

12-1-2017

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Alrefai, Hasan (2017) "There is no evidence for bone remodeling caused by transdermal calcium loss in sweat during Bikram Hot Yoga in premenopausal women," *Perpetua: The UAH Journal of Undergraduate Research*. Vol. 2: Iss. 1, Article 7.

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There is no evidence for bone remodeling caused by transdermal calcium loss in sweat during Bikram Hot Yoga in premenopausal women

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Abstract – It has been hypothesized that sweat loss during exercise causes a disruption in calcium homeostasis leading to bone resorption and low bone mineral density. There is substantial water and electrolyte loss in sweat during hot yoga, an exercise that is becoming increasingly popular with premenopausal women. A Bikram Hot Yoga session typically consists of 26 Hatha Yoga postures that are performed in 90 minutes inside of a room that is set to 105 °F and 40 % humidity. We measured sweat and plasma, electrolyte and water balance before and after a hot yoga session in a population of premenopausal women. Sweat was collected during the final yoga asana by saturating filter paper with sweat from the participants' thighs to estimate the total amount of electrolyte loss. There was no change in serum sodium or serum osmolality before or after Bikram Hot Yoga. Mean calcium concentration in serum increased after the hot yoga session. The concentration of parathyroid hormone (PTH) did not change from before to after the hot yoga session; however, a substantial amount of water was lost. This implies that the amount of parathyroid hormone in circulation decreased from before to after. Calcium loss in sweat loss did not trigger an increase in PTH secretion and did not initiate bone resorption. A disruption in calcium homeostasis was not observed in a bout of excessive sweating during a 90-minute Bikram Hot Yoga session. Bikram yoga appears to be a safe practice for premenopausal women as long as fluid and electrolytes are appropriately replenished after the Bikram Hot Yoga Session.

I. Introduction

Bikram Yoga is a method of Hatha Yoga created by Bikram Choudhury and is classified as hot yoga (Choudhury, 2007). Bikram Yoga is a sequence of 26 Hatha Yoga asana and two breathing exercises performed at 105 °F and 40 % humidity. Each posture

is repeated twice in a class lasting 90 minutes. There is significant sweat, water, and electrolyte loss due to the high temperature in a Bikram Yoga studio. Hot yoga is a non-loading exercise that is becoming increasingly popular with pre-menopausal women. Several studies have pointed towards many health benefits from practicing hot yoga, including increased strength and flexibility (Hewett et al. 2015; Field 2016), weight management (Pate & Buono, 2014), and improves arterial stiffness (Hunter et al. 2016). However, it has been hypothesized that large losses of calcium in sweat during exercise causes a disruption in calcium homeostasis, which may lead to bone resorption and low bone mineral density. Any potential risk for decreasing bone mineral density should not be taken lightly, as this population of women is at a higher risk of developing osteoporosis.

A large calcium loss in sweat would elicit a decrease in serum calcium concentration that would be detected by the parathyroid glands. In response, they will produce and release parathyroid hormone (PTH). PTH stimulates osteoclasts to break down bones, releasing calcium back into the extracellular fluid compartment. PTH also has effects on the kidneys and downstream effects on the gastrointestinal tract; it causes them to reabsorb and absorb more calcium, respectively. All of these processes function to increase the serum concentration of calcium (Fig. 1). Once serum calcium concentration reaches an appropriate level, negative feedback inhibition will prevent more PTH from being produced.

There is evidence to suggest that excessive dermal calcium loss in sweat leads to bone mineral content (BMC) decreases in young male athletes, with around 422 mg of calcium lost in sweat per basketball game. This leads to a 10.5% decrease in the BMC of their legs over the course of a basketball season, thus increasing the risk for bone breaks and fractures (Klesges et al. 1996). A study in female competitive

cyclists showed that physical-exercise induced calcium loss in sweat was associated with an increase in biomarkers of bone metabolism, such as PTH and CTX-I (Haakonssen et al. 2015). The increase in PTH during exercise could be attenuated by a pre-exercise high calcium meal (Barry et al. 2011). It was observed in athletes with similar training regimes that lower BMC was associated with an increased risk of stress fractures. Interestingly, these athletes also had a lower dietary calcium intake and menstrual irregularities (Myburgh et al. 1990).

Premenopausal women athletes may be at a much higher risk of bone fracture due to increased loss of calcium in sweat during exercise. Premenopausal women generally show a loss in bone mineral density of 0.3-1.3% per year at the femoral neck and lumbar spine, respectively, caused by hormonal changes that alter calcium levels and decrease BMC (Bainbridge et al 2002). Additional losses of calcium in sweat caused by multiple sessions of hot yoga per week may decrease the BMC and increase the risk for bone fractures unless accounted for by increased intake of calcium in the diet.

