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Maintaining Serum Ionized Calcium during Brisk Walking Attenuates the Increase in Bone Resorption in Older Adults

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Abstract

BACKGROUND: Endurance exercise can cause a decrease in serum ionized calcium (iCa) and increases in parathyroid hormone (PTH) and bone resorption, reflected by serum carboxy-terminal collagen crosslinks (CTX). We developed a calcium clamp to prevent the decrease in iCa during exercise, which attenuated increases in PTH and CTX during vigorous cycling in young men. The goal was to determine whether this occurs in older adults during brisk walking.

METHODS: Twelve older adults (6 men, 6 women) performed two identical 60-min treadmill walking bouts with Ca gluconate or half-normal saline infusion. Blood sampling for iCa, total calcium (tCa), phosphate (P), PTH, and CTX, occurred before, during, and for 4 hours after exercise.

RESULTS: iCa decreased during exercise with the saline infusion ($p=0.04$) and this provoked increases in PTH and CTX (both $p<0.01$). The Ca clamp prevented the decrease in serum iCa during exercise and attenuated the PTH and CTX responses.

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CONCLUSIONS: Preventing the exercise-induced decrease in iCa markedly attenuated the increases in PTH and CTX. The cause of the decrease in iCa during exercise remains unclear, but the increases in PTH and CTX are likely counter-regulatory responses to defend serum iCa. This contention is supported by previous observations that the disruption of Ca homeostasis during exercise occurs regardless of training status. It will be important to establish whether this acute catabolic effect of exercise diminishes the potential chronic anabolic effects of exercise on bone.

Keywords

exercise; biochemical markers of bone turnover; calcium

1. Introduction:

We and others have shown that vigorous cycling acutely increases a serum marker of bone resorption (1–8) for at least 4 hours after exercise in young adults.(1, 3) The acute activation of bone resorption also occurs with vigorous weight-bearing exercise (e.g., treadmill walking).(2, 7–10) The effect of vigorous exercise to transiently increase bone resorption could explain why skeletal adaptations to exercise tend to be small(11–15) or why bone mineral content and density decrease under certain exercise conditions.(16–18) We demonstrated that the disruption of calcium (Ca) homeostasis during exercise is a trigger for the acute catabolic effect of exercise on bone,(1, 2, 4, 5, 9, 19) and that oral Ca supplementation before and/or during exercise can attenuate the bone resorption response. (1–4, 6)

To understand the extent to which the decrease in serum ionized Ca (iCa) during exercise mediates the parathyroid hormone (PTH) and serum carboxy-terminal collagen crosslinks (CTX) responses, we developed a Ca clamp procedure to prevent the decrease in serum iCa during exercise using intravenous Ca infusion. The initial Ca clamp study was conducted in young men during 60 minutes of vigorous stationary cycling, and demonstrated that preventing the decrease in iCa during exercise attenuated increases in PTH and CTX by ~70%.(1) However, because older adults are at increased risk for osteoporosis, it is important to understand whether the results observed in young men also occur in older adults during brisk walking or if there are any age-related differences.

Accordingly, the aim of this study was to determine if Ca infusion to prevent the decrease in serum iCa during 60 minutes of brisk walking, a very common form of exercise for older adults,(20) attenuates the increases in PTH and CTX in older adults. We hypothesized that Ca infusion would significantly attenuate the increases in PTH (primary) and CTX (secondary) when compared with a volume-matched, half-normal saline infusion as the control condition.

2. Materials and Methods:

2.1. Participants.

Men (n=6) and women (n=6) aged 60 to 80 years who were accustomed to brisk walking were eligible to participate. Physical activity was evaluated by self-report and eligible

volunteers were those who reported brisk walking or running at least 2 days per week for at least 1 hour per session over the previous 6 months.

Exclusion criteria included: history of type 1 or type 2 diabetes mellitus; active cardiovascular disease (evidence of ischemic heart disease or serious arrhythmias at rest or during the graded exercise test); use of medications known to affect bone metabolism in the previous 6 months (e.g., osteoporosis medications, oral glucocorticoids); BMD T-score ≤ −2.5 at the total hip, femoral neck, or lumbar spine; renal impairment defined as an estimated glomerular filtration rate of <60 mL/min/1.73 m^2; hepatobiliary disease (aspartate transaminase or alanine transaminase concentration >1.5 times the upper limit of normal); uncontrolled hypertension, defined as resting systolic blood pressure >150 mmHg or diastolic blood pressure >90 mmHg; serum Ca <8.5 or >10.3 mg/dL; ultrasensitive TSH <0.5 or >5.0 mU/L; and serum 25(OH)D <20 ng/mL. Participants provided written informed consent and the study was approved by the Colorado Multiple Institutional Review Board and the study conformed to the Declaration of Helsinki. The study was registered at clinicaltrials.gov (NCT02580604).

2.2 Screening Visits.

Participants completed a medical history questionnaire and underwent a physical examination, fasted blood draw, dual-energy x-ray absorptiometry (DXA) scans, and a maximal treadmill exercise test to assess eligibilty.

2.2.1 DXA.—BMD at the lumbar spine (L1-L4), total hip, and femoral neck were measured to verify eligibility. A total body scan was performed to determine fat-free and fat mass. Scans were performed using the Discovery W (Hologic Inc; Waltham, MA).

2.2.2. Maximal exercise test.—An incremental treadmill walking test was used to assess peak aerobic power (VO_2peak) and screen for abnormal cardiovascular responses to exercise, such as evidence of ischemia or arrhythmias. During a 5-minute warm-up, treadmill speed was adjusted to elicit a heart rate that was roughly 70% of the age-predicted maximum. The test began at that walking speed and the grade was increased by 2% every 2 minutes until volitional fatigue. VO_2 was measured using the Parvo Medics TruMax 2400 Metabolic Cart (Parvo Medics Inc, Sandy, UT).

