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Journal

Medicine & Science in Sports & Exercise, 51(8)

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Publication Date

2019-08-01

DOI

10.1249/MSS.0000000000001967

Peer reviewed

HHS Public Access

Author manuscript

Med Sci Sports Exerc. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

Med Sci Sports Exerc. 2019 August ; 51(8): 1599–1605. doi:10.1249/MSS.0000000000001967.

Bone Biomarker Response to Walking under Different Thermal Conditions in Older Adults

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Abstract

Endurance exercise can cause a decrease in serum ionized calcium (iCa) and increases in parathyroid hormone (PTH) and c-terminal telopeptide of type I collagen (CTX), which may be due to Ca loss in sweat.

PURPOSE: To determine if exercise in a warm environment exaggerates the decrease in iCa and increases in PTH and CTX compared to a cool environment in older adults.

METHODS: Twelve women and men aged 61–78 y performed two identical 60-minute treadmill bouts at ~75% of maximal heart rate under warm and cool conditions. Serum iCa, PTH, and CTX were measured every 15 minutes starting 15 minutes before and continuing for 60 minutes after exercise. Sweat Ca loss was estimated from sweat volume and sweat Ca concentration.

RESULTS: Sweat volume was low and variable; there were no differences in sweat volume or Ca concentration between conditions. iCa decreased after 15 minutes of exercise and the change was similar in both conditions. Increases in PTH (Warm: 16.4, 95% CI: 6.2, 26.5 pg/mL; Cool: 17.3, 95% CI: 8.1, 26.4 pg/mL) and CTX (Warm: 0.08, 95% CI: 0.05, 0.11 ng/mL; Cool: 0.08, 95% CI: 0.01, 0.16 ng/mL) from before to immediately after exercise were statistically significant and similar between conditions. Adjusting for plasma volume shifts did not change the results.

CONCLUSION: The increases in PTH and CTX, despite the low sweat volume, suggest that dermal Ca loss is not a major factor in the decrease in iCa and increases in PTH and CTX observed during exercise in older adults.

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Author roles: Study design: SW, PW, RS, WK. Study conduct and data collection: SW, CS, TW, RB. Data analysis and interpretation: SW, PW, WK. Drafting manuscript: SW, PW, WK. Revising manuscript content: SW, CS, PW, TW, RB, RS, WK. Approving final version of manuscript: SW, CS, PW, TW, RB, RS, WK.

Conflict of Interest

We have no conflicts of interest to declare. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. Results of the present study do not constitute endorsement by ACSM.

Keywords

calcium homeostasis; parathyroid hormone; c-terminal telopeptide of type I collagen; treadmill exercise

Introduction

Regular physical activity that loads the skeleton is recommended for older adults to maintain bone health and prevent fracture.(1, 2) However, despite the documented beneficial effects of exercise on bone, there is emerging evidence that exercise training does not always lead to favorable bone adaptations.(3, 4) Indeed, bone mineral density (BMD) was observed to decrease in competitive cyclists over a year of training and competition(5, 6) and male NCAA basketball players were found to have a −10.5% decrease in leg bone mineral content (BMC) from preseason to late summer.(4) The underlying mechanisms that contribute to bone loss or lack of an increase in BMD with exercise training are not well understood.

One possible explanation is that exercise can cause a decrease in serum ionized calcium (iCa) that leads to increased bone resorption to maintain iCa. One hour of endurance exercise at 70–80% of maximal heart rate was found to decrease iCa and increase parathyroid hormone (PTH) and c-terminal telopeptide of type I collagen (CTX).(7–10) If this occurs with repeated exercise bouts, it could lead to impairments in the adaptive bone response to exercise training over time. The factors that lead to a decline in serum iCa loss (e.g., urinary excretion, dermal loss, muscle uptake) during exercise are unknown, but evidence from equine models suggests that sweating may play an important role. Like humans, horses lose Ca ions in sweat and serum Ca is decreased after strenuous exercise.(11, 12) Further, horses lose more Ca through sweat when exercising in a warm environment than a cool, dry environment, and the magnitude of loss can exceed normal daily Ca intake.(12) If humans experience similar high dermal Ca loss during exercise resulting in bone catabolism to maintain serum iCa, this may explain why bone does not always adapt favorably to exercise training. Our working model demonstrating the relationship between iCa, PTH, bone resorption, and the hypothetical impact on BMD has been previously published.(7)

There has been limited research to determine how dermal Ca loss in humans influences changes in iCa, PTH, and CTX during exercise. For older adults, who are recommended to engage in higher-intensity, weight-bearing exercise to maintain bone health,(13) it is important to understand the underlying mechanisms that contribute to the bone response to acute exercise; these mechanisms likely contribute to the adaptive bone response to exercise training. Therefore, the purpose of this study was to determine if exercise in a warm environment exaggerates the decrease in serum iCa and increases in PTH and CTX compared to a cool environment. Older adults completed 60 minutes of brisk treadmill walking under warm (targeted temperature 26°C) versus cool conditions (targeted temperature 16°C). We hypothesized that exercise in the warm condition would result in greater increases in serum PTH and CTX (primary outcomes) and a greater sweat volume (secondary outcome) when compared with the cool condition.

Methods

Subjects.

