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14. ABSTRACT

Batched analysis of serum/sweat samples from EXP1, regulatory approvals from EXP2, protocol amendments, and generation of forms were completed in Q1-4. Completion of 3 successful clamps occurred in October 2013 (EXP2-pilot) and EXP2 enrollment was initiated. Thereafter, the major goals for Year 2 were to complete approximately one-half of the Experiment 2 Study (EXP2) and obtain regulatory approval from EXP3-pilot. Approval of the Continuing Review application, which included the expanded description of EXP3-pilot, was received 28 Aug 2014. Isotopes for the protocol were ordered; delivery occurred 20 Oct 2014. A revised SOW for EXP3-pilot was submitted 10 June 2014 but an amended contract has not yet been received. We expect EXP3-pilot to begin in Q1 of Year 3. Wendy Kohrt, PhD, presented the full data on calcium disruption data from EXP1 at the 2014 Military Health System Research Symposium (Appendix A) and Vanessa Sherk, PhD, presented partial data from EXP1 at the 2014 American College of Sports Medicine Annual Meeting (Appendix B).

15. SUBJECT TERMS
calcium homeostasis, exercise, bone resorption, parathyroid hormone

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INTRODUCTION

The **Global Aim** of the proposed research is to investigate a novel mechanism for exercise-related bone loss. We postulate that the disruption of calcium (Ca) homeostasis during acute exercise is a trigger for the activation of bone resorption. The working model portends that excessive dermal Ca loss (i.e., sweating) causes a decline in serum ionized Ca (iCa; the unbound fraction) and triggers an acute increase in parathyroid hormone (PTH). PTH can defend serum Ca by reducing urinary Ca excretion, increasing intestinal Ca absorption, and increasing mobilization of skeletal Ca (bone resorption). If an increase in bone resorption occurs repeatedly over multiple exercise sessions (i.e., exercise training) and is not accompanied by appropriate loading forces to stimulate bone formation, we postulate that this could lead to a decrease in BMD over time. The **Specific Aims** are to **1)** determine whether the magnitude of dermal Ca loss during exercise is a determinant of the decline in iCa and increases in PTH and carboxy-terminal collagen crosslinks (CTX; marker of bone resorption); **2)** determine whether preventing the decline in serum iCa during exercise via intravenous Ca administration (i.e., iCa clamp) prevents an increase in serum PTH and CTX; and **3)** measure serum Ca flux and rate of Ca appearance during exercise and determine whether oral Ca loading before exercise attenuates the increase in serum CTX.

BODY

The following **major tasks** were proposed for Year 2:

- **Conduct recruitment, screening, and testing for EXP1**

Testing for EXP1 was completed on 1 Nov 2013. We met the goal of having 14 women and 14 men complete EXP1.

Progress on EXP1:

	Enrolled	Screen Failure	Withdrew Before Randomized	Randomized	Withdrew After Randomized	Completed
Women	18	2	1	15	1	14
Men	22	2	3	17	3	14
Total	40	4	4	32	4	28

Reasons for withdrawals:

Screening failures: TSH out of range (1), steroid use in the past 6 months (2), low vitamin D (1)

Before randomization: time constraints (2), injury not related to the study (1), stopped responding to correspondence (1)

After randomization: time constraints (3), stopped responding to correspondence (1)

- **Begin batched assays of serum and sweat samples in Q1**

The proposed assays for EXP1 have been completed. Data analyses are underway and the results may lead to additional assays being performed (e.g., total serum Ca concentration rather than just ionized Ca).

- **Hold medical monitor meeting in Q1**

This meeting was held 14 October 2013.

- **Get regulatory approvals for EXP2 and EXP3 pilot**

The local IRB application for EXP2 was submitted 13 November 2013. Although the methods proposed for EXP2-pilot were not modified for EXP2, the IRB chair deferred the protocol amendment to the full committee for review. Minor modifications were required after the initial review and the amendment to conduct EXP2 was approved 23 December 2013. The approved IRB documents were received by the PI on 9 January 2014 and submitted to the Army Human Research Protection Office (HRPO) for approval on 15 January 2014. The HRPO approved the amendment on 30 January 2014.

