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Authors

Kohrt, Wendy

Wolfe, Pamela

Sherk, Vanessa

et al.

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Dermal Calcium Loss is Not the Primary Determinant of PTH Secretion during Exercise

Wendy M Kohrt[1,2], **Pamela Wolfe**[3], **Vanessa D Sherk**[4], **Sarah J Wherry**[1,2], **Toby Wellington**[1], **Edward L Melanson**[1,2,4], **Christine M Swanson**[4], **Connie M Weaver**[5], **Rebecca S Boxer**[1,2]

[1]Department of Medicine, Division of Geriatric Medicine, University of Colorado Denver, Aurora, CO;

[2]Eastern Colorado VA Geriatric Research, Education, and Clinical Center, Aurora, CO;

[3]Department of Preventive Medicine and Biometrics, University of Colorado Denver, Aurora, CO;

[4]Department of Medicine, Division of Endocrinology, Metabolism and Diabetes, University of Colorado Denver, Aurora, CO;

[5]Department of Nutrition Science, Purdue University, West Lafayette, IN

Abstract

INTRODUCTION: Exercise can cause a decrease in serum ionized calcium (iCa) concentration, which stimulates parathyroid hormone (PTH) secretion and activates bone resorption. We postulated that dermal Ca loss during cycling exercise is the major determinant of the serum iCa, PTH, and bone resorption (C-terminal telopeptide of type 1 collagen; CTX) responses.

METHODS: To investigate this, women (n=13) and men (n=12) aged 18 to 45 years performed the same exercise bout under cool (18°C) and warm (26°C) conditions. Exercise was 60 minutes of cycling at ~75% of peak aerobic power. Sweat samples were obtained during exercise using a skin patch method, and blood samples were obtained before and during exercise and during 60 minutes of recovery.

RESULTS: Sweat volume and estimated sweat Ca loss were 50% higher for the warm condition than the cool condition. Despite this, there were no differences between thermal conditions in the changes (mean [95% CI]) in iCa (cool: −0.07 mg/dL [−0.16, 0.03]; warm: −0.07 mg/dL [−0.20, 0.05]), PTH (cool: 34.4 pg/mL [23.6, 45.2]; warm: 35.8 pg/mL [22.4, 49.1]), or CTX (cool: 0.11 ng/mL [0.08, 0.13]; warm: 0.15 ng/mL [0.11, 0.18]). Adjusting for exercise-related shifts in plasma volume revealed a marked decline in vascular iCa content in the first 15 minutes of

ADDRESS FOR CORRESPONDENCE: Sarah J Wherry, PhD, Campus Box B179, 12631 E 17th Avenue, Aurora, CO 80045, sarah.wherry@ucdenver.edu.

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.

exercise (cool: −0.85 mg/dL [−1.01, −0.68]; warm: −0.85 mg/dL [−1.05, −0.66]), before substantial sweat Ca loss had occurred.

CONCLUSION: This indicates that dermal Ca loss was not the primary trigger for the increases in PTH and CTX during exercise. Further research is necessary to understand the causes and consequences of the disruption in Ca homeostasis during exercise, and specifically the extra-vascular shift in iCa.

Keywords

sweat; parathyroid hormone; bone resorption; calcium; exercise

Introduction

Exercise can disrupt calcium homeostasis in a manner that increases parathyroid hormone secretion (PTH) and activates bone resorption, as reflected by an increase in serum c-terminal telopeptide (CTX).(1–10) The PTH response to high-intensity weight-supported and weight-bearing exercise lasting at least an hour is robust,(2, 4, 10), but more prolonged moderate-intensity cycling exercise also stimulates PTH secretion.(1, 5) A logical trigger for the increase in PTH during exercise is a decrease in serum ionized calcium (iCa) concentration,(2, 3, 7, 9–11) but PTH has been observed to increase during exercise even in the absence of a decline in iCa.(12–14) We previously demonstrated the importance of iCa as a trigger for the PTH response to exercise in a study that prevented a decline in iCa during exercise via intravenous infusion of Ca gluconate.(7) In the control condition (i.e., saline infusion), serum iCa concentration was unchanged during the first 15 minutes of exercise and then decreased steadily from 15 to 60 minutes of exercise. When the decrease in iCa was prevented by Ca infusion, the exercise-induced increase in serum PTH was attenuated by 65%, but not fully prevented.

Our working model for the disruption of Ca homeostasis by exercise portends that dermal Ca loss during exercise (i.e., sweating) causes a decrease in serum iCa concentration, which triggers an increase in PTH. PTH stimulates bone resorption to mobilize Ca from bone as a means of attenuating or preventing a further decline in serum iCa. In a series of experiments to challenge this model, we confirmed that vigorous endurance exercise causes a decrease in serum iCa concentration and increases in serum PTH and CTX.(1–3, 7, 10, 15) However, these experiments did not establish whether dermal Ca loss is the primary trigger for these responses.

In this context, the aim of the current study was to determine whether varying the thermal conditions during cycling exercise at ~75% VO_2peak (warm vs cool) to manipulate sweat rate and dermal Ca loss influences the iCa, PTH, and CTX responses to exercise. Healthy women and men accustomed to cycling exercise performed two identical 60-minutes bouts of cycling exercise in warm versus cool conditions. We postulated that the increases in serum PTH and CTX in response to exercise would be greater in the warm versus cool condition.