Healthy, recreationally active older women (n=5) and men (n=7) aged 60 to 80 y who were accustomed to brisk walking were eligible to participate. Volunteers were eligible if they reported walking, hiking, or running at least 1 hour per day at least 2 days per week in the previous 6 months. Exclusion criteria included: known disease or condition associated with intestinal malabsorption; moderate or severe renal impairment (estimated glomerular filtration rate of <60 mL/min/1.73m^2); use of medications known to affect bone metabolism (e.g., bisphosphonates, oral glucocorticoids, hormone replacement therapy) in the past 6 months; BMD T-score < −2.5 at the total hip, femoral neck or lumbar spine; chronic hepatobiliary disease; abnormal thyroid function (ultrasensitive TSH <0.5 or >5.0 mU/L); serum Ca <8.5 or >10.3 mg/dL; serum 25(OH)D <20 ng/mL; uncontrolled hypertension; history of type 1 or type 2 diabetes; and evidence of ischemic heart disease or serious arrhythmias at rest or during the graded exercise test (GXT). Volunteers with abnormal serum 25(OH)D or TSH values were allowed to participate if abnormal values were corrected with medical intervention. Participants who reported taking Ca supplements were asked to discontinue use for 24 hours before each exercise bout. All participants provided written informed consent, and the study was approved by the Colorado Multiple Institutional Review Board. The study was registered at ClinicalTrials.gov (NCT02468817).

Dual-energy X-ray Absorptiometry (DXA).

BMD of the lumbar spine (L1-L4), total hip, and femoral neck and trochanter regions of the hip were measured on a Discovery W DXA instrument (Hologic Inc; Waltham, Massachusetts) as a screening measure. Fat-free mass (FFM; kg), fat mass (FM; kg), and relative adiposity (fat mass as a percentage of body weight) were obtained from the total body scan.

Graded Exercise Testing.

Participants performed an incremental test on a treadmill for measurement of peak aerobic power (VO_2peak). The starting speed of the treadmill raised the heart rate to ~70% of age-predicted maximal heart rate (HR_{max}) and treadmill grade was increased by 2% every 2 minutes until volitional fatigue. VO_2 was measured using a Parvo Medics TruMax 2400 Metabolic cart (Parvo Medics Inc, Sandy, UT).

Pre-exercise Meal.

Participants fasted overnight and were then provided a breakfast that contained 575 kcal for men and 335 kcal for women. The macronutrient content of the meal was 50% carbohydrate, 35% fat, and 15% protein and contained <100 mg of Ca. Participants consumed the same meal 4 hours before each exercise bout.

Thermal Environment.

The room was pre-cooled or pre-warmed to the desired temperature (16°C or 26°C) and humidity was clamped at 50% before the participant arrived. Participants and study team

members remained in the room from 15 minutes before exercise until after the 5-minute cool-down. Temperature and humidity were continuously monitored during exercise. After exercise ended, the door was opened and recovery took place in ambient temperature and humidity.

Exercise Bouts.

The exercise bout was 60 minutes of treadmill walking at 70–80% of measured HR_{max}. Participants performed a 5-minute warm-up and 5-minute cool-down at a self-selected intensity; this was matched in duration and speed at both visits. For the first three participants, the order of the thermal conditions was randomized. However, due to a safety concern raised by the nursing staff regarding older adults experiencing cardiovascular symptoms while exercising in the heat, this was discontinued and the cool condition was performed first in the remaining participants. This allowed us to screen for any problems with exercise tolerance before exposing older adults to the higher temperature. The second exercise bout was performed 1 to 4 weeks after the first and the exercise protocol was replicated. Participants were instructed to wear the same clothing for both exercise bouts and the frequency and volume of water intake during and after exercise was repeated between visits.

Sweat and Dermal Ca Measurements.

Dermal Ca loss was estimated using the skin patch collection method used previously.(14) Six 42-mm filter paper discs were placed under a tegaderm dressing on the upper back (2), upper chest (2), and arms (2). Skin was thoroughly cleaned with deionized water and dried with sterile gauze prior to placement of the patches. Once saturated with sweat, the patches were placed in a 5-mL syringe and the sweat was expressed into a tube for determination of Ca concentration by VITROS microslide colorimetric assay (Ortho-Clinical Diagnostics, Inc., Rochester, NY). Total sweat loss was estimated from changes in body mass adjusted for fluid intake. Dermal Ca loss was estimated from sweat loss and sweat Ca concentration.

Blood Sampling and Analysis.

An indwelling, intravenous catheter was positioned before the start of exercise for serial blood sampling. Samples were obtained before (T=−15, 0), during (T=15, 30, 45, 60 min), and after (T=75, 90, 105, 120 min) exercise. Samples were analyzed for intact parathyroid hormone (PTH) by Immulite two-site EIA (Siemens, Erlangen, Germany) and c-terminal telopeptide of type I collagen (CTX) by chemiluminescence (Immunodiagnostics Systems, The Boldens, United Kingdom), and total Ca (tCa) by indirect ion sensitive electrode (Beckman Coulter, Inc., Brea, CA). However, due to a laboratory error, the tCa values were not valid. Laboratory-specific intra- and inter-assay coefficients of variation (CV) for the remaining outcomes are 2.4% and 3.7% for PTH and 3–4%−7.7% (for concentrations ranging from 0.201 ng/mL to 2.05 ng/mL) and 5.8%−8.6% (for concentrations ranging from 0.196 ng/mL to 2.08 ng/mL) for CTX. iCa and hematocrit (Hct) were measured using an iSTAT whole blood analyzer (Abbott Point of Care, Inc; Princeton, NJ). Because the iSTAT measures Hct based on conductivity, Hct values were not adjusted for trapped plasma, and no conversion from venous to whole body Hct was performed. iCa, PTH, and CTX values during and after exercise were corrected for plasma volume shifts using methods previously

described.(8, 15) This is done to correct for hemoconcentration, which may cause values to appear artificially elevated. Both unadjusted and adjusted values are presented. Values adjusted for plasma volume shifts are noted with an "ADJ" subscript (e.g. iCa_{ADJ}, PTH_{ADJ}, CTX_{ADJ}).