The purpose of this study was to measure the transdermal calcium loss from thermal sweating in premenopausal women participating in Bikram Hot Yoga. We will determine if this elicits a physiological response by measuring changes in PTH, a biochemical marker of bone remodeling.

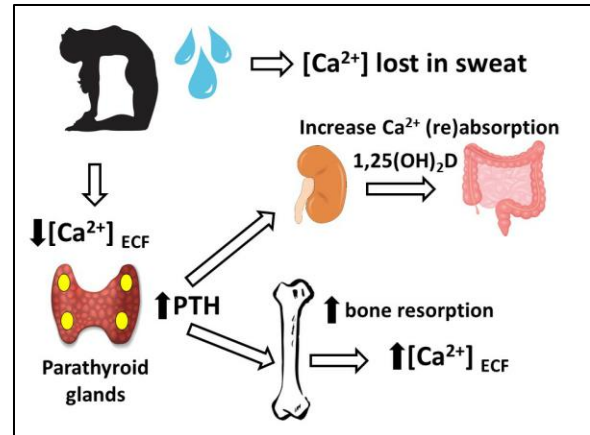


Figure 1. A schematic representation of the homeostatic response to low blood calcium.

Any excessive calcium loss in the sweat, urine, or feces would lower blood calcium in the extracellular fluid (ECF) from the normal range of 8.9-10.1 mg/dL. This is sensed by the calcium sensing receptor expressed in the parathyroid glands (yellow ovals) on the posterior side of the thyroid gland. They release parathyroid hormone (PTH) into the blood which acts on the kidneys to increase reabsorption of calcium from the urine, and on the gastrointestinal system to increase calcium absorption. In addition, the kidneys also release calcitriol, also known as 1,25(OH)₂D or Vitamin D, which increases absorption of calcium in the gastrointestinal tract. The most rapid effect of PTH is to stimulate bone osteoclasts to break down bone (resorption) and release calcium into the ECF increasing blood calcium concentrations. This can be detected by the calcium sensing receptor and result in negative feedback and decrease PTH secretion.



Figure 2. The 26 Asana of Bikram Yoga.

1. Half Moon (Arda-Chandrasana). 2. Hands to Feet (Pada-Hasthasana). 3. Awkward Pose (Utkatasana). 4. Eagle Pose (Garurasana). 5. Standing Head to Knee (Dandayamana-Janushirasana). 6. Standing Bow Pose (Dandayamana-Dhanurasana) 7. Balancing Stick (Tuladandasana) 8. Standing Separate Leg Stretching Pose (Dandayamana-Bibhaktapada-Paschimotthanasana). 9. Triangle Pose (Trikanasana). 10. Standing Separate Leg Head to Knee Pose (Dandayamana-Bibhaktapada-Janushirasana). 11. Tree Pose (Tadasana). 12. Toe Stand (Padangustasana). 13. Dead Body Pose (Savasana). 14. Wind-Removing Pose (Pavanamuktasana). 15. Sit-Up. 16. Cobra Pose (Bhujangasana). 17. Locust Pose (Salabhasana). 18. Full Locust Pose (Poorna-Salabhasana). 19. Bow Pose (Dhanurasana). 20. Fixed Firm Pose (Supta-Vajrasana). 21. Half Tortoise Pose (Ardha-Kurmasana). 22. Camel Pose (Ustrasana). 23. Rabbit Pose (Sasangasana). 24. Head to knee Pose and Stretching Pose (Janushirasana and Paschimotthanasana). 25. Spine-Twisting Pose (Ardha-Matsyendrasana). 26. Blowing in Firm Pose (Kapalbhati in Vajrasana). All postures are performed twice in a room heated to 105 °F at 40 % humidity over 90 minutes.

II. Materials and Methods

Study Population

Participants were female yogis selected by a flyer posted in the Bikram Hot Yoga of Huntsville studio (Madison, Al). A total of 5 subjects were recruited for this small viability study. Subjects with kidney disease, who use any medication known to affect bone metabolism, who are post-menopausal, and who use blood thinners were excluded from the study. Participants' mean age was 47.4 ± 4.7 years ($n = 5$). The subjects had attended a mean of 3.8 ± 1.4 Bikram yoga sessions per week for the last 2.0 ± 1.8 years. Sweat, pre and post yoga blood samples were collected from all 5 participants. This study was approved by the University of Alabama in Huntsville Human Institutional Review Board.