Methods

Participants.

Women (n=14) and men (n=14) aged 18 to 45 years who were accustomed to cycling exercise were recruited to participate. Exercise history was collected via self-report and reviewed with a member of the research team. Participants were eligible if they reported at least 2 days per week of moderate to vigorous cycling exercise at least 1 hour in duration. 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, thiazide diuretics, oral glucocorticoids); BMD T-score ≤ −2.5; moderate or severe renal impairment (estimated glomerular filtration rate of <60 mL/min/1.73 m^2); chronic hepatobiliary disease (aspartate transaminase or alanine transaminase >1.5 times the upper limit of normal); uncontrolled hypertension (systolic blood pressure >150 mmHg or diastolic blood pressure >90 mmHg); serum Ca <8.5 or >10.3 mg/dL; abnormal thyroid function (ultrasensitive TSH <0.5 or >5.0 mU/L); and serum 25-hydroxyvitamin D <20 ng/mL. All participants provided written informed consent, and the study was approved by the Colorado Multiple Institutional Review Board and the Army Human Research Protection Office.

Dual-energy x-ray absorptiometry (DXA).

Bone mineral density (BMD) of the total hip, femoral neck, trochanter, and lumbar spine (L1-L4) were measured using the Discovery W (Hologic, Inc., Waltham, MA) at screening. Participants were fasted and asked to be normally hydrated. Duplicate scans were conducted for every individual to verify eligibility criteria. Women completed a urine pregnancy test prior to their scan.

Graded exercise test.

Participants completed an incremental exercise test on a cycle ergometer for assessment of peak aerobic power (VO_2peak). Power output at the first stage was set to elicit a heart rate of ~70% of age-predicted heart rate maximum (HRmax). The wattage was increased by 15 W (women) or 25 W (men) every 2 minutes until volitional fatigue. VO_2peak was measured using the Parvo Medics TruMax 2400 metabolic cart (Parvo Medics Inc., Sandy, UT).

Controlled study meal.

Participants were provided a standardized meal that was consumed 4 hours prior to the start of exercise. The meal contained 25% of the estimated daily caloric need based on the sex-specific Mifflin equation (adjusted for height, weight, and an activity factor of 1.65).(16) The macronutrient content of the meal was 55% carbohydrate, 20% protein, and 25% fat and contained 90 to 100 mg of calcium. After consuming the study meal, participants remained fasted until the end of the visit.

Exercise bouts and thermal environment.

Participants completed two identical 60-minute exercise bouts on a cycle ergometer and both bouts were completed at the same time of day 1 to 4 weeks apart. Due to scheduling constraints for the temperature-controlled room, the study visits for women were not controlled for menstrual cycle phase and time of day was not controlled across participants. This resulted in some participants completing their exercise visits in the afternoon versus the morning, but both exercise bouts were completed at the same time of day for each participant. Cycling intensity was approximately 75% of VO_2peak at a self-selected cadence. Participants were allowed a 3- to 5-minute warm-up and cool-down that was duplicated at both exercise visits. All visits were conducted in a temperature-controlled room. The room was set to the desired temperature (18°C vs 26°C) and relative humidity (50%). The order of the thermal conditions was randomized and counter-balanced. Participants were instructed to wear the same clothing for both exercise visits and were permitted to drink water *ad libitum.* Participants were also instructed to refrain from caffeine the night before and the day of study visits and to have the same meal the night before both exercise visits.

Sweat and dermal Ca measurements.

Dermal Ca loss was estimated using the skin patch collection method used previously.(2) Six 42-mm filter paper discs (two per site) were placed under a tegaderm dressing on the upper back, upper chest, and arms. Skin was thoroughly cleaned with deionized water and dried with sterile gauze prior to placement of the patches. Once saturated with sweat, all patches were placed in a 5-mL syringe and the sweat was expressed into a single 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 urine production and fluid intake, and 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 min), during (T = 15, 30, 45, 60 min), and after (T = 75, 90, 105, 120 min) exercise. Blood samples at T-15, 75, 90, 105, and 120 min were taken in a semi-recumbent position. All other samples were taken while the participant was seated on the cycle ergometer. Intact PTH was measured using an Immulite two-site EIA (DPC; Los Angeles, CA). CTX was measured by ELISA (Nordic Bioscience; Copenhagen, Denmark). Ionized Ca (iCa), hematocrit (Hct), and pH were measured with an iSTAT whole blood analyzer (Abbott Point of Care, Inc; Princeton, NJ). iCa, PTH, and CTX values during and after exercise were adjusted for plasma volume (PV) shifts based on the methods of Van Beaumont, as previously described.(1, 17) PV-adjusted parameters are designated with a subscript (e.g., PTH_{ADJ}). Because the contraction of PV during exercise results in hemoconcentration, the adjustment for PV changes facilitated the assessment of changes in the vascular content of iCa, PTH, and CTX. Except for the whole blood used to measure iCa and Hct, samples were immediately frozen at −80°C after processing and all

analysis was done on the first thaw. Samples were analyzed in batches, with both visits for a single participant analyzed at the same time. Maximum storage time was 12 months.

