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ORIGINAL ARTICLE

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Bone turnover is altered during 72 h of sleep restriction: a controlled laboratory study

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Abstract

Purpose The objective of the study was to evaluate how controlled, short-term sleep restriction (SR; 72 h) alters markers of bone formation and resorption and urinary calcium (Ca) output.

Methods Ten healthy, sleep-adequate, male soldiers were housed in the research facility one day prior to and for the duration of SR. Diet was controlled to provide adequate energy balance and macronutrient distribution, meeting the recommended dietary allowance (RDA) for Ca. Subjects engaged in light activities to maintain wakefulness and were allowed 2 h of sleep per night (0430–0630 hours). Blood samples were collected each morning at 0 h (baseline) and 24, 48, and 72 h of SR. Serum was assayed for parathyroid hormone (PTH), bone alkaline phosphatase (BAP), tartrate-resistant acid phosphatase (TRAP), and C-terminal telopeptide of type I collagen (CTX). Urine was collected in 24 h increments during SR for measurement of Ca and creatinine (Cr).

Results BAP was reduced at 24 h ($P = 0.015$) and resorption markers TRAP and CTX were increased after 48 and 72 h of SR compared to baseline ($P < 0.05$). The ratio of BAP:TRAP was significantly lower ($P = 0.017$) at 48 and 72 h of SR. In contrast, total 24 h urinary Ca and Ca/Cr excretion were unchanged.

Conclusions Markers of bone formation and resorption are uncoupled in response to as little as 48 h of SR even when Ca intake is at the RDA. Sleep deprivation may be a risk factor for reduced bone health due to perturbations in bone turnover.

Keywords Sleep deprivation · Bone turnover · Biomarker · Military

Introduction

Both short-duration (<6.5 h per night) and long-duration (generally >8–9 h per night) sleep have been associated with indicators of poor bone health [1]. Cross-sectional studies have demonstrated lower volumetric bone mineral density (BMD) in women and torsional bending strength in men [2] who sleep less than 6.5 h/night during the weekdays. Others report increased osteoporosis and osteopenia risk in post-menopausal women who sleep less at night (i.e., <7 h) or who typically go to sleep at a later hour (after midnight) [3] when compared to those who sleep 8–9 h per night. Similar associations have been observed in European male and female populations when sleep quality was assessed [4]. Additionally, a recent review suggests that obstructive sleep apnea, which disrupts normal sleep patterns, may also be linked with indicators of poor bone health [5]. Increased total sleep (>10 h) has also been associated with increased osteopenia and osteoporosis risk [3]. Recently, two meta-analyses confirmed associations between both short- and long-duration sleep and increased osteoporosis risk [6, 7].

Animal studies have demonstrated acute effects of sleep restriction (SR) on both osteoblast and osteoclast function which may provide mechanistic insight into the long-term effects on bone tissue [8]. Long-term repeated SR in rats has been shown to dramatically reduce osteoid and osteoblast size and activity and increase the bone resorption marker tartrate-resistant acid phosphatase (TRAP), compared to sleep-adequate controls [9]. Another study in rats reported reduced bone morphology after 3 months of SR and reductions in both N-terminal cross-linking telopeptide of type I collagen (NTX), a marker of bone resorption, and serum N-terminal propeptide of type I procollagen (P1NP),

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a bone formation marker [10]. Thus, effects of SR on bone resorption and formation markers are not consistent across studies.

While there are cross-sectional associations between bone health and sleep and circadian patterns, there are few experimental or longitudinal studies in humans. An early human study reported that 24 h of total sleep deprivation lowered mean growth hormone values but did not alter the bone formation marker osteocalcin (OCN) [11]. In an attempt to discern the relative contributions of sleep versus body position, Ben-Sasson et al. [12] reported urinary Ca^{2+} excretion spiked after 63 h of SR and was also elevated when sleeping in a vertical position as compared to horizontal sleeping control subjects, suggesting a nightly period of skeletal unloading is important for bone health. More recently, Swanson et al. [13] sleep restricted (5.6/24 h) and disrupted the circadian pattern in young and old men over the course of 3 weeks. This intervention resulted in a 28% reduction in P1NP in younger men and an 18% reduction in older men, while the negative regulator of bone formation, sclerostin, was elevated by 22.9% in younger men only. The underlying mechanisms by which inadequate sleep affects bone turnover markers are not clear, but the etiology may include alterations in circadian patterns as bone turnover markers display circadian rhythms with resorption markers exhibiting the largest amplitude [14, 15]. Consistent with this hypothesis, BMD has been associated with positive rest-activity circadian rhythm patterns, but adjusting for lifestyle and comorbidities minimized this association [13, 16].