Study Measurements

Nude body weight was measured before and after the Bikram hot yoga session (Withings Body Scales, Withings, Inc., Cambridge, MA). Each participant's water bottle was weighed before and after the yoga session using a Philips Hipster Coffee Scale (Philip Ambrose, Five Points, Huntsville, AL) to calculate water volume consumed during a hot yoga session. Sweat was collected using a 5 cm x 5 cm square of filter paper (Whatman, No. 3) that was pressed against the thigh during the final Savasana posture. The thigh skin region was a good single representation of the whole body sweat sodium and chloride concentrations (Patterson et al. 2000). A blood sample was collected from all 5 participants immediately before and immediately after the 90-minute session of Bikram hot yoga.

Serum and Sweat Analysis

Blood was collected in a serum separator tube and allowed to clot. Serum was collected by centrifuging the blood at 1500 X g for 5 minutes at 4 °C. The serum was then aliquoted for analysis. Sweat was collected by centrifuging the sweat-loaded filter paper in a Salivette tube at 2500 x g for 2 minutes and was frozen for analysis (Sarstedt AG & Co., Nümbrecht, Germany).

Serum and sweat calcium concentration were measured using a Calcium Colorimetric Assay Kit Cat. No. K380 (BioVision Inc., Milpitas, CA). Serum sodium was measured using a colorimetric sodium assay Catalog No. DZ114B-K (Diazyme Laboratories, Inc., Poway, CA). Serum PTH was measured using

enzyme-linked immunoassay Catalog No. EIA-PTH (RayBiotech, Inc, Norcross, GA). Serum osmolarity was measured using a Micro-Osmometer Model 3300 (Advanced Instruments, Inc, Norwood, MA). Sweat sodium chloride concentration was calculated using a sweat conductivity analyzer Model 3120 (Wescor Inc, Logan, UT). In all cases manufacturer's instructions were followed for assays.

Body Volume Fluid Shift Calculations

For body volume fluid shift calculations total body water was assumed to be 50 % of body weight. The intracellular fluid compartment (ICF) was estimated to be 60% of total body water and extracellular fluid compartment (ECF) was estimated to be 40 % of total body water. Plasma volume was estimated to be 25% of the ECF. Theoretical changes in plasma sodium, calcium, and PTH levels were calculated using the water, sodium and calcium losses measured in sweat. The general assumptions were that intracellular solutes did not shift between the ECF and ICF compartments and that all electrolyte and fluid losses came from the extracellular fluid compartment.

Statistical Analysis

All data except PTH measurements are presented as mean \pm SD and were compared using a paired two-tailed Student's t test with a P value of $P < 0.05$ being considered as statistically significant. PTH measurements were presented in the same manner; however, a one-tailed Student's t test was performed with a P value of $P < 0.05$ being considered as statistically significant. All of the data was plotted and statistically analyzed using Prism 6.0 software. All assays were performed in triplicate.

III. Results

Nude body weight was initially 60.4 ± 5.5 kg and was 59.2 ± 5.4 kg ($n = 5$) after 90 minutes of Bikram Hot Yoga. A decrease of 1.2 ± 0.6 kg resulted in a decrease of 1.92 ± 0.90 % body weight. During the 90 minutes of hot yoga the yogis drank an average of 0.38 ± 0.22 ($n = 5$) liters of water. Adjusting for the amount of water drank during the 90 minute yoga session and assuming that all body weight loss was due to sweat production, the total sweat production during the 90 minute session was calculated as 1.54 ± 0.65 liters ($n = 5$). The volume of sweat lost and water consumed for all participants is shown in Figure 3.

First, we looked at body water and salt homeostasis. Serum osmolarity (Fig. 4A) was 276.4 ± 4.8 mOsm/L before yoga and 284.3 ± 7.6 mOsm/L after yoga ($P = 0.2089$, $n = 5$). Serum sodium (Fig. 4B) also did not change and was 137.5 ± 7.7 mmol/L before and 138.0 ± 5.4 mmol/L ($P = 0.9188$, $n = 5$) after hot yoga. Although 1.54 ± 0.65 liters ($n = 5$) of water (Fig. 3), and 164 ± 32 mmol/L of sodium chloride were lost in sweat, there was no change in body serum osmolarity and serum sodium concentration.

