Sleep Restriction With Circadian Disruption Negatively Alter Bone Turnover Markers in Women

Christine M. Swanson,[1] Steven A. Shea,[2,3] Wendy M. Kohrt,[4] Kenneth P. Wright, Jr.[1,5] Sean W. Cain,[6,7] Mirjam Munch,[8] Nina Vujović[6] Charles A. Czeisler,[6,9] Eric S. Orwoll,[10] and Orfeu M. Buxton[6,11]

[1]Division of Endocrinology, Metabolism and Diabetes, University of Colorado Anschutz Medical Campus, Aurora, Colorado, US; [2]Oregon Institute of Occupational Health Sciences, Oregon Health & Science University, Portland, Oregon, US; [3]OHSU-PSU School of Public Health, Portland, Oregon, US; [4]Division of Geriatric Medicine, University of Colorado Anschutz Medical Campus, and Eastern Colorado VA Geriatric Research, Education, and Clinical Center; Aurora, Colorado, US; [5]Department of Integrative Physiology, University of Colorado Boulder, Boulder, Colorado, USA; [6]Division of Sleep Medicine, Harvard Medical School, Boston, Massachusetts, US; [7]Monash Institute of Cognitive and Clinical Neurosciences, School of Psychological Sciences, Monash University, Clayton, Victoria, Australia; [8]Sleep/Wake Research Centre, Massey University Wellington, Wellington, New Zealand; [9]Division of Sleep and Circadian Disorders, Departments of Medicine and Neurology, Brigham and Women's Hospital, Boston, Massachusetts, US; [10]Division of Endocrinology and Bone and Mineral Unit, Oregon Health & Science University, Portland, Oregon, US; and [11]Department of Biobehavioral Health, Pennsylvania State University, University Park, Pennsylvania, US

ORCiD numbers: 0000-0003-1554-0862 (C. M. Swanson).

Purpose: The purpose of this work is to determine whether an uncoupling of bone turnover markers (BTMs) occurs in women exposed to the combination of sleep restriction with circadian disruption (SRCD), as previously reported in men.

Methods: Four bone biomarkers (N-terminal propeptide of type I procollagen [P1NP] and osteocalcin = bone formation; C-telopeptide [CTX] = bone resorption; sclerostin = bone formation inhibitor) were measured in bihourly samples over 24 hours at baseline and after approximately 3 weeks of sleep restriction (~5.6 hours of sleep/24 hours) with concurrent circadian disruption (SRCD, recurring 28-hour "day" in dim light). Maximum likelihood estimation in a repeated-measures model was used to assess the effects of SRCD and age on bone biomarkers.

Results: Five women were young (22 ± 2.8 years) and four were older (58 ± 1.8 years). Baseline bone biomarker levels did not differ by age (all $P \geq .07$). Bone formation markers were *lower* after SRCD (estimate ± SEE, ΔP1NP = –9.5 ± 2.8 µg/L, $P = .01$; Δosteocalcin = –2.3 ± 0.9 ng/mL, $P = .04$). The P1NP decline was greater in young women (ΔP1NP = –12.9 ± 3.7 µg/L, $P = .01$). After SRCD, CTX was significantly *higher* in young women (0.182 ± 0.069 ng/mL, $P = .04$) but did not change in older women.

Conclusions: These pilot data are similar to previous findings in men and suggest that SRCD negatively altered bone metabolism in women by decreasing markers of bone formation and, in young women, increasing a marker of bone resorption. If sustained, this pattern of BTM uncoupling may lead to bone loss and lower bone mineral density. **(*J Clin Endocrinol Metab* 105: 2456–2463, 2020)**

Freeform/Key Words: sleep, bone turnover markers, P1NP, CTX, circadian disruption, shift work

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 25 January 2020. Accepted 27 April 2020.
First Published Online 4 May 2020.
Corrected and Typeset 6 June 2020.

Abbreviations: BMD, bone mineral density; BTMs, bone turnover markers; CBT, core body temperature; CTX, C-telopeptide of type I collagen; CV, coefficient of variation; FD, forced desynchrony; P1NP, N-terminal propeptide of type I procollagen; SEE, standard error of the estimate; SRCD, sleep restriction with circadian disruption.

Night shift work for 20 or more years was associated with a 37% higher risk of hip and wrist fractures in postmenopausal women in the Nurses' Health Study (1). Night shift work induces circadian misalignment, which is a mismatch or desynchronization between an individual's internal master circadian clock and the external environment. It also shortens sleep duration because the quantity and quality of sleep are often diminished with daytime sleep as opposed to nocturnal sleep.