Data analysis for EXP1 was completed in January 2014 and two unexpected results suggested that the experimental approach for EXP2 should be expanded: 1) Despite a greater estimated dermal Ca loss in the warm exercise condition than the cool condition, the PTH responses were not significantly different. We added urine collections to EXP2 to evaluate the extent of urinary Ca loss during and after exercise; 2) As expected, the increase in PTH triggered an increase in a serum marker of bone resorption, but it was still increasing 1 hour after exercise. We expanded the post-exercise monitoring period from 1 hour to 4 hours. These modifications were based on discussions with the consultant for grant, Dr. Connie Weaver, who visited our institution February 19-21, 2014. An IRB amendment was submitted on 28 February 2014 and approved on 14 March 2014. A revised Statement of Work was submitted on 31 March 2014. An amended contract was received 19 June 2014.

We met with local experts in the modeling of calcium kinetics and drafted a plan for proceeding with EXP3-pilot. To avoid having two versions of documents in circulation with the IRB, the amendment to start EXP3-pilot was submitted after EXP2 was finalized. The local IRB application for EXP3 pilot was submitted 5 May 2014. The IRB chair deferred the protocol amendment to the full committee for review and it was approved with no modifications on 23 May 2014. The approved IRB documents were received by the PI on 3 June 2014. A revised Statement of Work was submitted to the Scientific Officer, Alexis Mosquera, on 10 June 2014. At this time, we have not yet received an amended contract. We submitted the Continuing Review report to the HRPO on 21 July 2014 that included the updated information for EXP3-pilot. The Continuing Review Acceptance and Amendment Approval was received on 28 Aug 2014. EXP3-pilot will start as soon as the amended contract is received.

Additional minor amendments to the protocol have been approved by the local IRB:

PAM007-3:

This was approved on 23 Dec 2013: Created new consent and HIPAA forms, recruitment materials, and screening tools for EXP2.

PAM008-2:

This was approved on 14 Mar 2014 for changes to EXP2: **1)** added urine sampling to measure Ca; **2)** extended blood sampling from 1 to 4 hours post-exercise; **3)** added 1-day diet log.

PAM009-1:

This was approved 23 May 2014: Created new consent and HIPAA forms, recruitment materials, and screening tools for EXP3 pilot.

PAM010-1:

This was approved 23 May 2014: **1)** Karen Shea, MD, was removed from the protocol. Rebecca Boxer, MD, and Sarah Wherry, PhD, were added as co-investigators. Toby Wellington was assigned as the new study contact; **2)** Recruitment and protocol materials were updated to reflect personnel changes.

- **Conduct recruitment, screening, and testing for EXP2**

We began recruitment for EXP2 in May 2014. Seven men have provided written informed consent and three have completed screening.

Progress on EXP2:

	Enrolled	Consented	Screen Failure	Withdrew	Completed
Men	13	13	2	3	6

- **Quarterly data quality assurance evaluations**

Screening data were generated for EXP2 and added to the database. During the development of the primary manuscript for EXP1, it was determined that measurement of total serum calcium (in addition to ionized serum calcium) would aid in the interpretation of results. The assays were performed and data were quality checked and merged into the database.

- **Submit EXP1 manuscript for publication**

Findings from EXP1 were presented at the American College of Sports Medicine Annual Meeting in May 2014 and at the Military Health Systems Research Symposium in August 2014. We expect to submit the primary EXP1 manuscript in Q1 of Year 3. Submission of the EXP1 manuscript has been delayed for two reasons: **1)** It was decided that total Ca response to exercise should be included so samples were analyzed for this outcome; and **2)** Careful scrutiny of the data during manuscript preparation revealed that the PTH response to exercise differed between those who did or did not have a decrease in pH during the first 30 minutes of exercise. It has not yet been determined whether a second manuscript will be needed to describe this phenomenon.

- **Prepare annual progress report in Q4**

This was accomplished.