Urine Collection and Analysis.

Urine samples were collected approximately 20 minutes before exercise and immediately after exercise. Samples were collected in a sterile container, weighed, and recorded. Ca concentration was measured by indirect ion sensitive electrode (Beckman Coulter, Inc., Brea, CA). Urine samples were acidified prior to testing. Estimated urine Ca loss was the product of urine Ca concentration and void volume.

Statistical Analyses.

We hypothesized that the increase in PTH during exercise would be greater in the warm vs cool condition because of greater sweat losses. The targeted temperatures were selected based on evidence that sweat rate is ~50% higher at 26 °C than at 18 °C.(16) We increased our targeted temperature difference to 10°C to allow for a greater difference in temperature exposure. We estimated that exercise in the 26 °C condition should result in a PTH response ~45±55 pg/mL higher than the 16 °C condition based on preliminary evidence from our lab that a 43% higher sweat rate was associated with a 230% greater increase in PTH during exercise.(7, 8) A participant sample size of 14 was selected to provide 80.8% power to detect a difference of approximately 45±55 pg/mL in the between-condition change in PTH using a paired *t* test. Due to funding limitations, we were able to study only 12 participants.

The effects of warm versus cool conditions on PTH and CTX responses were evaluated using linear contrasts in a repeated measures maximum likelihood model with all available data. This approach is conceptually identical to repeated measures analysis of variance but avoids the case-wise deletion of participants with missing assessments; estimates are unbiased under the assumption that missing data are missing at random. However, there were no missing data for the primary outcomes: PTH, CTX, and iCa. We estimated the variance for each treatment group separately to accommodate heterogeneity by condition (warm vs cool). Linear contrasts were used to estimate within- and between-group differences over the 60-minute exercise bout and the 60-minute recovery period. Secondary measures were evaluated in the same manner. Data are presented as mean±SD, unless otherwise specified. All analyses were conducted using SAS version 9.3 (SAS Institute Inc, Cary, NC, USA) and a p value ≤0.05 defined statistical significance.

Results

Participant characteristics are presented in Table 1. All participants were Caucasian. Seven participants had low bone mass (T-score ≤−1.0) at the lumbar spine (3 women, 4 men), 5 had low bone mass at the total hip (3 women, 2 men), 10 had low bone mass at the femoral neck (4 women, 6 men), and 5 had low bone mass at the trochanter (2 women, 3 men). Hip BMD was not measured in one woman due to bilateral hip replacement.

The desired difference in room temperature for the two conditions was not achieved. Room temperature averaged 21.6±0.7°C on the cool day and 28.0±1.2°C on the warm day. Sweat volume and dermal Ca loss were undetectable in 4 participants (1 man, 3 women) during at least one visit. These participants were excluded from all sweat comparisons for both visits. Among the participants who provided data at both visits, there were no significant differences between conditions in sweat volume (warm: 0.34±0.33 L; cool: 0.58±0.32 L; p=0.43), sweat Ca loss (warm: 11.3±13.4 mg; cool: 31.1±43.0 mg; p=0.37), or urine Ca loss (warm: 4.0±3.6 mg; cool: 4.0±4.1 mg; p=0.96). Average walking speed was 3.4±0.2 mph at a 0.3±1.1% grade. Average heart rate during exercise was 122±10 bpm during the cool condition and 124±8 bpm during the warm condition. Hematocrit did not change during exercise and decreased during recovery in both conditions ($p<0.05$), but there were no differences in plasma volume shifts between conditions during exercise (0 to 60 min) or recovery (60 to 120 min).

Serum iCa decreased by −0.16 mg/dL in both conditions (warm 95% CI: −0.28, −0.08 mg/dL; cool 95% CI: −0.24, −0.04 mg/dL) from before to after exercise (Figure 1A). The decrease began after 15 minutes of exercise and continued through 15 minutes of recovery (Figure 1). After adjusting for plasma volume shifts, the change in iCa_{ADJ} from before to after exercise was significant in the warm condition only (warm: −0.24 mg/dL, 95% CI −0.44, −0.04 mg/dL; cool: −0.04 mg/dL, 95% CI −0.28, 0.24 mg/dL; Figure 1B). During recovery (60–120 min), there was no change in iCa in either condition (warm: −0.04 mg/dL, 95% CI −0.12, 0.04 mg/dL; cool: 0.0 mg/dL, 95% CI −0.12, 0.12 mg/dL; Figure 1A). iCa_{ADJ} increased during recovery in both conditions (warm: 0.36 mg/dL, 95% CI 0.20, 0.56 mg/dL; cool: 0.44 mg/dL, 95% CI 0.20, 0.68 mg/dL). There were no between-condition differences in changes in iCa or iCa_{ADJ} during exercise or recovery.

PTH increased similarly during exercise in both conditions (warm: 16.4 pg/mL, 95% CI 6.2, 26.5 pg/mL; cool: 17.3 pg/mL, 95% CI 8.1, 26.4 pg/mL) and reached peak levels 15 minutes into recovery (Figure 1). The increase in PTH_{ADJ} during exercise was also similar between conditions (warm: 16.0 pg/mL, 95% CI 5.3, 26.7 pg/mL; cool: 18.1 pg/mL, 95% CI 8.8, 27.6 pg/mL). During recovery, PTH decreased in both conditions (warm: −12.3 pg/mL, 95% CI −22.4, −2.1 pg/mL; cool: −14.9 pg/mL, 95% CI −24.0, −5.8 pg/mL) and adjusting for plasma volume shifts did not change the results. There were no between-condition differences in changes in PTH or PTH_{ADJ} during exercise or recovery.