Sleep deprivation and disturbances are of particular interest to military populations because training and operations often require Warfighters to go for long time periods with little or disrupted sleep. Military populations also exhibit high rates of skeletal overuse injuries such as stress fracture [17]. However, since military training and operational environments contain multiple stressors, it is unknown if sleep disturbances are an independent factor in these injuries. In military personnel, the bone formation markers bone alkaline phosphatase (BAP) and OCN were suppressed, and the bone resorption marker TRAP increased following 8 weeks of US Army Ranger training where intensive physical demands are coupled with energy restriction and sleep deprivation (<4 h per 24 h) [18]. These findings suggest perturbations in bone metabolism in a multi-stressor environment, but the independent contribution of sleep deprivation could not be assessed.

The objective of this study was to comprehensively evaluate the independent effect of short-term SR (2 h sleep/night for 72 h) on markers of bone and calcium metabolism under laboratory conditions where confounders such as activity, psychological stress, and energy status were controlled. This study was undertaken as a secondary objective of a study examining the impact of SR on immune response [19]. We hypothesized that SR would increase bone resorption and urinary calcium excretion.

Methods

Subjects

Ten male subjects from the parent study [19] were used for this study. Based on the change in urinary Ca^{2+} (+170%) observed in 40% of the volunteers in the study by Ben-Sasson et al. [12], we estimated that 10 volunteers would provide adequate power to observe this level of change in urinary Ca^{2+} (alpha = 0.05 and power = 0.8) in the current study. The subjects represented a single cohort of a SR-only group in the parent study and were junior enlisted soldiers who were assigned to the Natick Soldier Systems Center, Natick, Massachusetts. Subject characteristics are presented in Table 1. Criteria for inclusion to the parent study were: ages between 19 and 35 years, generally healthy and not taking medications to include nonsteroidal anti-inflammatory drugs and aspirin, no history of psychiatric disorder or associated medication use, and regularly obtaining between 7 and 9 h of sleep per night at least 5 days per week. Subjects provided written informed consent after verbal explanation of the study objectives and methods. The study was approved by the Institutional Review Board, US Army Research Institute of Environmental Medicine. The investigators adhered to the policies for protection of human subjects as prescribed in Department of Defense Instruction 3216.02, and conducted in adherence with the provisions of 32 CFR Part 219. The Clinicaltrials.gov identifier is NCT02053506.

Design

Figure 1 represents the study timeline. The primary endpoint was change in urinary Ca with SR; secondary endpoints included markers of bone resorption and formation, intact parathyroid hormone (PTH), and cortisol (CORT). After a baseline testing period where sleep and food intake

Table 1 Subject demographic and anthropometric characteristics at baseline

Age, years	21.5 (1.5)
BMI, kg/m^2	26.3 (0.8)
Ht, m	1.75 (0.03)
Wt, kg	80.9 (2.9)
TDEE, kcal/day	2805 (104)

All data mean ± SE, $n = 10$. *BMI* body mass index, *TDEE* total daily energy expenditure

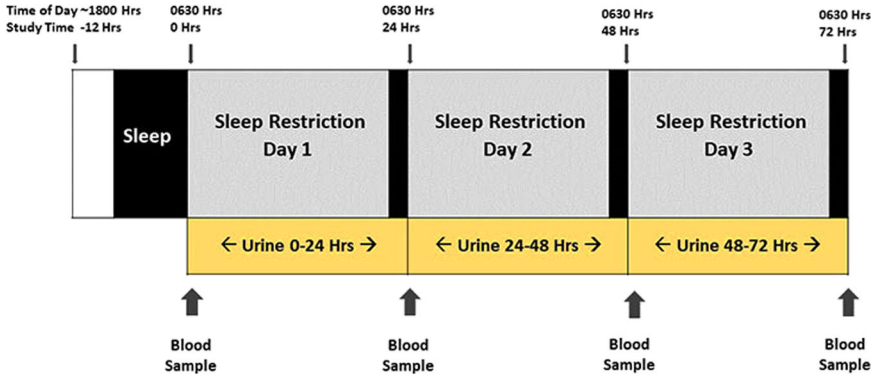

Fig. 1 Study design for 72 h of sleep restriction

were self-reported and reviewed daily for accuracy, subjects lived in the nutrition and sleep laboratory at the US Army Research Institute of Environmental Medicine for four consecutive days. Subjects reported to the laboratory 12 h prior to the SR period and were allowed to have a full 7–9 h of sleep that night. The SR period commenced upon waking the following morning at 0630 h. During the subsequent 72 h, subjects only slept 2 h/night (~0430 to ~0630 hours). To maintain wakefulness, the subjects engaged in a variety of activities (e.g. light exercise, video games, movies, etc.) similar to their regular activity patterns and were continuously monitored by study staff. Total activity was estimated during baseline and matched during the SR period. Subjects did not engage in heavy exercise during SR.