- **Data from EXP1**

As planned, 14 women and 14 men completed EXP1. The characteristics of the participants are in the following table (* p<0.01 women vs men):

	All (n=28)	Women (n=14)	Men (n=14)
Age (y)	32.9 ± 5.3	32.2 ± 5.2	33.6 ± 5.6
Height (m)	1.76 ± 0.10	1.69 ± 0.07*	1.84 ± 0.07
Weight (kg)	69.3 ± 13.5	58.9 ± 5.9*	79.8 ± 10.4
Fat-free mass (kg)	56.6 ± 11.6	46.7 ± 4.8*	66.5 ± 6.6
Fat mass (kg)	12.7 ± 3.9	12.2 ± 2.4*	13.2 ± 5.1
Lumbar spine T-score	-0.2 ± 1.0	-0.0 ± 1.1	-0.4 ± 1.0
Total hip T-score	-0.0 ± 0.8	0.2 ± 0.9	-0.3 ± 0.5
Femoral neck T-score	-0.1 ± 0.9	0.1 ± 1.1	-0.3 ± 0.7
Trochanter T-score	-0.1 ± 0.8	0.2 ± 0.9	-0.4 ± 0.6
Serum calcium (mg/dL)	9.3 ± 0.3	9.2 ± 0.2	9.4 ± 0.3
25(OH) Vitamin D (ng/mL)	33.3 ± 8.4	33.9 ± 9.7	32.6 ± 7.3
Maximal heart rate (bpm)	181 ± 7	180 ± 9	182 ± 6
VO_{2peak} (mL/min/kg)	52.4 ± 6.7	51.1 ± 6.0	53.8 ± 7.2

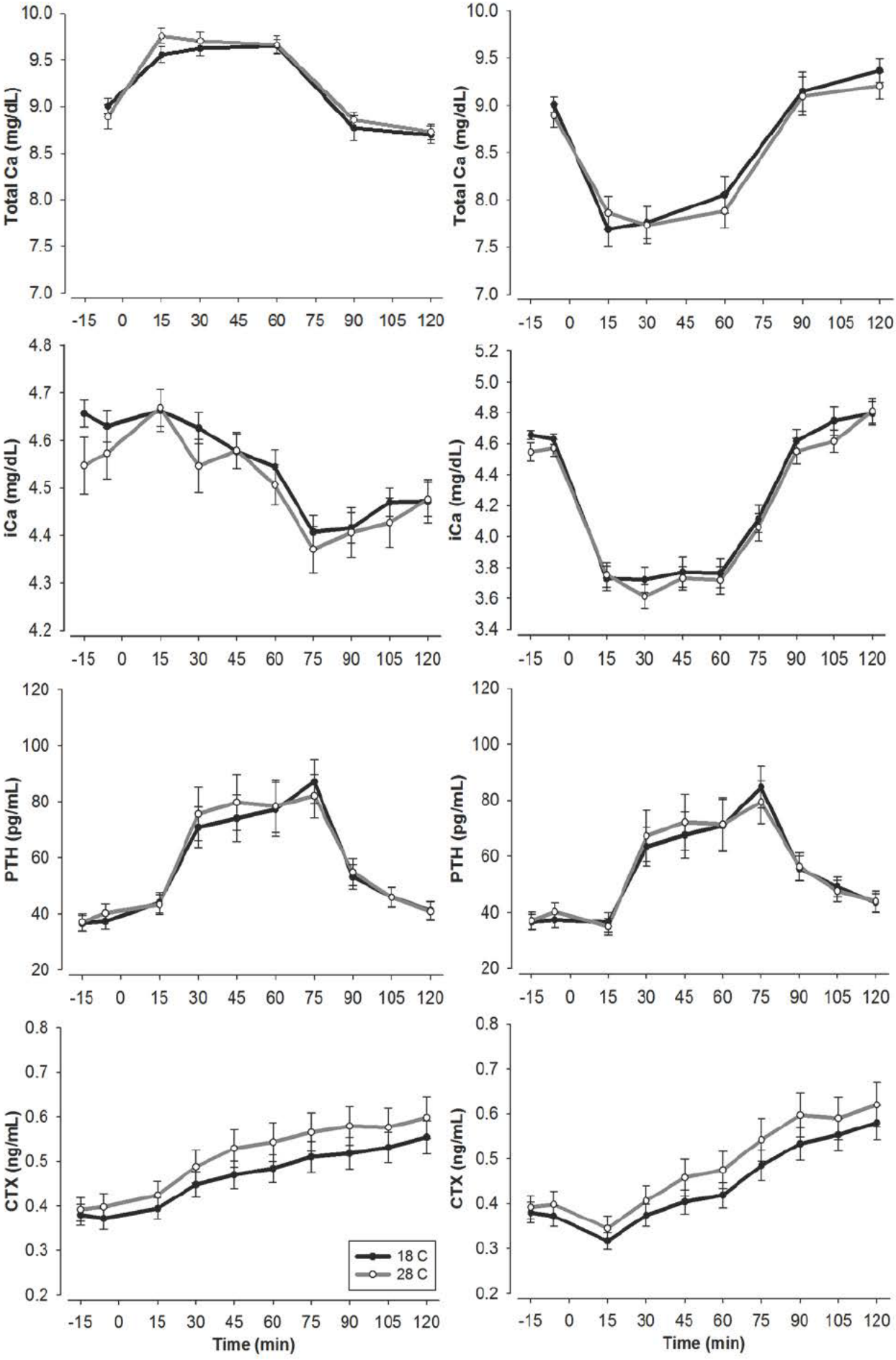
All participants completed two exercise bouts, under warm and cool thermal conditions. The ambient temperatures (26C and 16C) were selected to generate a sweat rate that was approximately 50% higher under the warm vs cool condition. As indicated in the following table, this difference was achieved (* p<0.05 warm vs cool; †p<0.05 women vs men):