CTX increased similarly during exercise in both conditions (warm: 0.08 ng/mL, 95% CI 0.05, 0.11 ng/mL; cool: 0.08 ng/mL, 95% CI 0.01, 0.16 ng/mL) (Figure 1E). Adjusting for plasma volume shifts did not change the results. During recovery, CTX increased in the warm condition only (warm: 0.09 ng/mL, 95% CI 0.06, 0.12 ng/mL; cool: 0.06 ng/mL, 95% CI −0.01, 0.13 ng/mL), but CTX_{ADJ} increased in both conditions (warm: 0.10 ng/mL, 95% CI 0.07, 0.13 ng/mL; cool: 0.09 ng/mL, 95% CI 0.02, 0.16 ng/mL). CTX and CTX_{ADJ} reached peak levels at the end of recovery in both conditions (Figure 1E, 1F). There were no between-condition differences for changes in either CTX or CTX_{ADJ} during exercise or recovery.

Discussion

Based on the evidence from equine models,(11, 12) our hypothesis was that dermal Ca loss is the main driver of the disruption in Ca homeostasis during exercise. The results do not support this hypothesis. A decrease in serum iCa began 15 minutes into exercise and PTH and CTX both began increasing early in exercise, which was likely before substantial sweating had occurred. The estimated sweat volume and dermal Ca loss were highly variable between participants and, in 4 participants, sweat loss was undetectable at one or both visits. Despite the low dermal Ca loss, there was a decrease in iCa and increases in PTH and CTX during exercise.

The increases in PTH and CTX during exercise were consistent with the results reported by our lab and others, indicating increased markers of bone resorption in response to endurance exercise lasting at least 1 hour.(8, 10, 17–20) PTH continued to increase throughout exercise and peaked at approximately 15 minutes after exercise, while iCa was the lowest at 15 minutes after exercise (Figure 1). PTH increases within minutes in response to small decreases in iCa,(21) and the timing of the peak in PTH with the nadir in iCa suggests PTH responded to the change in serum iCa concentrations. Interestingly, CTX continued to increase through recovery and was greatest at the end of the recovery period. We have shown previously that CTX remained elevated for at least 4 hours after the end of exercise in young, male cyclists.(18) Because of the shorter recovery window in the current study, it is not known if CTX also remains elevated for longer than 1 hour after exercise in older adults.

The sustained increase in CTX in this study and in previous studies indicates that endurance exercise can provoke an increase in bone resorption that is maintained during recovery. Thus, it is plausible that exercise disrupts bone metabolism in a manner that leads to bone loss under some conditions. For example, it could be an underlying mechanism for the bone loss that has been observed in competitive male cyclists(5) if cycling does not sufficiently activate bone formation to counteract the increase in resorption.(22) However, PTH can have either catabolic or anabolic actions on bone, depending primarily on the length of time it is elevated.(23) A chronic elevation in PTH (e.g., primary or secondary hyperparathyroidism) has catabolic skeletal effects, whereas intermittent increases in PTH are anabolic.(23) Teriparatide, a PTH analog used in the treatment of osteoporosis,(24) causes an increase in CTX that remains elevated ~3 hours after a single dose (25), but CTX decreases with daily therapy until it returns to baseline by 28 days.(26) After 28 days of teriparatide treatment, procollagen type 1 amino-terminal propeptide (P1NP), a marker of bone formation, increased by ~111%.(26) The net effect of teriparatide therapy is anabolic, as evidenced by an increase BMD with longer therapy.(27) It is unclear if the exercise-induced increase in PTH triggers only an acute catabolic response, as observed in the current study, or whether there is a net anabolic effect that occurs with repeated exercise-induced increases in PTH.

Bone remodeling is initiated by an increase in resorption, with a subsequent increase in formation.(28) In this context, it is possible that the exercise-induced increase in bone resorption is followed by an increase in bone formation,(28) and may have favorable long-term effects on bone metabolism. P1NP was not measured in the current study, so the effect of the exercise bout on bone formation was not assessed. However, we and others have

manipulated the PTH response to exercise through oral or intravenous Ca administration before or during exercise and measured CTX and P1NP.(17, 18) In these studies, supplemental Ca attenuated the increases in PTH and CTX when compared with placebo, but there was no difference between Ca and placebo conditions in the P1NP response. This suggests that the normal coupling of bone resorption and formation is disrupted by the exercise-induced increase in PTH, at least acutely. The more chronic effects of repeated exercise-induced increases PTH on bone resorption and formation are not clear.

Mode and Intensity of Exercise

Mode of exercise may be a determinant of the magnitude of changes in PTH and CTX in response to endurance exercise. We have conducted studies of both treadmill walking and stationary cycling at similar relative intensities and found that increases in PTH and CTX were higher in response to cycling.(7, 10) However, the participants in the treadmill exercise study were older and, therefore, exercised at a lower absolute exercise intensity; both of these factors may have contributed to the less robust PTH and CTX responses. The increases in PTH in response to moderate- and high-intensity treadmill running reported by Scott and colleagues(19) also appear to be attenuated when compared with our studies of moderate- and high-intensity cycling. (7, 8) It will be important to evaluate the effect of exercise mode using a within-subject experimental approach to determine whether mode is an important determinant of the PTH response to exercise.

Exercise intensity appears to be a direct determinant of the magnitude of the PTH response to exercise. We have found that high-intensity cycling results in a greater increase in PTH than moderate-intensity cycling, although the bouts were not matched for exercise duration. (7, 8) Further, Scott et al. demonstrated step-wise increases in both PTH and CTX with increasing exercise intensity during treadmill running.(19) However, to our knowledge, no studies have evaluated the effects of both exercise mode and intensity on the disruption of Ca homeostasis within the same study cohort. Further studies to better define the effects of exercise mode, intensity, and duration on the disruption of Ca homeostasis in different populations are warranted.