Power calculations and statistical analysis.

The hypothesis was that PTH would increase more from before to after exercise in the warm vs cool condition. The thermal conditions were selected to achieve a sweat rate during the warm condition that was ~50% higher than during the cool condition.(18) In our previous studies, the estimated rate of sweat loss during high-intensity exercise was 43% higher(2) than during moderate-intensity exercise,(1) and this was associated with a 230% greater increase in serum PTH (high intensity: +74 ± 63 pg/mL (mean ± SD); low intensity: +32 ± 36 pg/mL). Sample size calculations were based on the assumption that the difference in the PTH response between conditions (45 ± 55 pg/mL) was related to a difference in dermal Ca loss. At an alpha level of 0.05, a sample size of 14 provided 80% power to detect this magnitude of difference between conditions. It was conservatively assumed that rho = 0.5 in the power estimate. An exploratory aim was to evaluate whether there are sex differences in the disruption of Ca homeostasis by exercise. Therefore, we enrolled 14 women and 14 men. Pooling women and men provided 99% power to detect a difference in the PTH response between conditions of 45 ± 55 pg/mL.

The effects of warm versus cool condition 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 the same as a 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. PTH was missing at a single time point for two participants. CTX was missing at a single time point for four participants and was missing at two time points during the same exercise bout for one participant. 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 of ≤0.05 defined statistical significance.

Results

Participant characteristics (Table 1).

Three participants (1 woman, 2 men) were unable to complete the second exercise test at the same power output as the first test; they were excluded from analyses. Differences between women and men in characteristics included height, fat-free mass, serum total Ca concentration, and the peak power output attained during the maximal exercise test. Of the 25 participants, 6 (2 women, 4 men) had low bone mass (Z-score <−1.0) in the lumbar spine and 6 (3 women, 3 men) had low bone mass in the proximal femur.

Heart rate and sweating responses to exercise.

Room temperatures were 18.1 ± 0.9°C and 19.2 ± 1.1 °C at the beginning and end of the cool exercise session, and 27.8 ± 1.2°C and 27.1 ± 1.2°C at the beginning and end of the warm exercise session. Average power output during exercise was the same for both thermal conditions and averaged 144 ± 15 W for women and 185 ± 24 W for men. The average HR responses during exercise in cool and warm conditions were 90 ± 4 %HRmax and 91 ± 5 %HRmax for women, and 84 ± 4 %HRmax and 88 ± 5 %HRmax for men. Because of the relatively high intensity of the exercise, there was a transient increase in acidosis early in exercise. The peak change in pH (~−0.03) occurred at 15 minutes of exercise in women and men under both thermal conditions. The experimental approach was designed to result in a ~50% higher sweat loss during the warm condition when compared with the cool condition and this was achieved (0.9 ± 0.4 L vs 0.6 ± 0.4 L, $p<0.01$; Table 2). Because sweat Ca concentration was not different between conditions, the estimated dermal Ca loss during exercise was 50% higher in the warm vs cool condition (33 ± 19 mg vs 22 ± 15 mg, $p< 0.01$; Table 2).

iCa, PTH, and CTX responses during exercise and recovery (Figure 1).

iCa began decreasing 15 minutes into exercise for both conditions and there were nonsignificant decreases in serum iCa concentration from before to immediately after exercise in the cool (mean [95% confidence interval]; −0.07 mg/dL [−0.16, 0.03]) and warm (−0.07 mg/dL [−0.20, 0.05]) conditions. In both thermal conditions, there were increases in serum PTH (cool: 34.4 pg/mL [23.6, 45.2]; warm: 35.8 pg/mL [22.4, 49.1]) and CTX (cool: 0.11 ng/mL [0.08, 0.13]; warm: 0.15 ng/mL [0.11, 0.18]) from before to after exercise. The greatest increase in PTH occurred between 15 and 30 minutes into exercise (cool: 25.3 pg/mL [19.0, 31.7]; warm: 33.6 pg/mL [25.5, 41.8]) followed by a plateau from 30 minutes until the end of exercise for both conditions. There were no differences between thermal conditions in the changes in serum iCa, PTH, or CTX in response to exercise.

Serum iCa concentration continued to decline during the first 15 minutes of recovery and then increased gradually; responses were similar in cool and warm conditions. The peak in serum PTH occurred 15 minutes after exercise and then decreased similarly in both conditions (both $p<0.001$), with the largest decrease occurring between 15 and 30 minutes after exercise (cool: −30.4 pg/mL [−36.7, −24.0]; warm: −24.9 pg/mL [−33.1, −16.6]). Despite the decrease in PTH from 75 minutes to 120 minutes, serum CTX continued to increase during recovery in both conditions (warm $p=0.004$, cool $p<0.001$). There were no differences between thermal conditions in the changes in serum iCa, PTH, or CTX during recovery.

iCa_{ADJ}, PTH_{ADJ}, and CTX_{ADJ} responses during exercise and recovery (Figure 2).