Dietary control

Subjects were instructed to refrain from caffeine 3 days before the SR to avoid the effects of caffeine withdrawal during the study period. Subjects consumed controlled diets designed by registered dieticians to maintain energy balance and meet the Recommended Dietary Allowance (RDA) for calcium. The menus were composed of commercially prepared food items, and water was allowed ad libitum. No other food or beverages were allowed except those provided by the study staff. All food was analyzed for nutrient content using computer-based nutrient analysis software (Food Processor, ESHA Research); macronutrient breakdown was 9% kcal from protein, 26% kcal from fat, and 65% kcal from carbohydrate. Calcium intake was set at 1000 mg/day. Meals and snacks were provided at set times throughout the day.

Biological sampling

Overnight fasted blood samples were collected each morning by venipuncture approximately 30 min after waking at ~0700 hours. After clotting, blood was centrifuged and serum separated into aliquots and frozen at −80 °C until analysis. All urine was collected starting at 0 h of SR and continued as three 24 h pools for the duration of the study.

Blood biochemical analysis

Serum was assayed for BAP and TRAP using commercially available enzyme-linked immuosorbant assay (ELISA) kits from Quidel. C-terminal telopeptide of type I collage (CTX) was assayed using an ELISA kit from Immunodiagnostic Systems. PTH and CORT were assayed by chemiluminescence on a Siemens Immulite 2000 clinical analyzer. Interassay coefficients of variation were 8.2% for BAP, 5.0% for TRAP, 9.2% for CTX, 5.30% for PTH, and 5.2% for CORT.

Urine analysis

Twenty-four hours urine pools were measured for volume, and an aliquot was acidified and assayed for calcium (Ca) and creatinine (Cr) on a Siemens Dimension Xpand Plus clinical chemistry system.

Statistical analysis

Data were analyzed using repeated measures ANOVA using the Statistical Package for the Social Sciences (IBM SPSS, version 24). When a main effect of time was observed ($P< 0.05$), post hoc testing between time points was conducted with Fisher's LSD for multiple comparisons. All data are $n = 10$ except BAP and CTX which are $n = 9$. One subject was found to be a BAP hyper-responder (nearly 56% reduction from baseline to 24 h and >2SD from the mean response) and was removed from subsequent BAP analyses as an outlier. CTX data are $n = 9$ due to inadequate serum sample in one subject. All data are mean ± SE.

Results

BAP was lower at 24 h compared to baseline (36.0 ± 2.9 vs 37.5 ± 3.1 U/L, respectively, $P = 0.015$). The 48 and 72 h time points (37.3 ± 3.1 and 38.1 ± 3.3 U/L) were significantly different compared to 24 h ($P = 0.005$ and $P = 0.004$,