	All		Women		Men	
	Warm	Cool	Warm	Cool	Warm	Cool
Sweat loss (L)*†	0.93 ± 0.40	0.63 ± 0.41	0.68 ± 0.23	0.33 ± 0.17	1.18 ± 0.39	0.93 ± 0.35
Sweat Ca (mg/dL)	3.5 ± 1.6	3.6 ± 1.3	3.4 ± 1.7	3.2 ± 1.3	3.8 ± 1.6	3.9 ± 1.2
Ca Loss (mg)*†	33.3 ± 20.0	22.7 ± 16.7	24.9 ± 16.5	10.9 ± 6.1	43.2 ± 20.1	34.9 ± 15.1

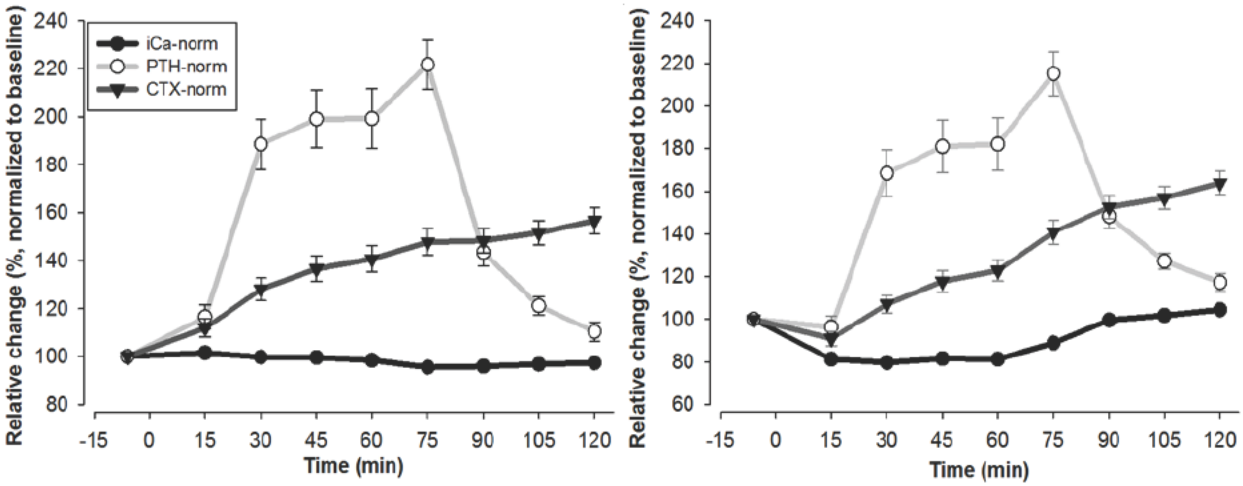
The primary objective was to determine if a higher sweat rate (warm condition) caused greater decreases in serum total and ionized Ca, a greater increase in PTH, and a greater increase in CTX. The table below depicts the mean changes in these outcomes from before to after exercise (0-60 min) and during the hour after exercise (60-120 min). Values are presented with and without correction for plasma volume shifts, estimated from change in hematocrit (* $p<0.01$, ** $p<0.05$, within-condition change):