Bone turnover markers (BTMs), particularly markers of bone resorption, display a 24-hour diurnal rhythm (2-4) that is likely important for normal bone remodeling. We previously reported that 10 healthy men exposed to approximately 3 weeks of a forced desynchrony (FD) protocol that simulates the stresses endured during rotating shift work (ie, cumulative sleep restriction with concurrent circadian disruption; SRCD) had a rapid, significant decline in a bone formation marker despite no change in a marker of bone resorption (5, 6). If sustained, this uncoupling of bone turnover could lead to bone loss over time and potentially increased fracture risk.

The effects of SRCD on BTMs in women are unknown. For the present analysis, we sought to describe the effects of the same approximately 3-week SRCD intervention (5, 6) on BTMs in women. We hypothesized that bone formation markers would decline in response to SRCD, as previously observed in men.

Materials and Methods

Study design and participants

The present analysis used serum samples from a previously performed clinical research study (7). Healthy women without a history of regular night shift work within 3 years and no travel across more than 2 time zones within 3 months age 18 to 60 years were recruited from the Boston, Massachusetts, area using paper and electronic advertisements (7). The study was performed between 2007 and 2010 in the Intensive Physiological Monitoring Unit of the Center for Clinical Investigation at Brigham and Women's Hospital.

For 3 weeks prior to admission to the inpatient unit, participants adhered to a regular sleep/wake schedule with a self-selected 10-hour sleep opportunity per day (Fig. 1 in Swanson et al [5, 6] and Buxton et al [7]). Sleep opportunities prior to admission were verified by sleep diary, time-stamped call-ins of bed and wake times, and wrist actigraphy. On admission, participants had 5 baseline nights with a minimum 10-hour sleep opportunity per night. The preadmission and baseline segments were designed to eliminate preexisting sleep debt and ensure participants were sleep satiated. After the baseline segment, participants were exposed to an FD protocol (5, 7, 8). The FD protocol was designed to desynchronize the internal circadian cycle from the environmental sleep-wake cycle (8). The protocol consisted of recurring scheduled 28-hour sleep-wake cycles (a 21.47-hour wake episode and a 6.53-hour sleep opportunity) that provided the equivalent of 5.6 hours of sleep opportunity per 24 hours. The FD segment in the 9 women reported here lasted approximately 22 ± 2 calendar days (~3 weeks) and induced SRCD (ie, misalignment between the internal circadian clock and external environment, similar to shift work or jet lag). All in-laboratory sleep opportunities and sampling intervals were performed relative to the midpoint of each woman's habitual sleep opportunity to account for different habitual bedtimes and to align each woman's data at baseline and after FD. Core body temperature (CBT) was continuously monitored to estimate circadian phase (9). CBT minimum varied minimally across all 9 women at baseline (greatest difference = 42 minutes) and therefore no further alignment between individuals was deemed necessary.

As previously described (5), serum samples were obtained hourly over two 24-hour sampling intervals. The first 24-hour collection was performed at baseline (night 5) when women were sleep replete and circadian aligned. The second 24-hour collection occurred approximately 3 weeks later at the end of the FD protocol (referred to as "postintervention"). The timing of the second 24-hour collection was carefully selected so the women were at a similar circadian phase compared to baseline (as assessed by CBT minimum). Thus, the comparisons between baseline and postintervention sampling intervals assess differences induced by accumulating sleep loss and a history of prolonged circadian disruption but without acute circadian misalignment (5). To facilitate blood draws throughout the protocol, heparinized intravenous tubing was used, except during a 1-week break from participants' intravenous catheters in the middle of FD. The study was performed in dim light (< 0.02 lux during sleep opportunity, < 15 lux maximum at ~183 cm with the sensor aimed toward the ceiling-mounted light fixture while awake).

During the inpatient protocol (baseline and FD), patients were ambulatory but exercise was prohibited. Individuals wore a wrist actigraphy device (Actiwatch-L; Mini Mitter) during the study. Each woman's actigraphically assessed wrist activity (counts/minute) was averaged for each condition (baseline and FD) and percentage change from baseline to FD was calculated for each woman and then averaged for the group. The 5 young women reported no regular exercise habits prior to admission. The 4 older women each reported regular endurance activities including walking, biking, gardening/yardwork, cross-country skiing, hiking, aerobics, and/or swimming on their screening forms. None of the 9 women were competitive athletes.