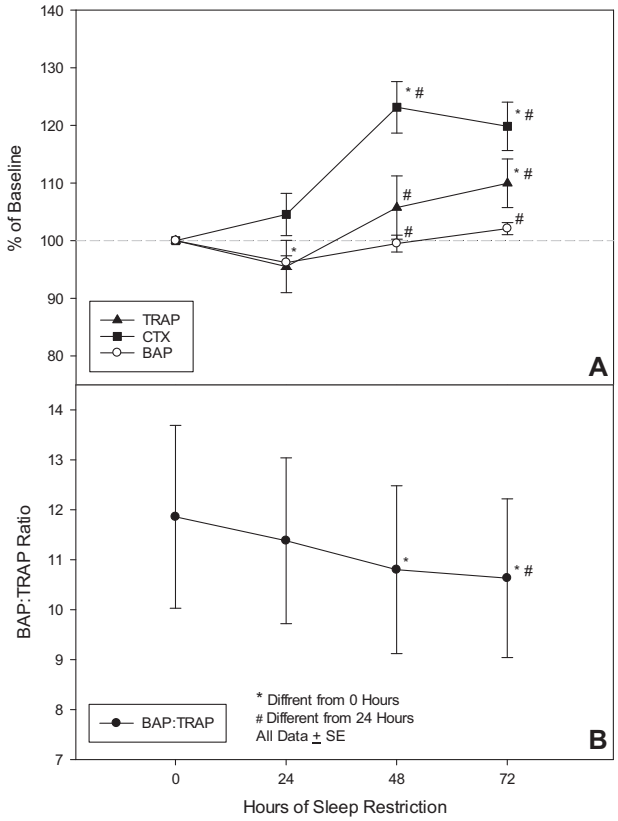

Fig. 2 Serum bone markers at 0 (baseline), 24, 48 and 72 h of sleep restriction of 2 h per night. All data are mean ± SE, $n = 10$ except BAP, BAP:TRAP, and CTX are $n = 9$. **a** Formation and resorption markers expressed as percent change from baseline. Open circle: Bone alkaline phosphatase (BAP), solid square: C-terminal telopeptide of type I collage (CTX), solid triangle: tartrate-resistant acid phosphatase (TRAP). **b** Solid circle: BAP:TRAP ratio. *Different from 0 h, #Different from 24 h, $P < 0.05$

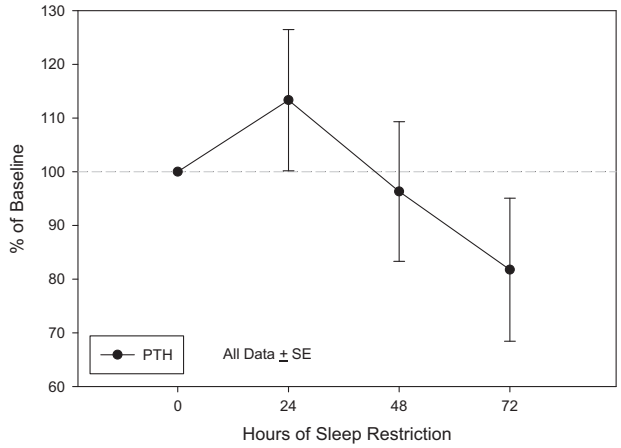

Fig. 3 Serum parathyroid hormone (PTH) at 0 (baseline), 24, 48, and 72 h of sleep restriction of 2 h per night expressed as percent change from baseline. All data are mean ± SE, $n = 10$. Solid cricle: PTH

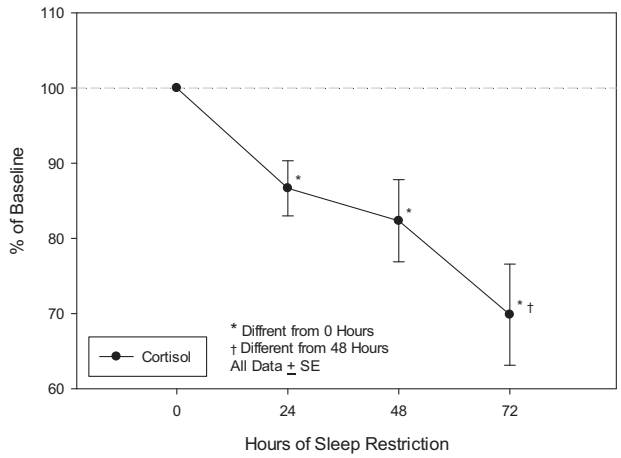

Fig. 4 Serum cortisol (CORT) at 0 (baseline), 24, 48 and 72 h of sleep restriction of 2 h per night expressed as percent change from baseline. All data are mean ± SE, $n = 10$. Solid circle: CORT. *Different from 0 h, †Different from 48 h, $P < 0.05$

respectively) but not different from baseline. Conversely, increases in resorption markers TRAP and CTX were observed at the same time points. TRAP increased over the 24 h time point (3.58 ± 0.50 U/L) at 48 and 72 h (4.04 ± 0.61 and 4.20 ± 0.62 U/L, $P = 0.029$ and $P = 0.003$, respectively) and CTX increased over baseline (1.43 ± 0.21 ng/mL) at 48 and 72 h (1.72 ± 0.23 and 1.69 ± 0.24 ng/mL, $P = 0.001$ and $P = 0.008$, respectively). The ratio of BAP to TRAP was calculated as a marker of overall bone formation to resorption and it was significantly lower at 48 h (10.8 ± 1.7, $P = 0.017$) and 72 h (10.6 ± 1.6, $P = 0.005$) compared to baseline (11.9 ± 1.8). Similar changes were detected when the data were expressed as a percent change from baseline (Fig. 2).