2.3. Exercise Sessions.

Participants performed two identical exercise sessions 1 to 4 weeks apart. To facilitate volume matching between the calcium and saline infusions, the Ca infusion always occurred during the first exercise session. The exercise protocol was 60 minutes of brisk walking at ~75% of the measured maximal heart rate. This intensity was chosen because this intensity was sufficient to induce an increase in both PTH and CTX in a similar population.(9) It is also a similar relative intensity to what has been performed in our previous experiments in young adults.(1, 19) There was a 3- to 5-minute warm-up before and a 3- to 5-minute cool-down after exercise, which was identical for both sessions. The walking speed and grade on the Ca infusion day was replicated on the saline infusion day. Participants were allowed unlimited deionized water during exercise.

2.3.1. Pre-exercise meal.—Participants fasted overnight for at least 8 hours and then consumed a standardized meal 4 hours before both exercise sessions. The meal contained 575 kcal for men and 335 kcal for women. Macronutrient content of the meal was 50% carbohydrate, 35% fat, and 15% protein and contained <100 mg of Ca. After the pre-exercise meal, participants consumed only water until after the final blood draw.

2.4. iCa clamp.

The Ca clamp procedure was performed as previously described (1). Briefly, Ca gluconate, at a concentration of 0.169 mg/mL of elemental Ca (Ca_{ELEM}), was infused intravenously beginning 15 minutes before exercise and continuing until the end of exercise. The infusion rate for the 15 minutes prior to exercise was calculated to deliver 0.5 mg Ca_{ELEM} per kg of body weight over 15 min. At the start of exercise, the infusion rate was set to deliver 2 mg CaELEM per minute. Blood samples were obtained every 5 minutes during exercise for measurement of iCa using the iSTAT analyzer (Abbot Point of Care, Princeton, NJ); iCa concentration was used to adjust the infusion rate to maintain iCa 0.1–0.2 mg/dL above the pre-infusion concentration. The half-normal saline infusion was conducted at the same time of day as the Ca infusion and the volume and timing of the infusion were matched to the Ca infusion. All infusion visits were conducted in an inpatient research unit with one-on-one nursing support. Infusion rates were monitored and adjusted by a physician based on the results of the real-time iCa. Safety protocols, including monitoring for signs of extravasation, were in place prior to any visits.

2.4.1. Ca Gluconate Preparation.—The infusate was prepared by the University of Colorado Hospital Investigational Pharmacy. A hypertonic Ca gluconate solution was diluted with half-normal saline to a final concentration of 2 g of Ca gluconate per 1100 mL (186 mg Ca_{ELEM}/1100 mL).(1)

2.5. Urinary Ca Loss.

Urine samples were collected at visit admission and immediately, 2 hours, and 4 hours after exercise. Urine volumes were recorded and 40-mL aliquots were sent for total Ca analysis by indirect ion selective electrode (Beckman Coulter, Inc., Brea, CA). Aliquots were acidified prior to testing. Urinary Ca loss was estimated as the product of Ca concentration and void volume.

2.6. Blood Collection.

An indwelling intravenous catheter was positioned ~30 minutes before exercise for serial blood sampling. Blood samples (~10 mL) were obtained before the start of the infusion (T = −15), immediately before exercise (T=0), and every 15 minutes during exercise (T=15, 30, 45, 60); blood sampling continued through 4 hours of recovery (T=75, 90, 120, 180, 240, 300). The first blood sample and all recovery blood samples were taken while participants were semi-recumbent. All remaining samples were taken while participants were upright. Samples were analyzed for parathyroid hormone (PTH) by Immulite two-site EIA (Siemens, Erlangen, Germany), serum carboxy-terminal collagen crosslinks (CTX) by chemiluminescence (Immunodiagnostics Systems, The Boldens, United Kingdom), total Ca (tCa) by indirect ion sensitive electrode (Beckman Coulter, Inc., Brea, CA) and phosphorus

(P) by colorimetry (Beckman Coulter, Inc.). Additional blood samples (~3 mL) were obtained at 5-minute intervals during exercise for measurement of serum iCa and hematocrit (i-STAT; Abbot Point of Care, Princeton, NJ). Laboratory intra- and inter-assay coefficients of variation (CV) for iCa, tCa, PTH, and CTX were published previously.(1) Manufacturer-determined intra- and inter-assay CVs for phosphate are 1.9% and 2.1% for concentrations near 2.0 mg/dL and 0.6% and 0.9% for concentrations of 5.7 mg/dL. PTH, CTX, iCa, tCa, and P values during and after exercise were adjusted for shifts in plasma volume as previously described, using T=0 as baseline.(4) Both unadjusted and adjusted data are presented; adjusted values are designated with a subscript (e.g., PTH_{ADJ}).

2.7. Statistical Analysis.

We hypothesized that preventing the decrease in the serum iCa concentration during exercise would attenuate the increase in CTX and PTH. The sample size required to evaluate the change in PTH from before to after exercise (primary outcome) was estimated from a previous study.(4) The estimate indicated that 10 participants would provide 91% power to detect a difference in the PTH response between the two experimental conditions of 74±63 pg/mL based on a 2-sided paired *t* test at an alpha level of 0.05. Sample size was increased to 12 to allow for attrition.