Plasma calcium concentration (Fig. 4C) increased from 10.4 ± 0.8 mg/dL before to 11.3 ± 0.8 mg/dL ($P = 0.0017$, $n = 5$) after hot yoga. There was no change in parathyroid hormone concentration, a marker of bone reabsorption (Fig. 4D). Serum PTH did not change and was 33.9 ± 3.3 pg/mL before hot yoga and 33.5 ± 3.3 pg/mL ($P = 0.6609$, $n = 5$) after hot yoga. The total calcium lost in sweat during the 90 minute period was estimated to be 41.3 ± 16.4 mg ($n = 5$).

In order to calculate changes in plasma volume, we used theoretical body water compartment calculations to determine if any of these changes could be explained by a decrease in plasma volume. The theoretical ECF concentration for calcium using the observed sweat loss and calcium lost in sweat was 11.6 ± 1.2 mg/dL. The theoretical measurement was not statistically different from the observed measurement 11.3 ± 0.8 mg/dL ($P = 0.3134$, $n = 5$); however, it was 10.1 % higher than the measured pre-yoga serum calcium concentration which was 10.4 ± 0.8 mg/dL ($P = 0.0206$, $n = 5$). This finding suggests that the observed increase in serum calcium concentration after hot yoga can be explained by the amount of water lost in sweat and the corresponding decrease in plasma volume.

The theoretical concentration of PTH was 36.4 ± 4.0 pg/mL after plasma volume reduction from sweating, whereas measured PTH concentration was only 33.5 ± 3.3 pg/mL. This implies that the amount of PTH in circulation decreased by about 15% ($P = 0.0251$).

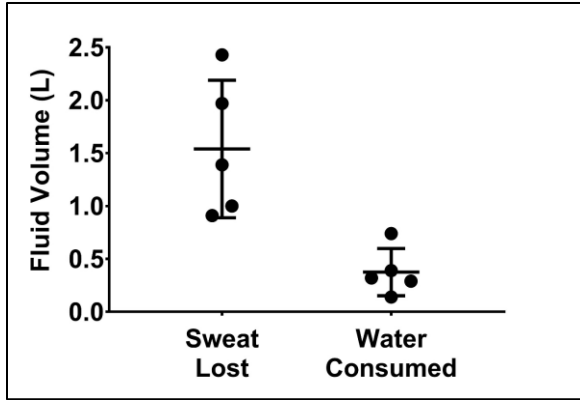


Figure 3. Fluid balance in yogis performing 90 minutes of Bikram Hot Yoga.

During the 90 minutes hot yoga session, the yogis lost 1.54 ± 0.65 liters ($n = 5$) in sweat. This loss was not completely replaced as the yogis drank only 0.38 ± 0.22 liters ($n = 5$) of water.

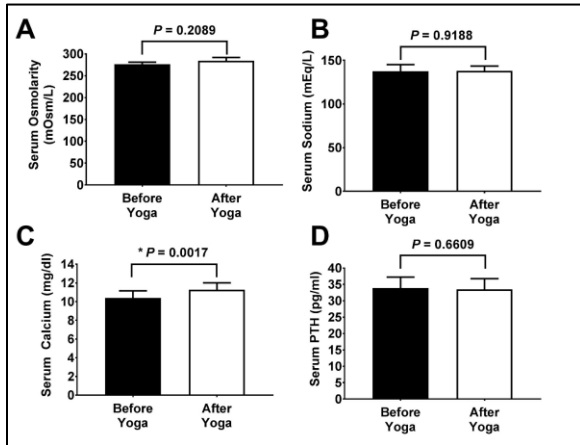


Figure 4. Measurement of electrolytes, osmolarity and PTH before and after Bikram Hot Yoga.

There was no difference in serum osmolarity (A) and serum sodium concentration (B) before or after yoga practice. However, serum calcium increased from 10.4 ± 0.8 mg/dL before to 11.3 ± 0.8 mg/dL ($P = 0.0017$, $n = 5$). There was no change in serum PTH concentration before and after a hot yoga session.

IV. Discussion

The primary goal of this study was to quantify the transdermal loss of calcium from sweat and measure potential markers of bone resorption during extracellular fluid calcium homeostasis. There is a large discrepancy (approximately 1.2 liters) between the volume of sweat produced and volume of water consumed during a 90 minute practice of

Bikram Hot Yoga. There is also a large quantity of sodium and a moderate amount of calcium lost in sweat. This raises the possibility of an electrolyte imbalance causing bone resorption to maintain the serum calcium levels at normal values. However, there was about 40 mg of calcium lost in the sweat. This small amount of calcium, in combination with the large amount of water lost can explain the increase in serum calcium concentration. Surprisingly, our data showed no change in serum sodium concentration or osmolarity before and after 90 minutes of hot yoga. This was due to the large amount of sodium lost in the thermal sweat during yoga.