PTH was unchanged with the exception of the 72 h time point when PTH was reduced compared to 24 h ($P = 0.009$). When expressed as percent change from baseline there were no changes in PTH (Fig. 3). Serum CORT was lower than baseline (16.0 ± 0.8 pg/mL) at 24 and 48 h (13.8 ± 0.9 and 13.0 ± 0.8 pg/mL, $P = 0.005$ and $P = 0.004$, respectively) and further reduced at 72 h of SR (11.2 ± 1.3 pg/mL, $P = 0.001$). Changes in CORT were similar when expressed as percent change from baseline. Serum CORT was approximately 70% of the baseline value at 72 h (Fig. 4).

Urine values for Ca and Cr are presented in Table 2 as 24 h pooled samples (0–24, 24–48, and 48–72 h). Urine Ca concentration and Cr were significantly increased in the 72 h pool. However, no changes in Ca excretion were observed when urine Ca was corrected for total urine volume and expressed as total Ca (mg/24 h) or Ca/Cr (mg/mg/24 h).

Discussion

The main finding of this study is that bone formation and resorption uncouple or become unbalanced during 72 h of

Table 2 Urine calcium marker concentrations in 24 h urine collections at 24, 48 and 72 h of sleep restriction of 2 h per night

	24 h	48 h	72 h
Ca, mg/dL	5.49 (1.11)	5.91 (0.77)	7.50 (1.28)*
Ca, mg/24 h	198.3 (27.5)	175.2 (21.1)	188.3 (28.0)
Cr, mg/dL	47.2 (5.4)	64.0 (9.8)	73.1 (4.7)*
Ca/Cr, mg/mg/24 h	0.115 (0.016)	0.101 (0.015)	0.100 (0.013)

All data mean ± SE, $n = 10$

*Different from 24 h, $P < 0.05$

SR in a laboratory setting. The bone formation marker BAP was slightly reduced as early as 24 h into SR and resorption markers TRAP and CTX were elevated at 48 and 72 h of SR. The ratio of BAP:TRAP has been previously reported to be a good indicator of bone metabolism [20, 21], and we found progressive declines in the BAP:TRAP indicating a shift in the bone turnover state to increased resorption. Even though the observed changes in circulating turnover makers were modest, the majority of our volunteers exhibited similar changes and these started to occur as soon as 24 h into SR.

This study illustrates some important aspects of bone metabolism in response to SR. Military personnel train regularly for sustained operations of up to 72 h while simultaneously being subjected to physical, psychological, nutritional, and sleep restricted stress. Previous studies examining physiological effects of military training and/or sustained operations have demonstrated dramatic hormonal alterations occur [22–24]. Likewise, bone turnover markers were altered following 8 weeks of US Army Ranger training and remain reduced for 2 weeks following training [18]. The current findings indicate that markers of resorption respond only after a few days of SR. The controlled nature of our study supports that SR is an independent contributor to short-term changes in bone metabolism and these effects are apparent as soon as 48 h into SR.

Bone metabolism and sleep

Previous studies examining the link between sleep and bone health have mainly been cross-sectional human studies or mechanistic work conducted in animal models. Most of cross-sectional studies have reported a relationship between indicators of reduced sleep, poor sleep quality, night-shift work, excessive sleep and various markers of bone health [2–5, 8, 16]. Animal models document that chronic sleep deprivation decreases both bone quality and markers of bone turnover [9, 10].

Relatively few well-controlled human interventional studies have been conducted to examine bone health and SR. Swanson et al. [13] showed that circadian rhythm disruption tips the bone formation/resorption balance to resorption. This study [13] collected serial blood samples over 24 h to characterize the full circadian pattern of bone turnover markers before and after 3 weeks of circadian disruption and circadian disruption was associated with a decrease in bone formation (P1NP) and no change in bone resorption (CTX). In the current study, SR was accompanied by a reduction in bone formation (BAP) and increases in two resorption markers (TRAP and CTX), but used a single time points versus serial measurements over the course of a day. CTX is known to be impacted by feeding and to display a diurnal pattern in humans with peak values seen in the early morning hours [15] and nadir in the afternoon. Given the timing of blood collection, it is likely we were capturing the peak value of CTX in the fasted morning sample. However, it is unclear from our single point data if bone formation/resorption circadian patterns shifted or were amplified or reduced as a result of the SR.