	Warm		Cool	
	0-60 min	60-120 min	0-60 min	60-120 min
Total Ca (mg/dL)[a]	0.77 ± 0.72*	-0.96 ± 0.31*	0.65 ± 0.48*	-0.96 ± 0.31*
Total Ca_{adj} (mg/dL)[a]	-1.01 ± 0.73*	1.34 ± 0.15*	-0.95 ± 0.96*	1.25 ± 0.15*
iCa (mg/dL)	-0.07 ± 0.21	-0.03 ± 0.13	-0.09 ± 0.18**	-0.08 ± 0.19
iCa_{adj} (mg/dL)	-0.86 ± 0.50*	1.09 ± 0.38*	-0.87 ± 0.43*	1.03 ± 0.37*
PTH (pg/mL)	38.2 ± 40.8*	-37.6 ± 39.3*	40.0 ± 44.5*	-30.4 ± 32.6*
PTH_{adj} (pg/mL)	31.2 ± 41.4*	-27.6 ± 37.9*	33.7 ± 43.8*	-21.7 ± 31.7*
CTX (ng/mL)	0.15 ± 0.10*	0.04 ± 0.08*	0.11 ± 0.08*	0.07 ± 0.06*
CTX_{adj} (ng/mL)	0.08 ± 0.09*	0.14 ± 0.08*	0.05 ± 0.09*	0.16 ± 0.06*
Hct (%)	3.7 ± 2.6	-5.6 ± 1.8	3.8 ± 2.2	-5.5 ± 1.5

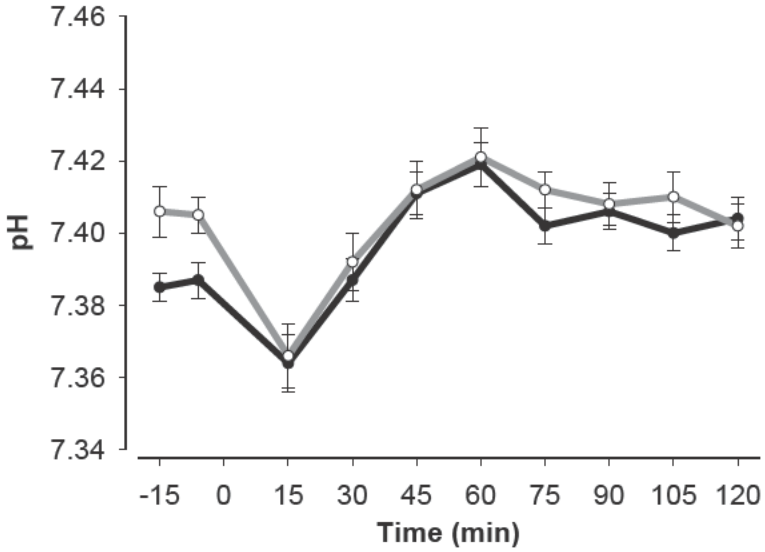
The following figures illustrate the changes in serum total Ca, iCa, PTH, and CTX. The left panels are unadjusted values and the right panels are adjusted for plasma volume shifts.