Vascular iCa content decreased markedly during exercise in both conditions, as reflected by changes in iCa_{ADJ} (cool: −0.85 mg/dL [−1.01, −0.68]; warm: −0.85 mg/dL [−1.05, −0.66]). The greatest decrease happened during the first 15 minutes of exercise (cool: −0.89 mg/dL [−0.99, −0.80]; warm: −0.84 mg/dL [−0.96, −0.72]) and concentrations remained low through throughout the duration of exercise. Increases in PTH_{ADJ} and CTX_{ADJ} from before to after exercise remained significant after adjustment for PV shifts (all $p<0.001$). There

were no differences between thermal conditions in the changes in serum iCa_{ADJ}, PTH_{ADJ}, and CTX_{ADJ} in response to exercise.

During recovery, iCa_{ADJ} increased back to pre-exercise levels in both thermal conditions (both $p<0.001$). The patterns of change in PTH_{ADJ} and CTX_{ADJ} during recovery were similar to those of PTH and CTX.

Sex-specific responses of iCa, PTH, and CTX to exercise and recovery (Figure 3).

Inferential statistical analyses to evaluate sex differences were not conducted because the study was not adequately powered for this. Within-sex changes in serum iCa, PTH, and CTX during exercise and recovery were similar in women and men (Figure 3). This was also true for iCa_{ADJ}, PTH_{ADJ}, and CTX_{ADJ} (data not shown).

Discussion

This study tested the hypothesis that the magnitude of Ca loss through sweating is the primary determinant of the changes in serum iCa, PTH, and CTX that have been observed in response to exercise.(1, 2, 4, 5, 7, 9) Participants performed two identical exercise sessions under warm and cool conditions. In contrast to the hypothesis, there were no differences between thermal conditions in the iCa, PTH, or CTX responses to exercise, despite a 50% greater estimated dermal Ca loss in the warm condition.

Rationale for the hypothesis

The hypothesis that dermal Ca loss is a mechanistic trigger for the disruption of Ca homeostasis during exercise was based on several observations. In humans, both long duration (1.5 to 2 h) moderate-intensity exercise(1, 5) and shorter duration (1 h) high-intensity exercise (2–4, 7, 10) result in increases in serum PTH and CTX. This raised the possibility that the magnitude of dermal Ca loss when sweat volume is high may be sufficient to cause a decrease in serum iCa during exercise, which would stimulate PTH secretion and activate bone resorption to mobilize Ca from bone. An additional observation was that proximal femur BMD was inversely related ($r = -0.72$) to the crude estimate of dermal Ca loss measured during a 2-h lab-based exercise session in young competitive male road cyclists.(1) This suggested that dermal Ca loss may be not only a determinant of the acute PTH-mediated mobilization of Ca from bone during exercise, but also a mechanism that contributes to the decline in BMD that has been observed in competitive cyclists during training and competition.(15, 19)

Equine investigations of the sweat Ca response to exercise also supported the plausibility of the hypothesis. In trained thoroughbreds, sweat volume during exercise and recovery was nearly 2-fold higher at ~34 °C than at ~21 °C, and Ca loss was also 2-fold higher in the hot condition.(20) The estimated rates of Ca loss through sweating were 1.1 and 2.8 mg/h per kg body weight in cool and hot conditions. Another equine study reported rates of sweat Ca loss during exercise of 0.7 to 1.1 mg/h per kg body weight.(21) Thus, both human and equine studies suggest that exercise can result in considerable Ca loss through sweating. We postulated this would cause a decrease in serum Ca, followed by the stimulation of PTH secretion and mobilization of Ca from bone to stabilize the serum Ca concentration.

Dermal Ca loss and disruption of Ca homeostasis during exercise

Two lines of evidence in the current study refuted the hypothesis that dermal Ca loss triggers the disruption of Ca homeostasis during exercise. First, the disruption of Ca homeostasis began early in exercise, before substantial sweat loss had likely occurred. The decrease in serum iCa concentration and increases in PTH and CTX were apparent after only 15 minutes of exercise; more frequent blood sampling would be necessary to better define the time course of these changes, as utilized by others.(9) Second, cool and warm exercise conditions were used to manipulate the sweat rate, with the intent of provoking greater dermal Ca loss during the warm condition. It had previously been reported that the sweat rate during exercise at 26 °C was 50% higher than during exercise at 18 °C.(18) Similar thermal conditions were achieved in the current study, and this resulted in the expected 50% higher sweat rate and dermal Ca loss in the warm condition. However, there was no signal for the decrease in serum iCa concentration or increases in PTH or CTX to be greater in the warm condition than the cool condition. These results provide evidence that dermal Ca loss is not the primary cause of the disruption of Ca homeostasis during exercise, but they do not rule out dermal Ca loss as a contributing factor.