PTH and calcium output

PTH was largely unchanged by SR, with the exception of a reduction at 72 h when presented as percent of baseline. Based on prior work [12], we hypothesized that SR would increase urinary Ca excretion, leading to reductions in circulating ionized Ca and stimulation of the PTH1–alpha-hydroxylase axis. Since PTH is a primary regulator of Ca handling, the observation that 24 h urine Ca output was unchanged is consistent with a lack of increase in serum PTH. Given the net increase in bone resorption as indicated by turnover markers, it stands that there may have been a net efflux of Ca from the bone into the circulation, thereby suppressing circulating PTH. Unfortunately the limitations of our study did not allow assessment of ionized Ca to confirm this hypothesis.

Potential mechanism and cortisol

Even though this study and other human trials have demonstrated alterations in bone turnover with SR and/or circadian rhythm disruption, the mechanisms of action are not clear. Swanson et al. [1] suggested that disruption of hormonal, inflammatory, cortisol, glucose, and/or sympathetic tone may play a role. Serum cortisol has emerged as a potential mediator due to its widespread catabolic effects on numerous organ systems and tissues and it exhibits a significant circadian pattern. This rhythm has also been shown to be associated with the circadian rhythm of osteocalcin [25, 26], a bone formation marker. The effect of SR on morning cortisol has been shown to exhibit either no change [27, 28], or a decrease [29]. One study reported that SR attenuated the circadian pattern of cortisol [30]. However, increases in evening cortisol and sympathetic system activity have been reported with SR [31]. Serum cortisol is

at its peak soon after awaking as described as the cortisol awakening response [32]. Our finding of increased bone resorption and decreased formation coupled with a progressive decline in morning cortisol indicates that serum cortisol is unlikely to be the contributing mechanism. However, since all of our blood sampling time points were at approximately 0630 hours, it is possible that the peak values of the diurnal variation had shifted. Another possibility is that the decline in cortisol was due to the baseline value being artificially high, but our reported value of 16.0 µg/dL falls well within the normal range of morning serum cortisol.

Although not assessed in the study, melatonin may also be a potential mediator of the circadian pattern of bone turnover markers and its alteration with SR. Melatonin promotes osteoblastic bone formation, decreases RANKL expression, and increases OPG [33]. The sum of these effects would be a more bone anabolic profile. Melatonin also has been shown to inhibit osteoclast differentiation [34]. In a human trial, 1 year of nightly melatonin supplementation was associated with higher femoral BMD [35]. Our study did not control the lighting conditions, but we might speculate, since room lights were on with the exception of a 2 h period each night, that melatonin levels may have been lower during the 72 h SR period.

Given that bone metabolism was a secondary outcome of a larger study, we were limited to one blood sample per day with inadequate sample for additional biomarker analyses. We also acknowledge the lack of a true control group or condition in this design, therefore we cannot fully exclude the possibility that unforeseen factors altered bone metabolism. However, the strengths of this investigation outweigh the limitations and address a gap in the literature relative to bone metabolism and SR. This study was conducted in a laboratory environment, thus allowed for control of several factors which are difficult to control in field or operational studies. For example, diet was maintained to achieve energy balance for activity levels and Ca intake was controlled at 1000 mg/day. Additionally, activity patterns during SR were controlled to light exercise only. Finally, when compared to military operational studies, it is likely the psychological stress of SR in the laboratory setting would be minimized.

Summary

This well-controlled laboratory study provides evidence that bone formation is reduced and bone resorption is increased when sleep is restricted to only 2 h per night for 72 h. There appears to be a direct effect of SR on bone metabolism when potential confounders including exercise, psychological stress, and energy balance are controlled. These findings support previous research indicating a long-term negative effect of sleep disruption on skeletal health. These findings are important for populations such as the military and first responders, as well as other populations who may experience repeated cycles of SR. Future studies using serial blood sampling and a full control group or condition should be undertaken to confirm these findings, and identify potential countermeasures that may reduce the negative impacts of SR on bone metabolism.

Disclaimer

The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or reflecting the views of the Army or the Department of Defense.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval *Research involving human participants*: All procedures were approved and performed in accordance with the ethical standards of the Institutional Review Board, US Army Research Institute of Environmental Medicine. The investigators adhered to the policies for protection of human subjects as prescribed in Department of Defense Instruction 3216.02, and conducted in adherence with the provisions of 32 CFR Part 219.

Informed consent Written informed consent was obtained from allindividual participants included in the study.

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