The effects of Ca versus saline infusion on PTH and CTX were evaluated using linear contrasts in a repeated measures maximum likelihood model with all available data. We utilized this approach in similar experiments,(1, 9, 19) 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. PTH was missing at a single time point for 3 participants and at 2 time points in the same visit for a single participant. CTX was missing at a single time point for 4 participants. Sample collections were not equally spaced over the study period, which was accounted for in the covariance structure. Linear contrasts were used to estimate within-condition changes and between-condition differences in changes over the 60-minute exercise bouts and the 4-h recovery periods. Within-condition estimates were evaluated to determine if responses to exercise were similar to what has been observed in young adults. The analyses were repeated for secondary outcomes. Data are presented as mean±SD, unless otherwise specified. All analyses were conducted using SAS software version 9.4 M4 (SAS Institute Inc, Cary, NC, USA) and a p-value ≤ 0.05 defined statistical significance.

3. Results

Participant characteristics are in Table 1. For the two exercise bouts, average walking speed was 3.4±0.2 mph (0% grade) for both. Average heart rate was 117±9 bpm and 116±13 bpm on the Ca and saline days, respectively. Hematocrit did not change during exercise for either Ca (p=0.42) or saline (p=0.21) and tended to decrease during recovery (Ca p=0.04; saline p=0.06), but there was no significant difference between conditions (p=0.72). The total amount of Ca infused during the entire clamp period (−15 to 60 minutes) was 134±16 mg and the amount infused during exercise (0 to 60 minutes) was 98±15 mg.

3.1 Responses to exercise

3.1.1. iCa and iCa_{ADJ}.—For iCa, the interaction (p=0.11) and time (p=0.20) effects were not significant, but there was a condition effect (p<0.01), whereby iCa was higher at all time points on the Ca clamp day. The Ca clamp successfully maintained serum iCa above baseline during exercise as expected, whereas iCa decreased by −0.15 mg/dL (95% CI: −0.29, −0.01 mg/dL) from immediately before to the end of exercise (Figure 1A). For iCa_{ADJ}, there were no interaction (p=0.35) or condition effects (p=0.07), but there was an effect of time (p=0.02) (Figure 1B).

3.1.2. tCa and tCa_{ADJ}.—Similar to iCa, the tCa interaction (p=0.76) and time (p=0.20) effects were not significant, but there was an effect of condition (p<0.01), whereby tCa was higher at all time points on the Ca day (Figure 1C). After adjustment for plasma volume shifts, the decrease in tCa_{ADJ} during the saline day was attenuated and there were effects of both condition (p=0.04) and time (p<0.01) (Figure 1D).

3.1.3. P and P_{ADJ}.—There were no interaction (p=0.33) or condition effects (p=0.79) for serum P, but there was an effect of time (p<0.01). P peaked after 30 minutes of exercise for both infusion conditions. Results were similar after adjusting for plasma volume shifts during exercise (Figure 1E and F).

3.1.4. PTH and PTH_{ADJ}.—For PTH, the interaction effect was not significant (p=0.13), but there were both condition (p<0.01) and time (p<0.01) effects. PTH was significantly higher during the saline versus the Ca infusion (all p<0.01). The increases from immediately before to the end of exercise were 3.2 pg/mL (95% CI: −1.3, 7.7 pg/mL) and 11.3 pg/mL (95% CI: −4.0, 19.6 pg/mL) on the Ca and saline days, respectively. Under both infusions, the exercise-induced increase in PTH peaked during recovery; it peaked shortly after exercise on the saline day, but did not peak until the end of recovery on the calcium day. The increase in PTH from immediately before exercise to 15 minutes after exercise was 8.5 pg/mL (95% CI: 3.5, 13.4 pg/mL) and 20.0 pg/mL (95% CI: 11.2, 28.7 pg/mL) on the calcium and saline days, respectively. Results were similar after adjustment for plasma volume shifts (PTH_{ADJ}) (Figure 2A and B).

3.1.5. CTX and CTX_{ADJ}.—For CTX, there were no interaction (p=0.11) or condition effects (p=0.76), but there was an effect of time (p<0.01). There was no change in CTX during the Ca day (0.01 ng/mL, 95% CI: −0.03, 0.06), but there was an increase during the saline day (0.11 ng/mL, 95% CI: 0.03, 0.18), although the difference between conditions was not significant. Results were similar for CTX_{ADJ} (Figure 2C and D).

3.2. Responses during recovery

3.2.1. iCa and iCa_{ADJ}.—For iCa, the interaction (p=0.07) and time effects (p=0.85) were not significant, but there was an effect of condition (p<0.01). The average iCa concentrations during the 4-h recovery were 4.9 mg/mL (95% CI: 4.8, 5.0) and 4.6 mg/dL (95% CI: 4.6, 4.7) on the Ca and saline days, respectfully. (Figure 1A). Adjustment for plasma volume shifts revealed an increase in iCa concentrations for both infusion conditions

beginning 30 minutes after exercise. There were no interaction (p=0.82) or condition effects (p=0.12), but there was an effect of time (p<0.01). (Figure 1B).

3.2.2. tCa and tCa_{ADJ}.—The interaction effect was not significant for tCa (p=0.59), but there were effects of both time (p=0.02) and condition (p<0.01). tCa concentration was higher for the Ca infusion than the saline infusion (p=0.003) (Figure 1C). After adjustment for plasma volume shifts, there were no interaction (p=0.69) or condition (p=0.11) effects, but there was an effect of time (p<0.01). tCa_{ADJ} increased during recovery for both conditions. (Figure 1D).

3.2.3. P and P_{ADJ}.—For P, there were no interaction (p=0.97) or condition (p=0.22) effects, but there was an effect of time (p<0.01). P decreased during recovery, returning to the pre-exercise level by 60 minutes after exercise (Figure 1E). Adjusting for plasma volume shifts revealed an increase in P_{ADJ} during recovery (Figure 1F).

3.2.4. PTH and PTHADJ.—For PTH, there was an interaction effect (p<0.01) and effects of time (p<0.01) and condition (p<0.01). PTH peaked 15 minutes after exercise following the saline infusion and did not return to the pre-exercise level. Under the Ca infusion, it remained below the pre-exercise baseline. The average PTH value was higher during recovery on the saline day (49.1 pg/mL, 95% CI: 43.5, 54.9) versus the Ca day (18.7 pg/mL, 95% CI: 13.2, 24.2; p<0.01). Results were similar for PTH_{ADJ} (Figure 2A and B).