Meal composition and timing were controlled throughout the protocol. Participants were provided a eucaloric, controlled-nutrient diet at standardized times relative to wake and ≥ 2.5 L of fluid per 24 hours (see Fig. 1 in Swanson et al [5] and Buxton et al [7]). Macronutrient composition was 55% to 60% carbohydrate, 15% to 20% protein, and 15% to 30% fat. Although the eucaloric diet adhered to current standards both for macronutrient and micronutrient content, calcium and vitamin D intake were not specifically controlled. Caffeine intake was not permitted.

The original study was approved by the Partners Human Research Committee and registered at ClinicalTrials.gov. All study procedures were conducted in accordance with the Declaration of Helsinki. All participants provided written

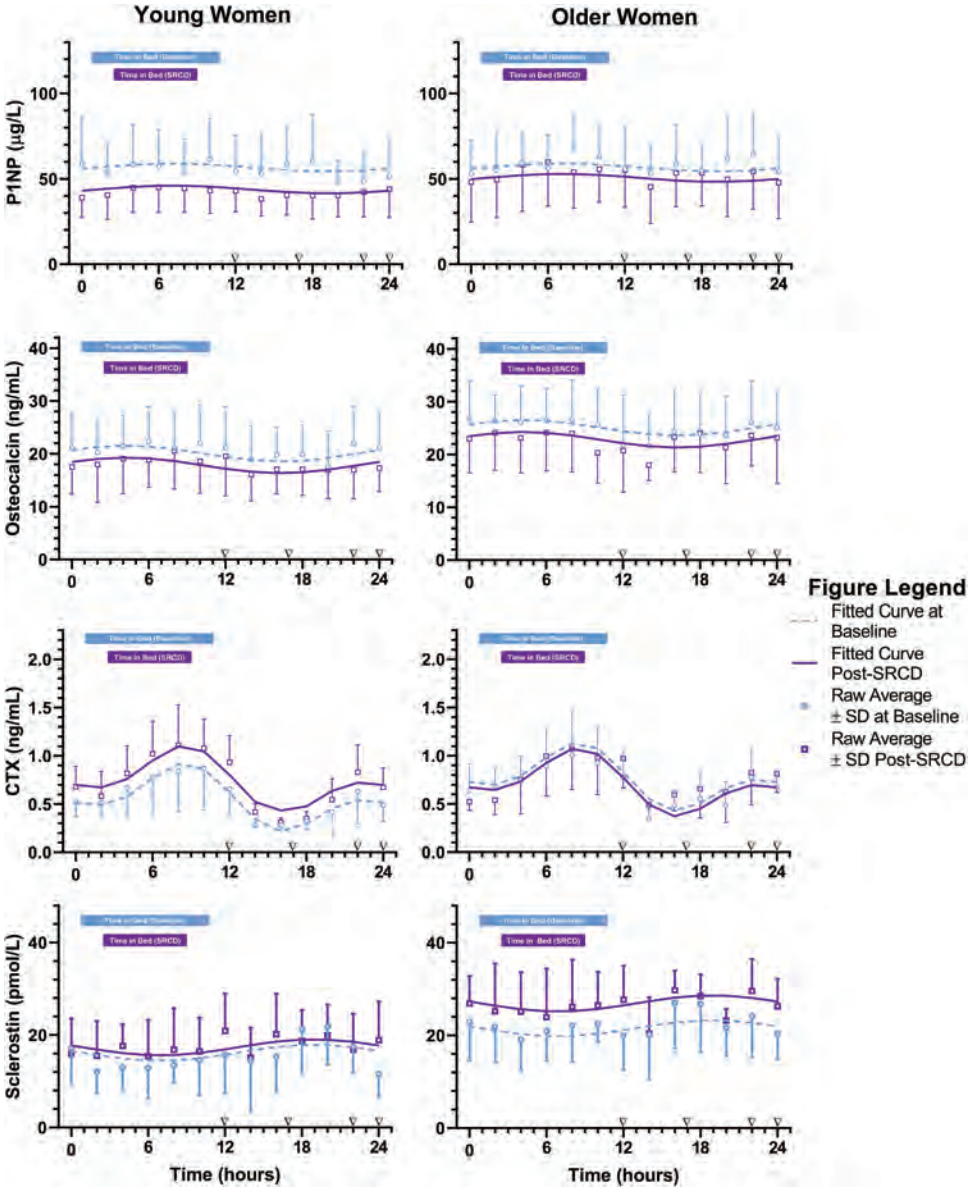

Figure 1. Twenty-four–hour serum profiles of bone biomarkers at baseline and post sleep restriction with circadian disruption intervention, by age group.