Exercise-induced acidosis and disruption of Ca homeostasis

It is possible that the increase in acidosis early in exercise stimulated the rise in PTH and masked subsequent effects of dermal Ca loss. For example, experimentally-induced metabolic acidosis for 60 minutes in dogs stimulated an increase in PTH within 10 minutes, when pH was decreased by ~0.05 units.(22, 23) In that study, serum iCa concentration was also increased by 10 minutes and continued to increase for the 60-minute experiment. As a result of feedback inhibition by the increase in iCa, serum PTH peaked at 30 minutes and then decreased for the remainder of the experiment. The continuous *increase* in iCa and the late *decline* in PTH during acute metabolic acidosis are clearly distinct from the *decline* in iCa and continuous *increase* in PTH during vigorous exercise in the current study. This indicates that factors other than exercise-induced acidosis contribute to the disruption of Ca homeostasis during exercise. We previously used a novel Ca clamp approach to demonstrate that preventing the decrease in serum iCa concentration during exercise attenuated the increase in PTH by 65%.(7) Further research will be necessary to determine whether acidosis accounts for the exercise-induced increase in PTH that is not attributable to the decrease in iCa concentration.

Decrease in serum iCa concentration during exercise

There was a steady decline in serum iCa after 15 minutes of exercise that reached a nadir 15 minutes after the cessation of exercise (Figure 1A). Although the decrease in iCa concentration from before to immediately after exercise of −0.7 mg/dL was not statistically significant, PTH secretion is highly sensitive to small changes in iCa.(24, 25) For example, preventing a ~0.5 mg/dL decrease in serum iCa during exercise via intravenous Ca gluconate infusion attenuated the PTH response by 65%.(7) Thus, the nonsignificant changes in serum iCa were likely of physiological relevance and probably mediated, in part, the increase in PTH.

As discussed above, the magnitude of dermal Ca loss does not appear to be the primary determinant of the decline in serum iCa during exercise. Urine Ca loss was not measured in the current study, but we demonstrated previously that it was very low (<10 mg/h) during and after a similar exercise bout.(7) When serum iCa levels were adjusted for the shifts in PV that are known to occur during exercise (Figure 2A),(26) there was a marked decrease in iCa_{ADJ} in the first 15 minutes of exercise of −0.85 mg/dL in both thermal conditions, which reflects a decrease in vascular iCa content. This was also observed in a separate cohort of young men who performed a similar exercise bout.(7) In that study, serum iCa_{ADJ} and total Ca_{ADJ} decreased by −0.7 mg/dL and −1.3 mg/dL, respectively, in the first 15 minutes of exercise, suggesting that the decrease in iCa content was not the result of an increase in Ca binding. Others have also observed a decrease in vascular Ca content during exercise.(12, 13, 27) Although Ca is essential for muscle contraction, it is not known whether increased skeletal muscle uptake of Ca during exercise could account for the decrease in vascular iCa. Multiple studies of rodents led to the conclusion in a review paper that there is very little change in the iCa content of muscle in response to exercise unless the exercise is prolonged. There is limited, but supportive, evidence for this in humans. Significant increases in muscle iCa content were found after 20-km (0.70 to 0.93 μmol/g WW) and 100-km (0.84 to 1.02 μmol/g WW) runs, but not after a 10-km run (0.81 to 0.91 μmol/g WW).(28, 29) It has also been demonstrated that muscle mitochondrial Ca content increases in response to exercise.(30) Further research will be necessary to understand systemic and tissue-specific Ca dynamics during exercise.

Preliminary evaluation of sex differences

Because many of the studies that investigated the disruption of Ca homeostasis by exercise included only men,(1, 2, 4, 6, 7, 9) women and men were studied to facilitate a preliminary evaluation of sex differences in the disruption of Ca homeostasis by exercise. The study was not powered to detect sex differences. Therefore, within-sex responses to exercise were evaluated but between-sex differences in responses were not. The decreases in iCa concentration and increases in PTH in warm and cool conditions appear to be were very similar in women and men (Figure 3A, B, D, E). The CTX responses (Figure 3C, F) suggest that the activation of bone resorption at a given PTH stimulus may be more robust in men than women, and may also be influenced by dermal Ca loss (warm vs cool conditions) to a greater extent in men. These preliminary findings should guide future investigations of sex differences.

Limitations

The regional patch technique for assessing sweat Ca concentration may not be as accurate as the whole-body wash-down approach.(31, 32) It is unlikely this influenced the conclusion that the magnitude of dermal Ca loss is not the primary determinant of the iCa, PTH, and CTX responses to exercise, because the same approach was used to assess dermal Ca loss under both thermal conditions. However, the reported levels of sweat Ca loss should be considered crude estimates.

Because the current study involved only one mode and one intensity of exercise in a cohort of young adults, it is possible the results are specific to vigorous cycling exercise in young

women and men. There is some evidence that PTH and CTX responses to treadmill exercise(4) are less robust than responses to cycling exercise,(1, 2) but this may reflect between-laboratory differences in experimental approach or differences in mechanical loading. Potential factors that influence the disruption of Ca homeostasis by exercise (e.g., mode/intensity/ duration of exercise, age, sex, menstrual cycle phase) should be investigated systematically in future studies.