3.2.5. CTX and CTX_{ADJ}.—For CTX, there were no interaction (p=0.07), time (p=0.13), or condition effects (p=0.06). CTX peaked 4 h after exercise for both infusions. The increase from the pre-exercise baseline to the end of recovery was 0.06 ng/mL (95% CI: −0.03, 0.15 ng/mL) on the Ca day compared to 0.25 ng/mL (95% CI: 0.11, 0.39 ng/mL) on the saline day. Results were similar for CTX_{ADJ} (Figure 2C and D).

3.3. Urinary Ca Excretion

There was a condition-by-time interaction for urinary Ca loss (p<0.01), as well as condition (p<0.01) and time (p<0.01) effects. Ca loss during the saline infusion was low and did not change over time (Figure 3). Urinary Ca loss on the Ca day was higher for all collections when compared to the saline day (all p<0.05). The excess urinary Ca loss on the Ca day accounted for approximately 25% of the infused Ca.

4. Discussion

Based on our previous findings in young men during cycling exercise,(1) we hypothesized that preventing the decrease in iCa in response to brisk treadmill walking in older adults would attenuate increases in PTH and CTX. The results partially supported this hypothesis. iCa concentration remained above the pre-exercise concentration on the Ca day, and this was accompanied by markedly attenuation of the PTH during exercise and recovery. The CTX responses also appear to be partially attenuated, although the difference between conditions was not significant. Similar to our findings in young men, under the half-normal saline control infusion, there was a significant change in CTX concentration from baseline to the

end of recovery, and CTX concentration remained elevated throughout the 4-h recovery despite the decrease in PTH after exercise.

4.1. Changes in iCa, tCa, PTH, and CTX on the control day

During the saline infusion, both iCa and tCa decreased steadily after 15 minutes of exercise (Figure 1A and C). The decrease in iCa concentration during exercise could be related to increased Ca binding to albumin, but the effect of acute endurance exercise on calcium binding during exercise has not been fully investigated. Our data from young men suggested that decreases in iCa were not attributable to increased Ca binding.(1) The current results also support this because there were similar patterns of change in iCa and tCa during and after exercise.

During the saline condition, the decrease in iCa after only 15 minutes of exercise was temporally aligned with the initial increase in PTH and CTX, suggesting the increases in PTH and CTX reflect mechanisms to defend serum iCa. The rapid PTH response to iCa was expected because PTH can react within minutes to small changes in iCa.(21) The increase in PTH resulted in increased bone resorption to stabilize iCa,(22, 23), as reflected by the increase in CTX. However, it is not known which bone cell (osteocyte versus osteoclast) is responsible for the increase in CTX observed with endurance exercise. The osteocyte is believed to be responsible for minute-to-minute regulation of mineral homeostasis,(24–26) but, to the best of our knowledge, there have been no studies in humans to determine if osteocytic osteolysis is the mechanism by which Ca is quickly mobilized from bone during exercise. This information could be valuable for understanding how osteoporosis medications, specifically medications that target osteoclast function, influence skeletal adaptations to exercise.

Interestingly, CTX did not follow the same temporal pattern as iCa and PTH during recovery. Despite the decrease in PTH early in recovery, CTX remained elevated for the entire 4-h recovery interval during the saline condition. There is evidence that the half-life of CTX is only ~1 hour,(27) so the sustained increase in CTX suggests that the rate of bone resorption remains elevated for hours after exercise has ended. This was similar to what we observed in young adults.(1) If the prolonged increase in CTX is truly reflective of increased bone resorption without concurrently increased bone formation, strategies to attenuate this could enhance the bone-building benefits of exercise. However, a marker bone formation was not measured in this study. In our previous Ca clamp study of young men, there was an increase in a marker of bone resorption from before exercise to the end of recovery, but there was no difference in the response between the Ca and saline infusion days. This suggested that the markedly greater increase in PTH on the saline day compared with Ca day did not stimulate bone formation. (1)

4.2 Anabolic versus Catabolic Actions of PTH: Implications for Bone Formation

Evidence from the current study and others indicates that endurance exercise results in an acute activation of bone resorption triggered by the increase in PTH,(1–4, 6–10, 19, 28) but it is not clear whether there is a subsequent activation of bone formation. PTH has paradoxical actions on bone; a sustained increase in PTH is catabolic to bone, but repeated

transient increases such as those induced by pharmacologic agents (e.g., teriparatide, abaloparatide) promote bone formation and result in increased bone mineral density.(29–31) The main criterion that determines whether PTH has anabolic or catabolic actions is the length of time it is elevated, with 4 to 5 hours being the threshold.(31) Based on the timeframe PTH was elevated in response to exercise (<2 hours) in the current study, we would expect to observe an increase in bone formation following an acute exercise bout.

We and others have measured changes in markers of bone resorption(1–3, 7–10, 19, 28), but few studies have also assessed markers of bone formation in response to an acute exercise bout(1, 3, 4, 8). In the studies that measured a marker of bone formation (procollagen type I n-terminal propeptide (P1NP) or bone specific alkaline phosphatase (BAP)), it does not appear that the increase in PTH in response to an acute exercise bout also triggers a bone formation response.(1, 3, 4, 8) Bone formation may be activated hours to days later in response to a stimulus, (32) but it is not clear how soon after exercise bone formation could be activated by the increase in PTH. It is possible the sampling window in our previous calcium clamp study (4 hours) was insufficient to detect a change in bone formation.(1) Markers of bone formation were not assessed in this study or our previous studies of older adults due to funding limitations,(6, 9) so it is unclear if exercise generated an acute anabolic response in older adults. We cannot rule out the possibility that the exercise-induced increase in PTH stimulates bone formation hours to days following an acute exercise bout in young or older adults. Future research is needed to address whether the increase in PTH in response to an acute exercise bout is anabolic to bone.