The X-axis represents number of hours into the 24-hour sampling profile. Data from the 5 young women are displayed in the left column and the 4 older women in the right column. Twenty-four–hour fitted profiles depicted for A, N-terminal propeptide of type I procollagen (P1NP), B, osteocalcin, C, C-telopeptide of type I collagen (CTX), and D, sclerostin at baseline (dashed light blue) and post sleep restriction with circadian disruption (SRCD) (solid purple) by age group. Raw averages (±SD) are plotted for every–2-hour samples. Sleep opportunities for the respective phases of the protocol are represented by horizontal light blue (baseline) or purple (post-SRCD) bars at the top of each graph. The SRCD protocol consisted of recurring scheduled 28-hour sleep-wake cycles (21.47-hour wake episode and a 6.53-hour sleep opportunity) that provided the equivalent of 5.6-h of sleep opportunity per 24 hours. Upside-down triangles along the x-axis represent meal times of breakfast (hour 12), lunch (hour 17), dinner (hour 22), and snack (hour 24). The 2 serum profiles were aligned by the midpoint of sleep.

informed consent (7). The present analysis used deidentified serum and was deemed nonhuman subject research by the Colorado Multiple Institutional Review Board (COMIRB number 16–0032).

Assays

Assays were performed by the Oregon Clinical and Translational Research Institute laboratory at Oregon Health and Science University to be consistent with our prior work (5). Samples were stored at less than –70 °C until thawed for assay. Each woman's samples were analyzed in the same assay to minimize variability. Samples were analyzed in duplicate and an average taken as the final result.

Four bone biomarkers were measured on bihourly serum samples across the two 24-hour intervals. The intact trimer of N-terminal propeptide of type I procollagen (P1NP) was measured using the Orion Diagnostica radioimmunoassay. Interassay coefficient of variations (CVs) for P1NP were 3.3% (low control)/10% (high control) and the average percentage difference between duplicates was 7.7%. Osteocalcin and C-telopeptide of type I collagen (CTX) were measured using Immunodiagnostics Systems enzyme-linked

immunosorbent assays (ELISAs) with interassay CVs of 4.3% (low control)/2.9% (high control) and 6.3% (low control)/7.7% (high control), respectively. The average percentage differences between duplicates for these assays were 4.7% for osteocalcin and 4.6% for CTX. Sclerostin was measured using ALPCO's ELISA Biomedica assay with interassay CVs of 5.2% (low control)/7.6% (high control) and average percentage difference between duplicates of 13.9%. P1NP and osteocalcin were used as markers of bone formation, CTX as a marker of bone resorption, and sclerostin as an inhibitor of bone formation and a marker of mechanical loading/unloading as sensed by the osteocyte. As previously reported (7), cortisol and insulin were measured using Beckman Coulter Inc chemiluminescent immunoassays with interassay CVs of 6.4% to 7.9% (cortisol) and 3.1% to 5.6% (insulin), and intra-assay CVs of 4.4% to 6.7% (cortisol) and 2.0% to 4.2% (insulin).

Statistical analysis

We previously observed a 10 to 20 µg/L decrease in P1NP in 10 men exposed to the same protocol (5). We hypothesized that we would see a similar P1NP decrease in women because previous animal studies observed similar BTM changes in females and males exposed to sleep restriction (10, 11). With our predetermined sample size of 9 women, we had 85% power to detect a mean 13.9 µg/L difference in P1NP levels from baseline to post-SRCD. This was conservatively estimated using a paired *t* test, assuming an SD of 12 µg/L, a correlation between repeated measures on the same individuals to be at least 0.5 (both based on preliminary data (5)) and 2-sided α of .05. Maximum likelihood estimation in a repeated-measures model was used to assess the effects of the sleep and circadian intervention and age on bone biomarkers. A cosinor model was used to estimate the circadian rhythm characteristics of baseline bone biomarker levels (5, 12). Rhythmicity was included in the repeated-measures model if a significant diurnavariation in a bone biomarker was identified in this or other analyses (eg, CTX, osteocalcin, P1NP [4]). Paired *t* tests were used to determine whether the change in physical activity, weight, or fasted morning cortisol or insulin from baseline to post-SRCD was statistically significant. A change in fasted morning cortisol and insulin levels with SRCD intervention was assessed by calculating the difference between previously obtained (7) levels at baseline and at the end of FD for each woman and then averaged for the group. We used Spearman correlations to evaluate whether changes in P1NP correlated with changes in weight or physical activity. Descriptive variables are presented as mean ± SD. Data from the repeated measures model are presented as estimate ± standard error of the estimate (SEE) unless otherwise stated. A *P* value of less than .05 was considered statistically significant, and no adjustment was made for multiple comparisons because this study was intended to be exploratory and hypothesis generating.

