with a member of the research team to a) have the study and what is expected of them explained in detail; b) discuss their reasons for wanting to participate to determine whether they are realistic; c) discuss any practical problems (e.g., scheduling conflicts) that could interfere with participation; d) have their questions answered; and e) demonstrate their ability to provide informed consent by describing their understanding of the major study goals and what is expected of participants.

Volunteers are given a signed copy of the consent form and a signed copy is maintained in the study chart for each participant. The HIPAA regulations are also explained at this session. The member of the research group who will obtain consent and HIPAA authorization from volunteers will have complied with IRB and HIPAA education requirements. The PI will have the responsibility of ensuring that research personnel are prepared to convey information to volunteers specific to the study protocol.

b. Protections against risk

Plans to minimize risks.

Exercise testing: The risks of exercise testing will be minimized by adhering to 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 severe fatigue that forces cessation of exercise.

The criteria that will be used to stop the exercise test before volitional fatigue include thedevelopment 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.

Training of personnel: Study personnel assisting with the administration of screening cycling tests will have BLS certification. A physician will be immediately available in the facility if not directly administering these screening tests. A physician will interpret ECG and BP responses for all screening cycling 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. Exercise tests are terminated if any of the ACSM absolute or relative stopping criteria are met. 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 Exercise Research Laboratory operates as a 911 emergency response facility, as do all other UCH outpatient clinics.

Submaximal exercise tests: The intensity for the submaximal, 1-hour exercise tests for all experiments will be individualized, based on performance during the maximal exercise tests, such that the relative intensity is similar for all participants. Eligible participants will be accustomed to the usual discomforts associated with vigorous exercise. For all experiments, and particularly for the experiment in a warm environment, participants will be allowed (and encouraged) to drink water *ad libitum*.

Venipuncture: The risks of hematoma and infection are minimized by having trained personnel perform the procedures using sterile techniques.

Confidentiality and privacy: The risks will be minimized by not including personal identifying information on the forms, when possible, and by conducting interviews and collection of personal information in a private setting.

Potential benefits to the subjects and others

The benefits to an individual participant in the study are not known. There is potential benefit from the assessment of health status by medical history and physical examination, and screening for cardiovascular disease. The potential benefits of the study to women and men in general could be significant. The study is expected to lead to the scientific discovery of the role that calcium metabolism during exercise affects changes in bone mineral density (BMD). This may lead to the development of strategies to maximize improvements in BMD with exercise. In light of these potential benefits, the risks to subjects are reasonable.

Importance of knowledge to be gained

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 whether vigorous exercise also activates bone resorption. Importantly, the proposed mechanisms to be investigated are thought to be triggered by the dermal loss of calcium during exercise (i.e., sweating), which has not been addressed in preclinical studies because most laboratory animals do not lose calcium through this route. This line of research could lead to strategies to enhance the benefits of exercise on osteoporosis prevention.

4.4 Data and safety monitoring plan

The data and safety monitoring for the proposed experiments will be carried out by the research team and an independent Research Monitor. Kerry Hildreth, MD, Assistant Professor of Medicine in the Division of Geriatric Medicine, will serve as the Research Monitor.
Responsibilities and Authorities of the Research Monitor

The Research Monitor will: 1) approve the protocol and any changes to the protocol that have an impact on participant; 2) monitor recruitment, consenting, enrollment, and testing of study participants; 3) review anticipated and unanticipated problems; 4) review study progress and data

quality; and 5) submit reports on these activities and recommended action items to the PI, local IRB, and sponsor (if applicable). Meetings with the Research Monitor will be held at least annually, or more frequently if requested by the IRB, Sponsor, or Monitor.

Because of the nature of the studies (i.e., mechanistically-driven physiological experiments, not clinical trials), the Research Monitor will monitor safety only and not efficacy. In the event the Research Monitor determines an experiment should be stopped for reasons of safety, this will be communicated to the PI, IRB, and Sponsor.

The investigators will promptly report all anticipated (i.e., identified in the consent form) and unanticipated problems involving risk to subjects or others to the Research Monitor for review. The monitor will confirm whether the investigators have appropriately categorized the problem as anticipated or unanticipated. The Research Monitor will have the authority to discuss the research protocol with the investigators, interview human subjects, and consult with others outside of the study about the research.

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