4.3. Factors Contributing to Differences in Magnitude of iCa, PTH, and CTX Responses between Young and Older Adults in Current Compared to Prior Studies

In the current and our previous studies, (1, 9, 19) we documented similar patterns but lower magnitudes of change in iCa, PTH, and CTX in older adults compared to young adults. For example, older adults in the current study had a 0.11±0.08 ng/mL increase in CTX after 60 minutes of brisk walking compared to a 0.24±0.03 ng/mL increase in young men after 60 minutes of high-intensity cycling.(1) In our other studies, increases in CTX ranged from 0.11 to 0.25 ng/mL in young adults (2, 4, 19) and 0.08 to 0.15 ng/mL in older adults.(6, 9) These differences seem to be primarily driven by the change in iCa. Older adults had a smaller decrease in iCa, so smaller increases in PTH and CTX are expected. However, the similarities in patterns of change between young and older adults suggests that the mechanisms that drive the activation of bone resorption during exercise are sustained with aging.

The difference in the magnitude of decrease in iCa we observed between young and older adults during exercise could be related to other differences between studies, such as absolute exercise intensity or mode of exercise. Relative exercise intensity was similar between the current study and our previous study of young men(1), but absolute exercise intensity was not. The young men exercised at an intensity that approximated 9 metabolic equivalents (METs), whereas the exercise intensity for the older adults was approximately 4 METs. Indeed, exercise intensity is a determinant of the PTH and CTX responses,(8) so it is plausible that this could explain the differences in the magnitude of the decrease in iCa and

the changes in other biomarkers between the studies. The underlying mechanisms for why exercise intensity may be associated with changes in iCa, PTH, and CTX are not known. We previously hypothesized that the decrease in iCa during exercise was due to dermal calcium loss from sweat (i.e., greater decline in iCa with increased sweat rate) but our studies did not support this hypothesis.(9, 19)

Additionally, we used weight-bearing exercise (walking) in older adults versus weight-supported exercise (cycling) in young adults. Although the effect of exercise mode has not yet been evaluated in a within-subject approach, studies that evaluated PTH and CTX responses to cycling(1, 19) have generally found higher concentrations of PTH and CTX than studies that measured responses to running.(8, 28) The differences in PTH and CTX responses to cycling versus running may reflect lab-to-lab differences, but this should be tested by measuring changes in bone biomarkers during treadmill and cycling exercise in the same participants.

4.4. Changes in iCa, tCa, PTH, Phos and CTX on the Ca infusion day

As in our study of young men,(1) the Ca clamp was successful in preventing the decrease in iCa during exercise in older adults. Adjustment for plasma volume shifts in young men revealed a ~15% decrease in iCa_{ADJ} during the first 15 minutes of exercise, and this was attenuated but not prevented by Ca infusion (1). This decrease was confirmed in a second study of young women and men during one hour of vigorous cycling exercise.(19) This was not the case in older adults. We did not observe a contraction of plasma volume during exercise in this study, which was consistent with our previous study of older adults.(9)

During recovery, increases in iCa_{ADJ} and tCa_{ADJ} were similar to those observed during the saline condition and were likely a result of plasma volume shifts, as observed in previous studies of older adults.(9) We observed plasma volume expansion in young adults after exercise,(1, 19) which causes unadjusted concentrations to be artificially low. After correcting for plasma volume shifts, a steep increase in both iCa_{ADJ} and tCa_{ADJ} over the recovery interval was revealed.(1) Young adults had a contraction of plasma volume during exercise, and plasma volume expansion during recovery brought iCa and tCa concentrations back toward baseline.(1) In contrast, older adults did not have a significant contraction of a plasma volume during exercise, but had an expansion of plasma volume after exercise. After adjusting for this fluid shift, there was an increase in iCa_{ADJ} and tCa_{ADJ} during recovery (Figure 2; Panels B, D).

It was not clear why there was an absence of plasma volume contraction during exercise in the current study because others have found similar reductions in plasma volume during exercise in young and older adults,(33, 34). This may be partially dependent on both temperature and humidity(35); sweat rates were similar between young and older adults in a warm-humid environment (37° C, 60% relative humidity), but young adults produced more sweat in a hot-dry environment (48° degrees C, 15% relative humidity).(35) There is some evidence that older adults drink enough fluid during endurance exercise to maintain fluid balance during exercise, (36) and the ability to maintain fluid balance may also be easier in older adults due to low sweat rates.(36–38) Participants were allowed to drink water during exercise in the current study and also received fluids (600 to 800 mL) through the saline

and Ca infusions. The combination of fluid administration and low sweat rate may have minimized the contraction of plasma volume.(1, 19)

Ca infusion attenuated, but did not prevent, the increase in PTH during exercise. The increase was less than 5 pg/mL during the Ca infusion in older adults, compared to an increase of approximately 10 pg/mL during Ca infusion in young adults.(1) PTH secretion can be stimulated by metabolic acidosis (39, 40), which could explain the increase in PTH we have observed early in exercise, before there is a measurable decrease in iCa in both young(1) and older adults.(9) PTH secretion may also be stimulated by P.(10) Townsend et al. found that changes in P and PTH during exercise were directly correlated, and the increase in P preceded the increase in PTH.(10) The authors concluded that iCa was the major stimulus of PTH secretion, but that P was a contributing factor. In the current study, the increase in P during exercise was similar on the saline and Ca infusion days and could have contributed to the increase in PTH before the decrease in iCa on the saline day. High levels of P can bind Ca and increase PTH in patient with disorders that impact P clearance, such as chronic kidney disease, (41, 42) but it is not known whether the increase in P observed during exercise could cause similar calcium binding and subsequent increases in PTH. We did not find that an increase in P preceded the increase in PTH, but more frequent blood sampling may have been necessary to detect this.(10)

In contrast to what we observed in young adults,(1) CTX and CTX_{ADJ} did not increase during exercise, despite the small increase in PTH.(22, 43) It is not clear why CTX increased in the young adults but not in the older adults, although it may be related to the small absolute change in PTH in the older adults compared to young adults. It is also possible this was due to the differences between the studies discussed earlier, such as exercise intensity and mode of exercise.