Ca Binding

Ca-sensing receptors in the parathyroid glands respond to changes in serum iCa concentration.(29) A change in serum iCa concentration could occur as a result of changes in plasma volume (e.g., hemoconcentration with plasma volume contraction) or a change in Ca binding. We had intended to measure both tCa and iCa to assess whether the decrease in iCa concentration during exercise could reflect an increase in Ca binding. However, due to technical error in the laboratory that analyzed the samples, the tCa values were not valid. In young, male cyclists, iCa and tCa, unadjusted and adjusted for changes in plasma volume, had similar patterns of change during exercise, suggesting there was no effect of exercise on Ca binding.(18) This contradicted a previous study that reported a decrease in iCa of ~1mg/dL after 21 minutes of running exercise in young men, but no change in tCa.(30) However, it was not clear whether one or both of the Ca outcomes had been corrected for changes in plasma volume. Further research will be needed to resolve the question of whether a change in Ca binding contributes to the changes in serum iCa during exercise.

Role of Dermal and Other Sources of Ca Loss

We were unable to maintain the targeted temperatures for the two exercise conditions. Once participants began exercising, the temperature rose steadily and the system was unable to counter-balance the heat generated. This resulted in a 6.4°C temperature difference instead of the 8°C temperature difference on which the study was based(16) or the targeted 10°C difference. An 8°C temperature difference was previously shown to result in a 50% greater sweat rate at the higher temperature.(16) We did not observe a difference in sweat rate in the current study, but it was not clear whether this was because the targeted temperature differential was not achieved or because the sweat rate was low in older adults. The low sweat rate was not unexpected, because older adults produce less sweat than young adults in response to the same thermal stimulus.(31, 32). However, even though dermal Ca loss was low, there was a decrease in iCa and increases in PTH and CTX that occurred independently of the thermal stimulus. This suggests dermal Ca loss is not the primary determinant of these responses and it is unlikely that achieving the desired thermal conditions would have changed the results. We cannot rule out that dermal Ca loss contributes to the disruption of Ca homeostasis during exercise.

Another potential source of Ca loss during exercise is urinary excretion. We previously found that urinary Ca excretion during and after exercise was low (<10 mg/h) and did not account for the decrease in serum iCa during exercise.(18) Urinary Ca excretion was also low in the current study.

Because Ca is essential for muscle contraction, it seems plausible that the decrease in serum Ca during exercise could reflect muscle uptake. In a review paper that included a discussion of muscle iCa content and movement, it was argued that net iCa content of muscle can increase with repeated contractions, but that there is usually very little change unless the exercise is prolonged.(33) Although most of the studies that led to this conclusion were conducted in rodents, there is supporting evidence from humans. There was a nonsignificant increase in muscle iCa content (0.81 to 0.91 μmol/g WW) in response to a 10-km run, but significant increases in response to 20-km (0.70 to 0.93 μmol/g WW) and 100-km runs (0.84 to 1.02 μmol/g WW).(34, 35) These findings suggest that skeletal muscle metabolism may contribute to the disruption of serum Ca homeostasis during exercise.

Limitations

We acknowledge the limitations of estimating sweat loss from the change in body weight and the use of skin patches to quantify dermal Ca loss.(36, 37) Methods were identical at each visit, so any error in the estimation of dermal Ca loss should have been similar for both conditions. Because the changes in serum iCa, PTH, and CTX started early in exercise, when sweat loss was minimal, other mechanisms for the decrease in serum iCa during exercise should be investigated.

We had planned to complete testing on 14 participants (7 men, 7 women). Due to funding limitations, we were able to complete only 12. Our initial power analysis indicated that a difference of 45±55 pg/mL in change in PTH between the two thermal conditions was necessary to detect a significant difference with a sample size of 14. The actual difference in

change in PTH between the two conditions was less than 5 pg/mL; completing an additional 2 participants would not have changed the results.

Conclusion

A decrease in serum iCa during exercise triggers increases in PTH and CTX in both young and older adults.(7, 8, 10, 17, 18) Equine studies(11, 12) suggested this may be due to dermal Ca loss during exercise, but our results do not support this conclusion. The decrease in iCa and the increases in PTH and CTX occurred with low sweat volume. Further, the changes began soon after the onset of exercise. It is possible that dermal calcium loss contributes to the disruption in calcium homeostasis during exercise, but it does not appear to be the primary trigger. Future research should investigate potential mechanical and metabolic factors that may contribute to the decrease in serum iCa and the increases in PTH and CTX. Specifically, sex, mode of exercise, exercise intensity, and time of day may contribute to magnitude of the PTH and CTX response to exercise.

Acknowledgements

This study was supported in part by NIH awards UL1 TR002535, T32 AG000279, K23 AR070275 and P30 DK048520 and the VA Eastern Colorado Geriatric Research, Education, and Clinical Center (GRECC).

Funding Sources: This study was supported in part by NIH awards UL1 TR002535, T32 AG000279, K23 AR070275 and P30 DK048520 and the VA Eastern Colorado Geriatric Research, Education, and Clinical Center (GRECC). Contents are the sole responsibility of the authors and may not represent official views of the NIH or VA. The authors have no conflicts of interest to declare.