PTH levels increase in response to low blood calcium levels. An increase in PTH triggers bone resorption, an increase in kidney calcium reabsorption, and an increase in intestinal calcium absorption. All of these mechanisms cooperate to increase extracellular fluid compartment calcium concentrations (Fig. 1). In our study, serum calcium concentration increased after hot yoga which would not cause an increase in PTH secretion from the parathyroid glands (Fig. 5). Furthermore, it was shown that the amount of PTH in circulation decreased.

Transdermal calcium loss from sweat leads to an increase in serum PTH levels and is associated with decreases in bone mineral density (BMD) in cyclists (Sherk et al. 2014), but not in yoga (Lu et al. 2016). This may be due to the effects of weight-bearing exercise on bones. Cycling is not considered a weight-bearing exercise and does not stimulate bone growth while yoga is a body weight-bearing exercise and may cause bone remodeling resulting in an increase in BMD. In a recent study 12 minutes of Hatha Yoga daily for 2 years resulted in an increase in BMD of the spine and femur and reversed osteoporotic bone loss (Lu et al. 2016). The release of PTH by modulators other than serum ionized calcium is poorly understood. Increased phosphate release from muscle during intense exercise has been shown to stimulate PTH secretion (Townsend et al. 2016). There is no information available on the effect of other byproducts of moderate muscle usage, such as increased carbon dioxide, serum lactate levels, decreased pH, and increased temperature have on the release of PTH from the parathyroid glands.

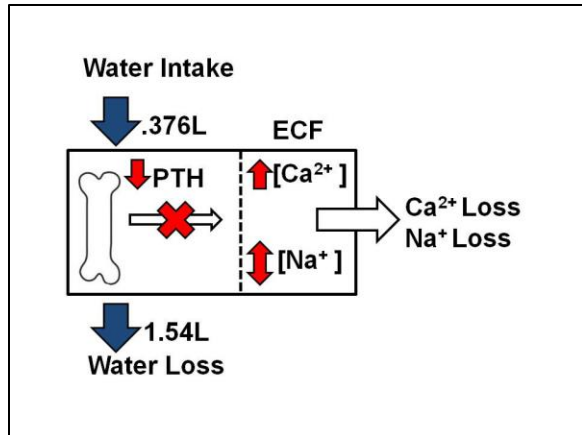


Figure 5. Model of water, salt, and calcium loss during 90 minutes of Bikram Hot Yoga.

In this schematic, it is shown that the water consumed was insufficient to replace the total amount of water lost in the sweat. Roughly 1.2 liters of water was lost. Due to the decrease in volume of extracellular fluid compartment, serum calcium concentration increased from before to after hot yoga, which was sensed by the parathyroid gland. In response, PTH secretion decreased, which implies that no bone remodeling occurred to replace the moderate amount of calcium lost in sweat. A large amount of sodium was lost in the sweat. Serum sodium concentration remained the same before and after the hot yoga session.

Our findings show that there is no evidence that the moderate amount of calcium lost in the sweat during Bikram Hot Yoga decreases serum calcium levels and initiates bone resorption through release of

PTH. Surprisingly, the decrease in plasma volume, together with moderate calcium losses in the sweat increases the apparent calcium concentration in the circulation, and produces a decrease in PTH secretion from the parathyroid gland (Fig. 1 & Fig. 5). This would set the necessary conditions for bone growth, although we would need conclusive evidence from BMD measurements to make this statement for a certainty. Future work includes measuring BMD, other minerals, and electrolytes lost in sweat during yoga and other bone-loading exercises.

In summary, despite the loss of calcium in sweat during yoga, there is no evidence that Bikram Hot Yoga stimulates bone loss to maintain extracellular fluid calcium levels. Bikram yoga appears to be a safe practice for premenopausal women. Our data does suggest that a large amount of fluid and sodium is lost, and a moderate amount of calcium is lost, and these should be replenished when rehydrating throughout and after hot yoga practice.

VI. Acknowledgements

This preliminary study was funded by a UAH CCFR grant to Shannon Mathis (Kinesiology) and Gordon MacGregor (Biological Sciences). Our thanks go to Carmeladell Watkins, owner of Bikram Hot Yoga Huntsville for allowing this study to take place and teaching the classes used in this study. We are forever grateful to the yogini who gave their blood, sweat and spiritual tears for this study. Namaste.

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