4.5. Conclusions

Preventing the decrease in serum iCa during brisk treadmill walking in older adults markedly attenuated the increases in PTH and CTX, as previously observed in young men during vigorous cycling exercise. Under the saline condition, CTX remained elevated throughout recovery, suggesting the rate of bone resorption was elevated for at least 4 hours after the cessation of exercise. Our findings add to the growing evidence that vigorous endurance exercise, regardless of whether is it weight-bearing or weight-supported, has an acute catabolic effect on bone in young and older adults that is mediated by an increase in PTH. What remains unknown and will be important to investigate is whether transient exercise-induced increases in PTH also generatea more chronic anabolic response in bone, similar to the pharmacologic effects of PTH analogs.

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Highlights:

- Calcium infusion during exercise prevents the decrease in serum ionized calcium.
- Preventing the decrease in ionized calcium attenuates increase in bone resorption.
- Pattern of change in calcium and bone biomarkers similar in young and older adults.

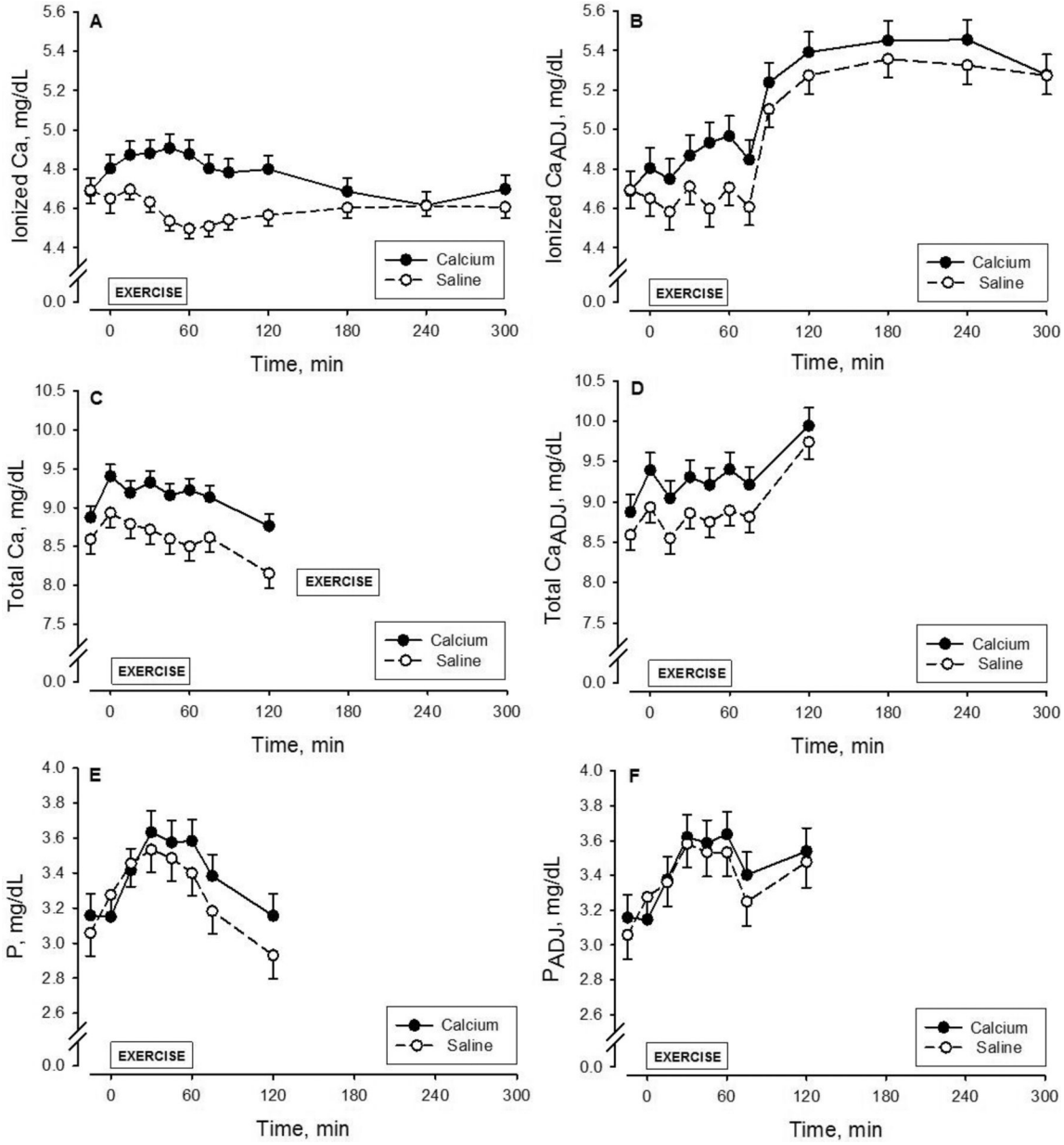

Figure 1.