All analyses were conducted using SAS software version 9.4 (SAS Institute). GraphPad Prism 8.0 (GraphPad Software) was used to generate all figures.

Results

Of the 9 women in this study, 5 were young and premenopausal (age 22 ± 2.8 years, range, 18-24 years) and 4 were older and postmenopausal (age 58 ± 1.8 years, range, 56-60 years). All 9 women were white and non-Hispanic by self-report. The average body mass index was 22.9 ± 3.3 kg/m^2.

Baseline bone biomarker levels did not differ by age (all $P \geq .07$; Table 1), although osteocalcin, CTX, and sclerostin were somewhat higher in the older compared to young women. At baseline, CTX and osteocalcin displayed significant 24-hour diurnal rhythms (see Fig. 1; both $P < .01$).

After exposure to the sleep restriction with concurrent circadian disruption in the FD protocol, mean levels of both bone formation markers (P1NP, osteocalcin) were significantly lower (see Table 1, Fig. 1). On average, P1NP declined –9.5 ± 2.8 µg/L ($P = .01$). Young women had a greater decline in P1NP than older women ($P = .01$; –12.9 ± 3.7 µg/L, $P = .01$ vs –6.1 ± 4.1 µg/L, $P = .19$, respectively). Osteocalcin declined 2.3 ± 0.9 ng/

Table 1. Bone biomarker baseline values and change after exposure to sleep restriction with circadian disruption

	Baseline biomarker concentration for all women and by age group (estimate ± SEE)	Effect of SRCD for all women and by age group, estimate ± SEE, *P*
P1NP, µg/L	56.7 ± 4.6 µg/L	**–9.5 ± 2.8 µg/L, *P* = .01**
Older	56.7 ± 6.8 µg/L	–6.1 ± 4.1 µg/L, *P* = .19
Young	56.7 ± 6.1 µg/L	**–12.9 ± 3.7 µg/L, *P* = .01**
Osteocalcin, ng/mL	22.6 ± 1.5 ng/mL	**–2.3 ± 0.9 ng/mL, *P* = .04**
Older	25.1 ± 2.3 ng/mL	–2.3 ± 1.3 ng/mL, *P* = .13
Young	20.1 ± 2.0 ng/mL	–2.2 ± 1.2 ng/mL, *P* = .10
CTX, ng/mL	0.656 ± 0.049 ng/mL	0.063 ± 0.052 ng/mL, *P* = .27
Older	0.761 ± 0.073 ng/mL	–0.056 ± 0.077 ng/mL, *P* = .49
Young	0.550 ± 0.066 ng/mL	**0.182 ± 0.069 ng/mL, *P* = .04**
Sclerostin, pmol/L	18.9 ± 1.3 pmol/L	3.3 ± 1.5 pmol/L, *P* = .07
Older	21.6 ± 2.0 pmol/L	5.4 ± 2.2 pmol/L, *P* = .05
Young	16.2 ± 1.8 pmol/L	1.2 ± 2.0 pmol/L, *P* = .58

Abbreviations: CTX, C-telopeptide of type I collagen; P1NP, N-terminal propeptide of type I procollagen; SEE, standard error of the estimate. Bold indicated statistically significant changes.

mL from baseline ($P = .04$). The declines in bone formation markers occurred despite no change (group) or an increase (young women) in the bone resorption marker CTX (see Table 1, Fig. 1). In young women, the relatively greater decline in P1NP was accompanied by a 0.182 ± 0.069 ng/mL increase in CTX from baseline ($P = .04$). Sclerostin increased after SRCD, particularly in older women, but was not statistically significant (see Table 1).