REFERENCES

1. Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC, Birge SJ Jr. Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women. Ann Intern Med. 1988;108(6): 824–8. Epub 1988/06/01.. [PubMed: 3259410]
2. Khan K, McKay H, Kannus PA, Bailey DA, Wark J, Bennell K. Physical activity and bone health: Human Kinetics; 2001.
3. Guadalupe-Grau A, Fuentes T, Guerra B, Calbet JA. Exercise and bone mass in adults. Sports Med. 2009;39(6):439–68. Epub 2009/05/21. doi: 10.2165/00007256-200939060-00002. [PubMed: 19453205]
4. Klesges RC, Ward KD, Shelton ML, Applegate WB, Cantler ED, Palmieri GM, et al. Changes in bone mineral content in male athletes. Mechanisms of action and intervention effects. JAMA. 1996;276(3):226–30. Epub 1996/07/17. doi: 10.1001/jama.1996.03540030060033. [PubMed: 8667568]
5. Barry DW, Kohrt WM. BMD decreases over the course of a year in competitive male cyclists. J Bone Miner Res. 2008;23(4):484–91. Epub 2007/12/13. doi: 10.1359/jbmr.071203. [PubMed: 18072875]
6. Sherk VD, Barry DW, Villalon KL, Hansen KC, Wolfe P, Kohrt WM. Bone loss over 1 year of training and competition in female cyclists. Clin J Sport Med. 2014;24(4):331–6. Epub 2013/12/12. doi: 10.1097/JSM.0000000000000050. [PubMed: 24326929]
7. Barry DW, Hansen KC, van Pelt RE, Witten M, Wolfe P, Kohrt WM. Acute calcium ingestion attenuates exercise-induced disruption of calcium homeostasis. Med Sci Sports Exerc. 2011;43(4): 617–23. Epub 2010/08/28. doi: 10.1249/MSS.0b013e3181f79fa8. [PubMed: 20798655]
8. Barry DW, Kohrt WM. Acute effects of 2 hours of moderate-intensity cycling on serum parathyroid hormone and calcium. Calcif Tissue Int. 2007;80(6):359–65. Epub 2007/06/06. doi: 10.1007/s00223-007-9028-y. [PubMed: 17549534]

9. Sherk VD, Wherry SJ, Barry DW, Shea KL, Wolfe P, Kohrt WM. Calcium Supplementation Attenuates Disruptions in Calcium Homeostasis during Exercise. Med Sci Sports Exerc. 2017;49(7): 1437–42. Epub 2017/03/02. doi: 10.1249/MSS.0000000000001239. [PubMed: 28248693]
10. Shea KL, Barry DW, Sherk VD, Hansen KC, Wolfe P, Kohrt WM. Calcium supplementation and parathyroid hormone response to vigorous walking in postmenopausal women. Med Sci Sports Exerc. 2014;46(10):2007–13. Epub 2014/03/01. doi: 10.1249/MSS.0000000000000320. [PubMed: 24576866]
11. Soliman MK, Nadim MA. Calcium, sodium and potassium level in the serum and sweat of healthy horses after strenuous exercise. Zentralblatt fur Veterinarmedizin Reihe A. 1967;14(1):53–6. Epub 1967/01/01.
12. McCutcheon LJ, Geor RJ. Sweat fluid and ion losses in horses during training and competition in cool vs. hot ambient conditions: implications for ion supplementation. Equine veterinary journal Supplement. 1996(22):54–62. Epub 1996/07/01.
13. Kohrt WM, Bloomfield SA, Little KD, Nelson ME, Yingling VR, American College of Sports M. American College of Sports Medicine Position Stand: physical activity and bone health. Med Sci Sports Exerc. 2004;36(11):1985–96. Epub 2004/10/30. [PubMed: 15514517]
14. Barry DW, Hansen KC, Van Pelt RE, Witten M, Wolfe P, Kohrt WM. Acute calcium ingestion attenuates exercise-induced disruption of calcium homeostasis. MedSciSports Exerc. 2011;43(4): 617–23.
15. Van Beaumont W Evaluation of hemoconcentration from hematocrit measurements. J Appl Physiol. 1972;32(5):712–3. Epub 1972/05/01. [PubMed: 5038863]
16. Reneau PD, Bishop PA, Ashley CD. Comparison of a military chemical suit and an industrial usage vapor barrier suit across two thermal environments. American Industrial Hygiene Association journal. 1997;58(9):646–9. Epub 1997/09/18. doi: 10.1080/15428119791012432. [PubMed: 9291562]
17. Haakonssen EC, Ross ML, Knight EJ, Cato LE, Nana A, Wluka AE, et al. The effects of a calcium-rich pre-exercise meal on biomarkers of calcium homeostasis in competitive female cyclists: a randomised crossover trial. PLoS One. 2015;10(5):e0123302 Epub 2015/05/15. doi: 10.1371/journal.pone.0123302. [PubMed: 25970439]
18. Kohrt WM, Wherry SJ, Wolfe P, Sherk VD, Wellington T, Swanson CM, et al. Maintenance of serum ionized calcium during exercise attenuates parathyroid hormone and bone resorption responses. J Bone Miner Res. 2018 Epub 2018/03/25. doi: 10.1002/jbmr.3428.
19. Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. The role of exercise intensity in the bone metabolic response to an acute bout of weight-bearing exercise. J Appl Physiol (1985). 2011;110(2):423–32. Epub 2010/12/04. doi: 10.1152/japplphysiol.00764.2010. [PubMed: 21127210]
20. Scott JP, Sale C, Greeves JP, Casey A, Dutton J, Fraser WD. The effect of training status on the metabolic response of bone to an acute bout of exhaustive treadmill running. J Clin Endocrinol Metab. 2010;95(8):3918–25. Epub 2010/06/04. doi: 10.1210/jc.2009-2516. [PubMed: 20519353]
21. Schmitt CP, Schaefer F, Bruch A, Veldhuis JD, Schmidt-Gayk H, Stein G, et al. Control of pulsatile and tonic parathyroid hormone secretion by ionized calcium. J Clin Endocrinol Metab. 1996;81(12):4236–43. Epub 1996/12/01. doi: 10.1210/jcem.81.12.8954021. [PubMed: 8954021]
22. Kohrt WM, Barry DW, Schwartz RS. Muscle forces or gravity: what predominates mechanical loading on bone? Med Sci Sports Exerc. 2009;41(11):2050–5. Epub 2009/10/09. doi: 10.1249/MSS.0b013e3181a8c717. [PubMed: 19812511]
23. Silva BC, Costa AG, Cusano NE, Kousteni S, Bilezikian JP. Catabolic and anabolic actions of parathyroid hormone on the skeleton. J Endocrinol Invest. 2011;34(10):801–10. Epub 2011/09/29. doi: 10.3275/7925. [PubMed: 21946081]
24. Cosman F Parathyroid hormone treatment for osteoporosis. Curr Opin Endocrinol Diabetes Obes. 2008;15(6):495–501. Epub 2008/10/31. doi: 10.1097/MED.0b013e32831a46d6. [PubMed: 18971677]
25. Zikan V, Stepan JJ. Marker of Bone Resorption in Acute Response to Exogenous or Endogenous Parathyroid Hormone. Biomark Insights. 2008;3:19–24. Epub 2008/01/01. [PubMed: 19578490]