The study evaluated the disruption of Ca homeostasis after only a single bout of exercise. The results suggest that the exercise-induced increase in PTH has a catabolic effect on bone, as evidenced by the increase in CTX. Markers of bone formation were not measured, but previous studies demonstrated that intravenous(7) or oral(2, 5) Ca administration before and during exercise attenuated the increase in a marker of bone resorption, but not formation. However, it is possible there is a delayed increase in formation following the activation of resorption because of the serial nature of the coupling between bone resorption and formation. Future studies should include detailed temporal maps of the changes in markers of bone metabolism that occur with multiple exercise sessions. The importance of such an approach is heightened by the known paradoxical actions of PTH on bone, which are catabolic when elevated chronically, but anabolic when increased transiently on a regular basis.(33) Indeed, two drugs used to treat osteoporosis, teriparatide and abaloparatide, are analogs of PTH that have net anabolic effects on bone. A single dose of teriparatide provoked an increase CTX (bone resorption) that lasted for at least 3 hours; P1NP (bone formation) remained stable during this interval.(34) In contrast, 28 daily doses of teriparatide resulted in a 111% increase in P1NP and no change in CTX(35). Future studies should determine whether repeated exercise-induced increases in PTH have similar effects on bone metabolism as multiple doses of PTH analogs.

Finally, changes in plasma volume were calculated from hematocrit values obtained from the iSTAT whole blood analyzer, which measures hematocrit by conductivity. In a pilot study to compare hematocrit measured by the iSTAT and the reference method (i.e., manual spinning), we found a high level of agreement between the methods ($R^2 = 0.93$), which was consistent with previous findings.(36) It is unlikely that use of the iSTAT to measure hematocrit altered the results.

Summary and conclusion

Studies from multiple laboratories provide evidence that exercise can disrupt Ca homeostasis, resulting in increased PTH secretion and activation of bone resorption.(1–10, 37) The knowledge gap addressed by the current study was whether the magnitude of Ca loss through sweating is the primary stimulus of these responses. This was investigated by having participants perform the same exercise under cool and warm conditions. Estimated dermal Ca loss was 50% higher during the warm condition, but this did not translate into exaggerated PTH or CTX responses. As demonstrated previously,(7) the decline in serum iCa concentration during exercise is the major stimulus of the PTH response, but the decline was not influenced by sweat Ca loss in the current study. Both the current and previous(7) studies found a marked decline in vascular iCa content in the first 15 minutes of exercise, but the factors that regulate this extra-vascular shift in iCa and why it occurs remain unclear.

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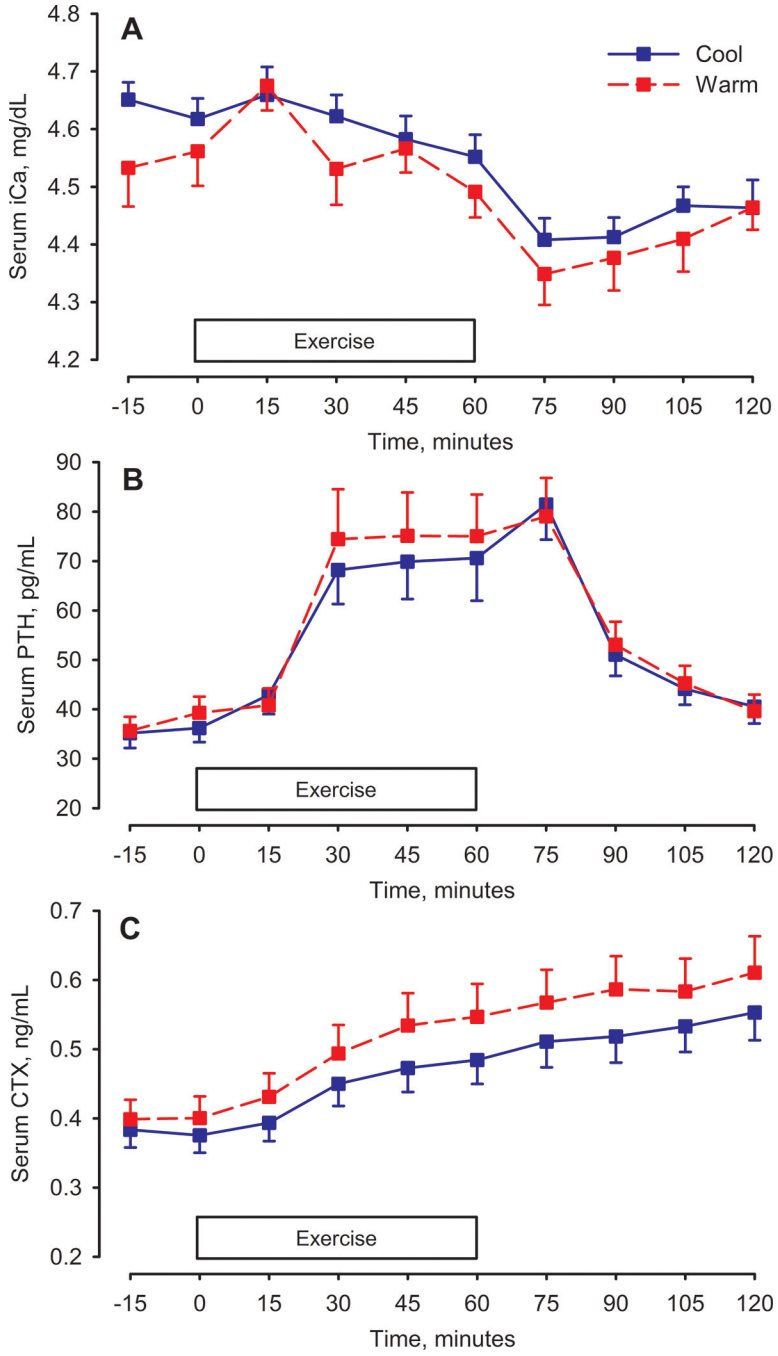

Figure 1.