Serum ionized calcium (A), total calcium (C), and phosphate (P) concentrations before, during, and after 60 min of brisk treadmill walking during conditions of saline (open circles, dashed line) and calcium infusion (closed circles, solid line). Outcomes were adjusted for changes in plasma volume (iCa_{ADJ}: B; tCa_{ADJ}: D; P_{ADJ}: F). Results for the condition-by-time interaction and main effects of condition and time for the exercise interval: (A) iCa: condition-by-time ($p=0.11$), condition ($p<0.01$), time ($p=0.20$); (B) iCa_{ADJ}: condition-by-time ($p=0.35$), condition ($p=0.07$), time ($p=0.02$); (C) tCa: condition-by-time ($p=0.76$), condition ($p<0.01$), time ($p=0.20$); (D) tCa_{ADJ}: condition-by-time ($p=0.99$), condition ($p=0.04$), time ($p<0.01$); (E) P: condition-by-time ($p=0.33$), condition ($p=0.79$), time ($p<0.01$); and (F) P_{ADJ}: condition-by-time ($p=0.63$), condition ($p=0.93$), time ($p<0.01$).

Statistical results for the condition-by-time interaction and main effects of condition and time for the recovery interval: (A) iCa: condition-by-time ($p=0.07$), condition ($p<0.01$, time ($p=0.85$); and (B) iCa_{ADJ}: condition-by-time interaction ($p=0.82$), condition ($p=0.12$), time ($p<0.01$).

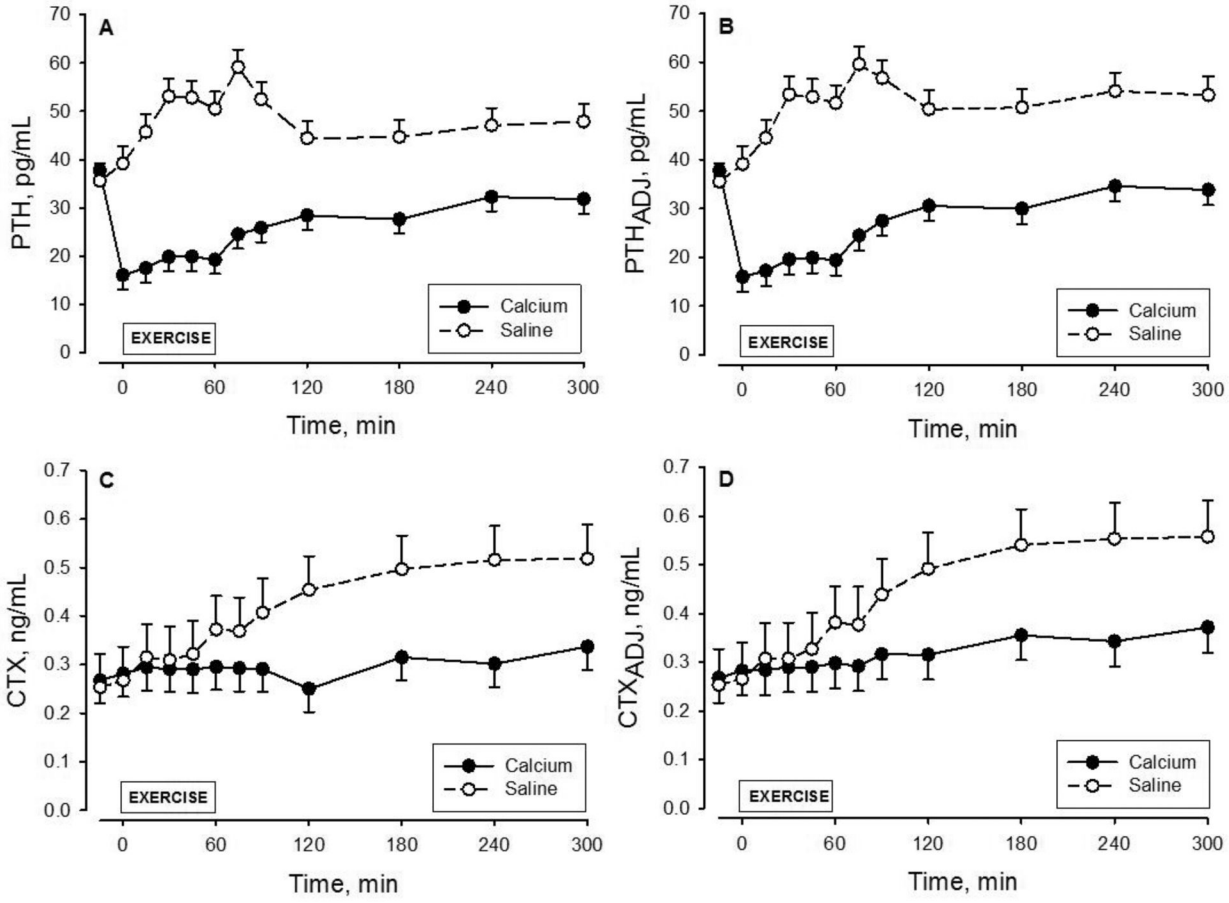

Figure 2.

PTH (A) and CTX (C) concentrations before, during, and after 60 min of brisk treadmill walking during conditions of saline (open circles, dashed line) and calcium infusion (closed circles, solid line). Outcomes were adjusted for changes in plasma volume (PTH_{ADJ}: B; CTX_{ADJ}: D). Results for the condition-by-time interaction and main effects of condition and time for the exercise interval: (A) PTH: condition-by-time ($p=0.13$), condition ($p<0.01$), time ($p<0.01$); (B) PTH_{ADJ}: condition-by-time ($p=0.11$), condition ($p<0.01$), time ($p<0.01$); (C) CTX: condition-by-time ($p=0.11$), condition ($p=0.76$), time ($p<0.01$); and (D) CTX_{ADJ}: condition-by-time ($p=0.07$), condition ($p=0.74$), time ($p=0.02$). Statistical results for the condition-by-time interaction and main effects of condition and time for the recovery interval: (A) PTH: condition-by-time ($p<0.01$), condition ($p<0.01$), time ($p<0.01$); (B) PTH_{ADJ}: condition-by-time ($p=0.05$), condition ($p<0.01$), time ($p<0.01$); (C) CTX: condition-by-time ($p=0.08$), condition ($p=0.06$), time ($p=0.13$); and (D) CTX_{ADJ}: condition-by-time ($p=0.35$), condition ($p=0.08$), time ($p<0.01$).