Total Ca (mg/dL)
iCa (mg/dL)
PTH (pg/mL)
CTX (ng/mL)
Time (min)
18 C
28 C

Despite the greater sweat rate and greater estimated dermal Ca loss in the warm condition compared with the cool, there were no significant differences between conditions in the decline in total or ionized Ca or the increases in PTH and CTX. When pooled across conditions, it appears that a decline in serum iCa is a trigger for the increases in PTH and CTX. The figure below depicts the relative changes in these outcomes without (left panel) and with (right panel) adjustment for plasma volume shifts.



However, it is possible that another stimulus of PTH secretion, such as metabolic acidosis, contributed to the robust increase in PTH and masked the ability to detect the effects of sweat rate. As evidence for this, pH decreased markedly early in exercise under both conditions, as shown in the figure below (black, cool condition; gray, warm condition):



EXP2 will provide insight on the relative contributions of the decrease in serum iCa and the increase in metabolic acidosis on the PTH response, because it involves Ca infusion during exercise to prevent the decrease in iCa.

KEY RESEARCH ACCOMPLISHMENTS

The analyses to date do not support our first hypothesis that extent of dermal calcium loss during exercise is the primary determinant of the PTH response. However, the declines in serum ionized and total calcium concentration during exercise are large (almost 1 mg/dL). EXP2 and EXP3 are expected to provide insights on

whether the decline in serum ionized calcium fully accounts for the increase in PTH and on the magnitude of calcium flux during exercise.

REPORTABLE OUTCOMES

Reportable outcomes will be available after the first manuscript undergoes review.

CONCLUSION

No conclusions can yet be drawn.

REFERENCES

The PI is not aware of any published studies that inform this research beyond those included in the grant application.

APPENDICES

APPENDIX A

Abstract from Military Health System Research Symposium

Disruptions in Calcium Homeostasis During Exercise Under Different Thermal Conditions

BACKGROUND: Endurance exercise has been observed to cause a decrease in serum ionized calcium (iCa) and increases in parathyroid hormone (PTH) and a marker of bone resorption (C-terminal telopeptides of type I collagen; CTX). It is not known whether this disruption in Ca homeostasis during exercise is caused by the dermal loss of Ca. Accordingly, the objective was to determine whether exercise under a warm condition exaggerates the increases in PTH and CTX, when compared with a cool condition, as a result of greater dermal Ca loss.
METHODS: Women (n=14) and men (n=14) aged 18 to 45 years performed two 60-minute bouts of high intensity cycling under cool (18°C) and warm (28°C) conditions. The order of the conditions was randomized and power output was matched across conditions. Serum iCa, PTH, and CTX were measured every 15 minutes from 15 minutes before to 60 minutes after the end of exercise. Dermal Ca loss was estimated from sweat loss and sweat Ca concentration. Changes in iCa, PTH, and CTX were adjusted for plasma volume shifts.
RESULTS: Estimated dermal Ca loss was ~50% greater ($p<0.01$) in the warm condition (33.3 ± 20.0 mg) than the cool condition (22.7 ± 16.7 mg). Despite this, there were no differences between conditions in the adjusted changes from before to after exercise in serum iCa (warm vs cool: -0.86 ± 0.50 vs -0.87 ± 0.43 mg/dL), PTH (31.2 ± 41.4 vs 33.7 ± 43.8 pg/mL), or CTX (0.08 ± 0.09 vs 0.05 ± 0.09 ng/mL). Under both conditions, serum PTH declined markedly by 60 minutes after the end of exercise but CTX was still increasing.
CONCLUSIONS: The large decrease (~19%) in serum iCa during exercise likely triggered the increases in PTH and CTX. Although dermal Ca loss may contribute to the decline in serum iCa, it does not appear to be the major determinant of the disruption in Ca homeostasis during exercise. Further research will be needed to determine whether exercise-induced increases in PTH and CTX have beneficial or harmful skeletal effects.

Learning objectives

Attendees will be made aware of the potential of exercise to stimulate PTH secretion and acutely activate bone resorption.