Changes in serum ionized calcium (iCa; A), parathyroid hormone (PTH; B), and C-terminal telopeptide of type 1 collagen (CTX; C) in 25 women and men in response to exercise performed under cool (solid line) and warm (dashed line) conditions.

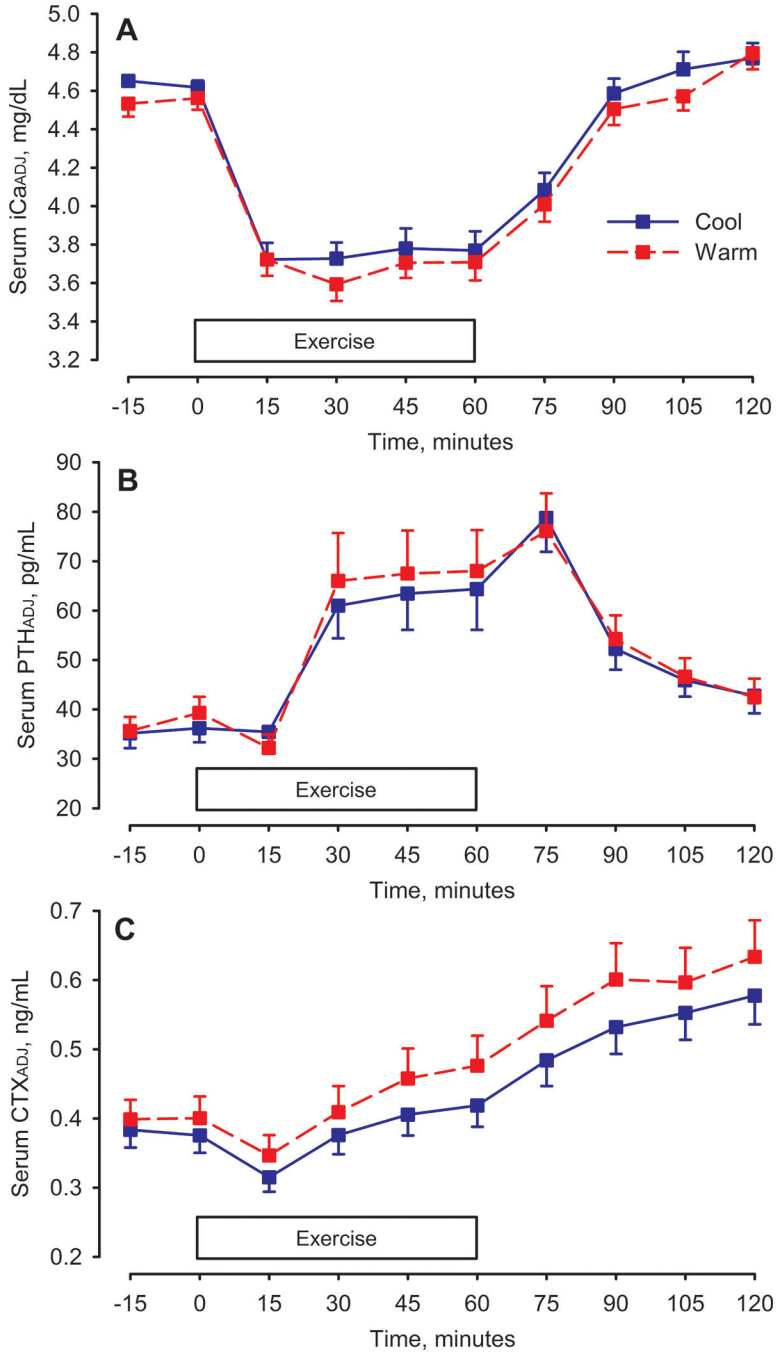

Figure 2.

Plasma volume-adjusted changes in serum ionized calcium (iCa_{AJD}; A), parathyroid hormone (PTH_{ADJ}; B), and C-terminal telopeptide of type 1 collagen (CTX_{ADJ}; C) in 25 women and men in response to exercise performed under cool (solid line) and warm (dashed line) conditions.