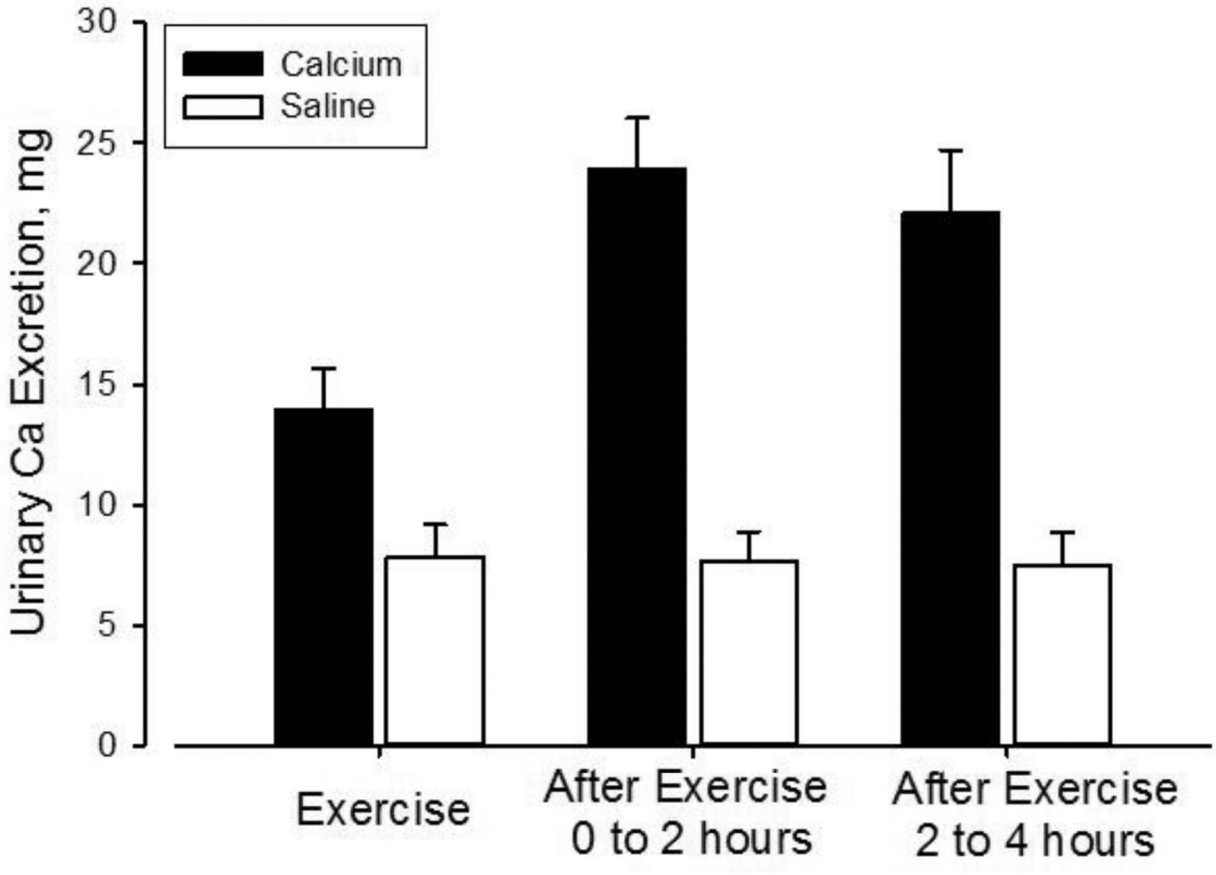

Figure 3.

Urinary Ca excretion during exercise and through the 4 hours of recovery for the calcium infusion (black bars) and the saline infusion (white bars). There was a significant condition-by-time interaction ($p<0.01$), condition ($p<0.01$) and time ($p<0.01$) effect for urinary Ca loss.

Table 1.

Descriptive characteristics (mean ± SD)

Variable	All (n = 12)	Women (n = 6)	Men (n = 6)
Age (y)	65.5 ± 5.7	62.5 ± 1.6	69.0 ± 6.6
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.0
Weight (kg)	75.1 ± 12.8	66.1 ± 8.7	84.1 ± 9.4
Fat-free mass (kg)	52.9 ± 10.8	44.4 ± 6.0	61.4 ± 6.8
Fat mass (kg)	22.2 ± 5.2	21.6 ± 5.5	22.7 ± 5.4
Lumbar spine T-score	−0.5 ± 1.3	−0.7 ± 1.6	−0.2 ± 1.1
Total hip T-score	−0.4 ± 1.0	−0.6 ± 1.3	−0.3 ± 0.9
Femoral neck T-score	−0.8 ± 1.3	−1.0 ± 1.6	−0.7 ± 1.0
Serum Ca (mg/dL)	9.3 ± 0.2	9.3 ± 0.3	9.3 ± 0.2
Serum 25-hydroxyvitamin D (ng/mL)	34.6 ± 9.1	34.2 ± 9.3	35.0 ± 9.8
Maximal heart rate (bpm)	157 ± 13	156 ± 14	158 ± 14
VO_2peak (mL/min/kg)	29.0 ± 4.6	29.0 ± 5.5	29.1 ± 4.0