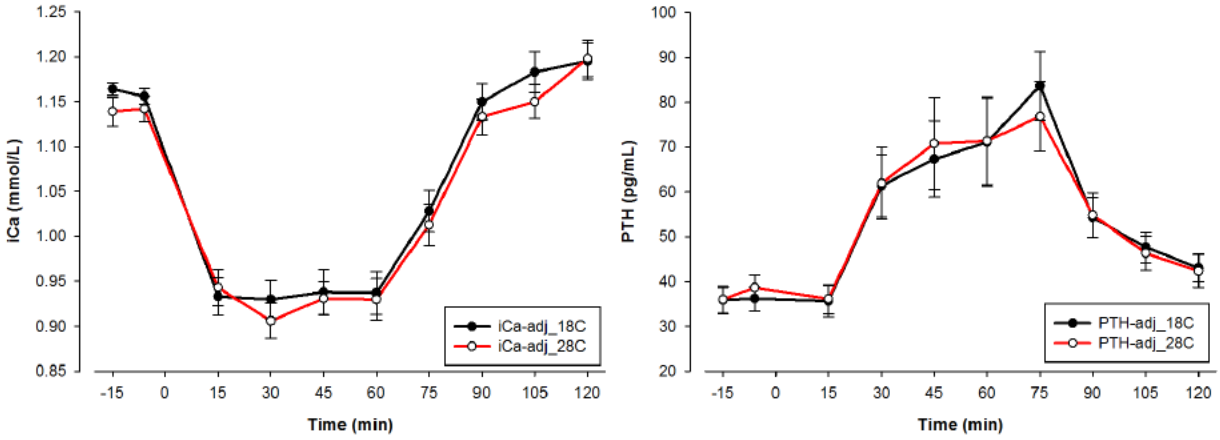
Attendees will be made aware of the complexities of interpreting the potential clinical consequences of acute disruptions in calcium homeostasis and bone metabolism by exercise.

APPENDIX B

Abstract from American College of Sports Medicine Annual Meeting

Disruptions in calcium homeostasis during high intensity exercise under different ambient conditions

Decreases in serum ionized calcium (iCa) and increases in parathyroid hormone (PTH) have been observed in response to high intensity exercise. However, it is not known to what extent the magnitude of dermal Ca loss influences these changes.
PURPOSE: To determine whether the magnitude of dermal Ca loss during exercise is a determinant of the decline in iCa and increase in PTH.
METHODS: Healthy cycling-trained women (n=14) and men (n=13) aged 18 to 45 years performed two 1-hour bouts of high intensity cycling exercise at different ambient temperatures: 18 C and 28 C. The order of the conditions was randomized and rides were matched for power output. Participants stopped taking Ca supplements 48 hours before each bout and ate a standardized breakfast containing <100 mg Ca 4 hours before exercise. PTH and iCa were measured every 15 minutes immediately before to 1 hour after the end of exercise. PTH and iCa values during and after exercise were adjusted for plasma volume shifts. Dermal Ca loss was estimated from sweat loss and sweat Ca concentration.
RESULTS: Estimated dermal Ca loss was greater in the 28 C condition (33±20 vs. 23±17 mg, $p<0.01$; mean±SD); sweat Ca concentration was not different (28C: 3.53±1.32; 18C: 3.56±1.32 mg/dL). The increase in PTH from before to 60' was not different between conditions (28C: 32.7±42.0; 18C: 34.9±44.2 pg/mL), nor was the decrease in PTH from 60' to 120' different between conditions (28C: -29.0±39.4; 18C: -22.1±32.0 pg/mL). There was no difference between conditions in either the decrease in iCa from before to 60' (28C: -0.21±0.05; 18C: -0.22±0.11 mmol/L) or the increase from 60' to 120' (28C: 0.27±0.09; 18C: 0.26±0.09 mmol/L).
CONCLUSION: The decrease in iCa during high intensity exercise is a likely trigger for the increase in PTH. However, the mechanisms underlying the decline in serum iCa during exercise remain unclear, as it did not appear to be influenced by the magnitude of dermal Ca loss.



SUPPORTING DATA

none