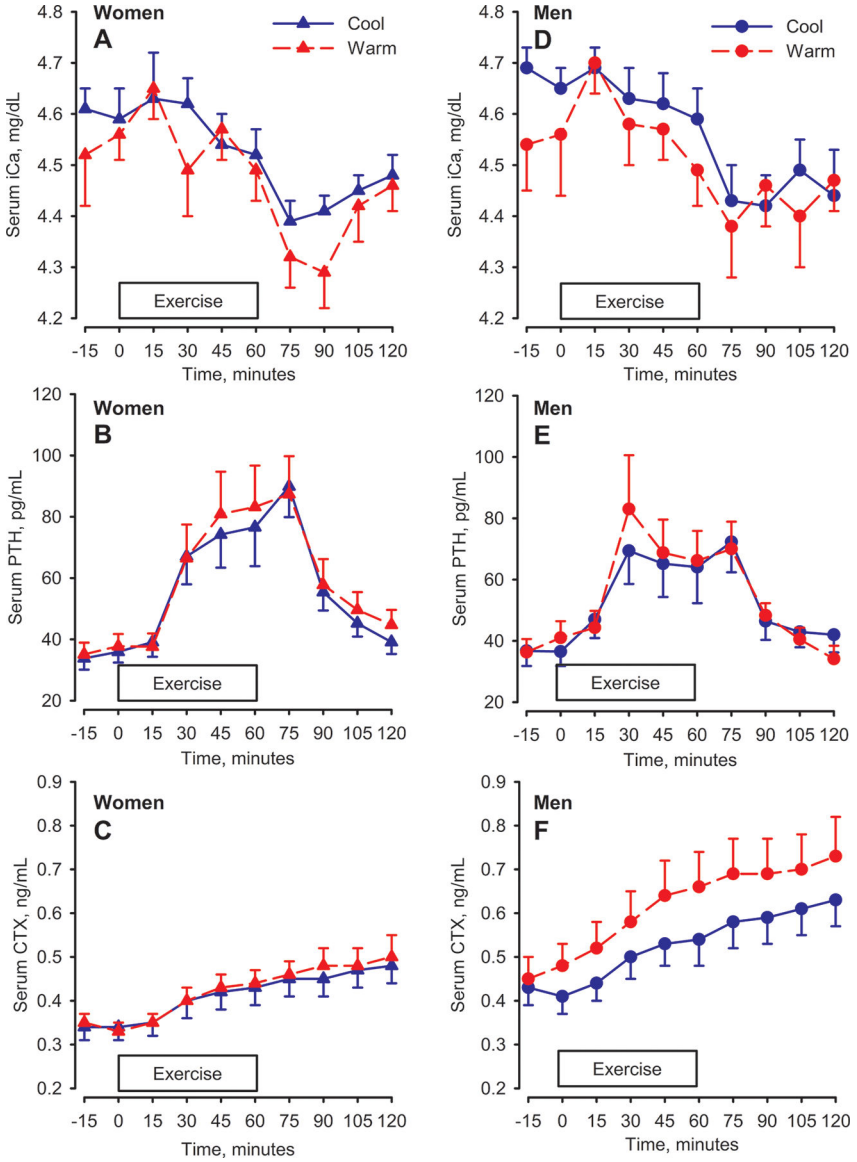

Figure 3.

Sex-specific changes in serum ionized calcium (iCa; A, D), parathyroid hormone (PTH; B, E), and C-terminal telopeptide of type 1 collagen (CTX; C, F) in 13 women and 12 men in response to exercise performed under cool (solid line) and warm (dashed line) conditions.

Table 1.

Participant characteristics

	All (n=25)	Women (n=13)	Men (n=12)
Age, y	33.5 (5.3)	32.6 (5.2)	34.5 (5.5)
Height, m	1.76 (0.09)	1.70 (0.06) *	1.83 (0.07)
Weight, kg	68.4 (12.0)	59.7 (5.3) *	77.7 (9.8)
Fat-free mass, kg	56.0 (10.8)	47.2 (4.6) *	65.4 (6.4)
Fat mass, kg	12.4 (3.4)	12.5 (2.1)	12.3 (4.6)
Lumbar spine T-score	−0.24 (0.98)	0.06 (1.10)	−0.57 (0.74)
Total hip T-score	0.02 (0.80)	0.35 (0.95)	−0.35 (0.37)
Femoral neck T-score	−0.13 (0.90)	0.19 (1.05)	−0.48 (0.56)
Trochanter T-score	−0.03 (0.78)	0.32 (0.89)	−0.42 (0.41)
Serum total calcium, mg/dL	9.3 (0.3)	9.2 (0.2) **	9.5 (0.3)
Serum 25-hydroxyvitamin D, ng/mL	33.1 (8.9)	33.9 (10.1)	32.3 (7.7)
Peak heart rate, beats per minute	181 (7)	179 (8)	182 (6)
Peak aerobic power, mL/min/kg	51.7 (6.4)	50.0 (4.6)	53.6 (7.6)
Peak power output, W	261 (48)	227 (25) *	298 (39)

Women vs men,

* $p < 0.01$,

** $p = 0.02$

Table 2.

Sweating responses to exercise

	All		Women		Men	
	Warm	**Cool**	**Warm**	**Cool**	**Warm**	**Cool**
Sweat volume, L *†	0.9 (0.4)	0.6 (0.4)	0.7 (0.2)	0.3 (0.2)	1.1 (0.4)	0.9 (0.4)
Sweat calcium concentration, mg/dL	3.6 (1.6)	3.5 (1.2)	3.5 (1.7)	3.3 (1.3)	3.7 (1.7)	3.6 (1.2)
Estimated sweat calcium loss, mg *†	33 (19)	22 (15)	26 (16)	11 (6)	40 (20)	32 (14)

*warm vs cool, $p < 0.01$;

†women vs men, $p < 0.01$;

n=24 for sweat ca and sweat ca loss