26. Glover SJ, Eastell R, McCloskey EV, Rogers A, Garnero P, Lowery J, et al. Rapid and robust response of biochemical markers of bone formation to teriparatide therapy. Bone. 2009;45(6): 1053–8. Epub 2009/08/15. doi: 10.1016/j.bone.2009.07.091. [PubMed: 19679211]
27. Eriksen EF, Keaveny TM, Gallagher ER, Krege JH. Literature review: The effects of teriparatide therapy at the hip in patients with osteoporosis. Bone. 2014;67:246–56. Epub 2014/07/24. doi: 10.1016/j.bone.2014.07.014. [PubMed: 25053463]
28. Eriksen EF. Cellular mechanisms of bone remodeling. Reviews in endocrine & metabolic disorders. 2010;11(4):219–27. Epub 2010/12/29. doi: 10.1007/s11154-010-9153-1. [PubMed: 21188536]
29. Brown EM. Calcium receptor and regulation of parathyroid hormone secretion. Reviews in endocrine & metabolic disorders. 2000;1(4):307–15. Epub 2001/11/15. [PubMed: 11706745]
30. Bouassida A, Zalleg D, Zaouali Ajina M, Gharbi N, Duclos M, Richalet JP, et al. Parathyroid hormone concentrations during and after two periods of high intensity exercise with and without an intervening recovery period. Eur J Appl Physiol. 2003;88(4–5):339–44. Epub 2003/01/16. doi: 10.1007/s00421-002-0721-2. [PubMed: 12527961]
31. Anderson RK, Kenney WL. Effect of age on heat-activated sweat gland density and flow during exercise in dry heat. J Appl Physiol (1985). 1987;63(3):1089–94. Epub 1987/09/01. [PubMed: 3654456]
32. Inoue Y, Shibasaki M, Ueda H, Ishizashi H. Mechanisms underlying the age-related decrement in the human sweating response. European journal of applied physiology and occupational physiology. 1999;79(2):121–6. Epub 1999/02/24. doi: 10.1007/s004210050485. [PubMed: 10029332]
33. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008;88(1):287–332. Epub 2008/01/16. doi: 10.1152/physrev.00015.2007. [PubMed: 18195089]
34. Overgaard K, Fredsted A, Hyldal A, Ingemann-Hansen T, Gissel H, Clausen T. Effects of running distance and training on Ca2+ content and damage in human muscle. Med Sci Sports Exerc. 2004;36(5):821–9. Epub 2004/05/06. [PubMed: 15126716]
35. Overgaard K, Lindstrom T, Ingemann-Hansen T, Clausen T. Membrane leakage and increased content of Na+ -K+ pumps and Ca2+ in human muscle after a 100-km run. J Appl Physiol (1985). 2002;92(5):1891–8. Epub 2002/04/19. doi: 10.1152/japplphysiol.00669.2001. [PubMed: 11960939]
36. Baker LB, Stofan JR, Lukaski HC, Horswill CA. Exercise-induced trace mineral element concentration in regional versus whole-body wash-down sweat. IntJSport NutrExercMetab. 2011;21(3):233–9.
37. Palacios C, Wigertz K, Weaver CM. Comparison of 24 hour whole body versus patch tests for estimating body surface electrolyte losses. IntJSport NutrExercMetab. 2003;13(4):479–88.

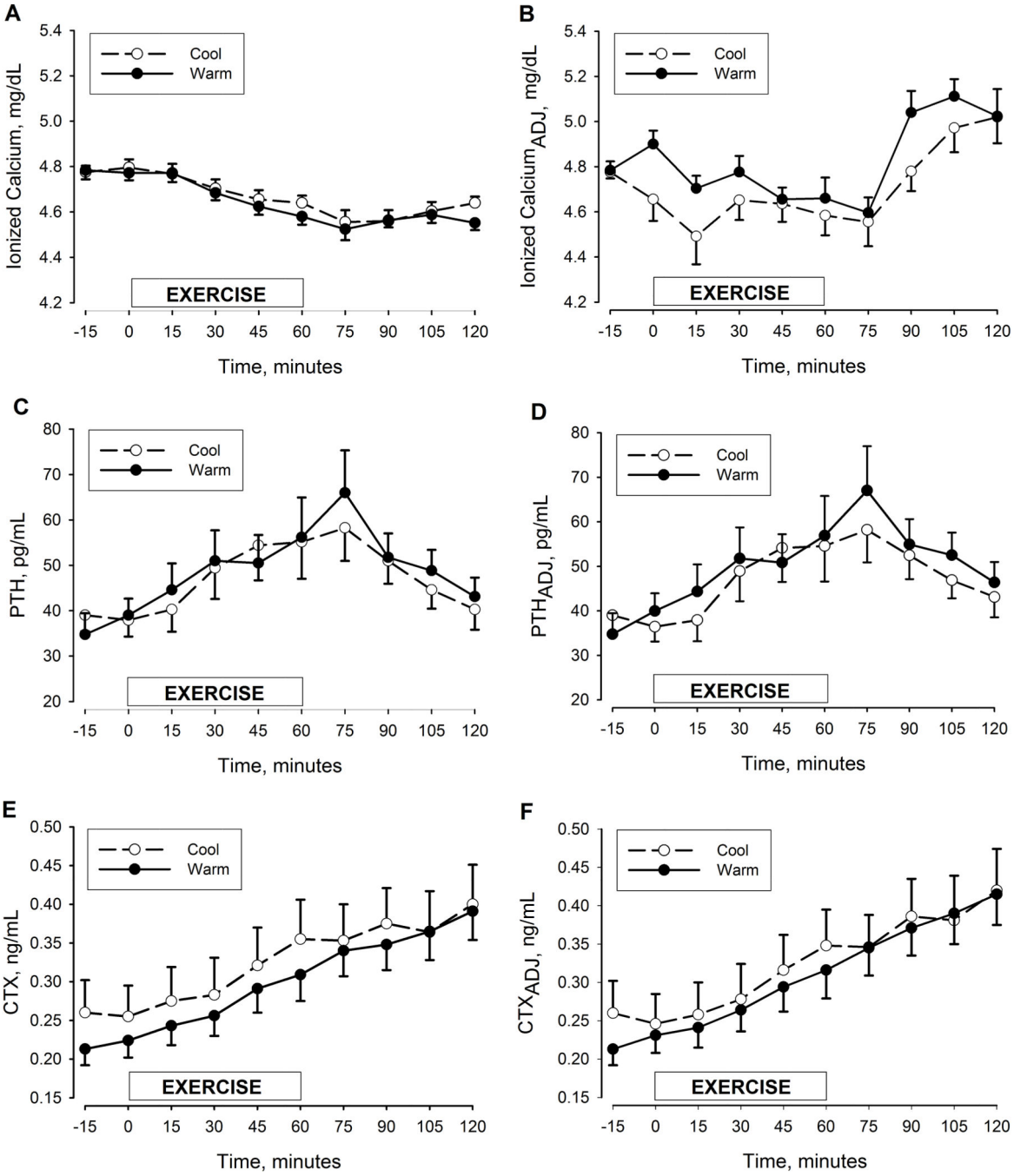

Figure 1.

Serum ionized calcium (panel A), parathyroid hormone (panel C), and c-telopeptide (panel E) concentrations before, during, and after 60 minutes of brisk treadmill walking during the cool (open symbols, dashed lines) and warm (closed symbols, solid lines) conditions. Adjustment for plasma volume shifts (iCa_{ADJ}, panel B; PTH_{ADJ}, panel D; CTX_{ADJ}, panel F) reflects changes in vascular iCa, PTH, and CTX content. Statistical results: Panel A) iCa decreased during exercise in both conditions ($p<0.01$); changes in iCa during recovery were not significant; Panel B) iCa_{ADJ} decreased during exercise in the warm condition ($p=0.02$),

but the between-group difference in the change was not significant ($p=0.11$); iCa_{ADJ} increased similarly during recovery in both conditions ($p<0.001$); Panel C) PTH increased similarly during exercise in both conditions ($p<0.01$) and decreased similarly during recovery in both conditions ($p<0.05$); Panel D) adjusting PTH for plasma volume shifts did not change the results; Panel E) CTX increased similarly during exercise in both conditions ($p<0.001$), and continued to increase during recovery in the warm condition only ($p<0.001$); changes were not different between conditions; Panel F) CTX_{ADJ} increased during exercise and recovery in both conditions ($p<0.05$); changes were not different between conditions

Table 1.

Participant characteristics (mean ± SD)

Variable	All (n = 12)	Women (n = 5)	Men (n = 7)
Age (y)	67 ± 5	67 ± 5	67 ± 6
Height (m)	1.7 ± 0.1	1.6 ± 0.0	1.8 ± 0.0
Weight (kg)	67.7 ± 15.9	53.3 ± 4.2	78.0 ± 12.6
Fat-free mass (kg)	49.4 ± 10.5	38.0 ± 2.4	57.6 ± 3.4
Fat mass (kg)	18.3 ± 8.0	15.3 ± 3.9	20.4 ± 9.7
Lumbar spine T-score	−0.4 ± 1.6	−0.7 ± 1.7	−0.3 ± 1.6
Total hip T-score *	−0.9 ± 0.9	−1.4 ± 0.6	−0.6 ± 0.9
Femoral neck T-score *	−1.6 ± 0.6	−1.9 ± 0.4	−1.4 ± 0.6
Serum calcium (mg/dL)	9.2 ± 0.3	9.2 ± 0.3	9.2 ± 0.3
Serum 25-hydroxyvitamin D (ng/mL)	34.1 ± 6.8	34.4 ± 7.8	33.9 ± 6.7
Maximal heart rate (bpm)	162 ± 12	159 ± 14	164 ± 11
VO_2peak (mL/min/kg)	29.9 ± 6.0	29.0 ± 6.2	30.6 ± 6.2

* One woman excluded due to bilateral total hip replacement