Actigraphically assessed wrist activity increased 63.5% ± 29.6% from baseline to FD ($P < .01$). Young women had slightly greater percentage increases in wrist activity (young women 71.6% ± 30.3% vs older women 53.4% ± 25.2%). However, absolute activity counts were approximately 35% higher at both time points in older women. There was no correlation between changes in P1NP and actigraphically assessed wrist activity ($r = 0.28$, $P = .46$). The women had a statistically nonsignificant 0.88 ± 1.12 kg decline in weight from baseline to the end of FD ($P = .06$). Change in P1NP significantly and inversely correlated with change in weight ($r = –0.72$, $P = .03$). The P1NP changes reported in the preceding paragraph were unaffected by adjusting for change in weight. Change in weight was not correlated with change in CTX ($r = –0.28$, $P = .46$). There was no significant change in morning fasted cortisol or insulin from the first 24-hour collection at baseline to the second 24-hour collection at the end of FD in these 9 women (Δcortisol: 0.25 ± 4.43 mg/dL; $P = .88$; Δinsulin: –0.43 ± 2.18 μIU/mL; $P = .59$).

Discussion

Approximately 3 weeks of cumulative cumulative sleep restriction with circadian disruption, in the absence of acute circadian misalignment, significantly decreased markers of bone formation (P1NP ~17%, osteocalcin ~10%) in this relatively small study of healthy women, despite an increase (young women) or no change (older women) in a bone resorption marker (CTX). During midadult life, bone formation and resorption are typically coupled, increasing or decreasing together to maintain bone health over time. However, the pattern of acute BTM uncoupling observed in the present study, if sustained, could limit attainment of optimal peak bone mass in young individuals and/or lead to accelerated age-related bone loss in older individuals. We hypothesize that these changes could over time lead to an increased risk for osteoporosis and fracture. The present study did not include any long-term bone health measures such as bone mineral density (BMD) or fracture. However, the BTM changes observed in this pilot study 1) closely parallel those observed in sleep-restricted animals that did translate into lower BMD (10, 11), and 2) could underlie and help explain the increased fracture risk in night shift workers reported in the Nurses' Health Study (1).

The detrimental BTM changes in response to SRCD were of greater magnitude in the young, premenopausal women. The young women appeared to have a "double hit" with a greater (~23%) *decline* in P1NP (ie, less bone formation) and an approximately 33% *increase* in CTX (ie, more bone resorption). This pattern of unfavorable BTM changes in women exposed to SRCD was similar to that observed in men, in whom there were relatively greater declines in P1NP in young individuals with no change in CTX (5). Taken together, these data may indicate that younger age, not higher BTM levels at baseline, may confer greater risk for impaired bone formation with sleep and circadian disruptions. Given the critical window of bone mass accrual in early adulthood, we hypothesize that sleep and circadian disruption during this time could limit attainment of optimal peak bone mass if the unfavorable, clinically significant (13) changes in bone metabolism are sustained. Additional studies are needed to determine whether it is age or perhaps other hormonal or environmental conditions present in young age that diminish or change with aging (eg, sex hormone levels, circadian rhythm robustness, inflammation) that predispose to greater BTM changes in response to sleep restriction and circadian disruption. Average P1NP levels after SRCD in men (5) and women were reduced to similar levels (~40 ng/mL). It is possible that the impairment in bone formation observed with SRCD reaches a certain basement level, beyond which further decline does not occur.

The patterns of BTM change observed in women (and previously in men [5]) closely paralleled previous circadian disruption and chronic sleep-restriction studies in animals (10, 11, 14). Longer exposure to sleep restriction or constant light exposure in those studies demonstrated that the unfavorable BTM responses preceded the development of lower BMD and poorer bone microarchitecture (10, 11). Interestingly, when Lucassen et al reversed the constant light exposure and resumed normal light/dark cycles, the deficits in trabecular bone microarchitecture reversed (14). Sleep and circadian intervention studies that examine bone outcomes in humans are limited but have similar results to the present study. First, the magnitude of CTX increase observed in the present study is similar to that observed in male soldiers who habitually slept 7 to 9 hours per night exposed to sleep restriction (2 hours/night) for 3 nights (15). Those men, who were similar in age (average

21.5 ± 1.5 years) to the young women in the present study, also had a decrease in the bone formation marker bone specific alkaline phosphatase after 24 hours (15). Similar declines in bone formation markers with an increase in the bone resorption marker tartrate-resistant acid phosphatase (but not CTX) were also observed by Hughes and colleagues in 22 men after 8 weeks of US Army Ranger Training that included a 4-hours-per-night sleep restriction combined with energy restriction (16). The Hughes study suggested that some BTMs take 2 to 6 weeks to recover after resolution of multistressor military training that included sleep restriction (16). The ability of BTMs to recover with resumption of normal sleep/wake cycles outside the other stresses inherent in military training and the time course of that recovery in humans are currently unknown and require additional research.

The mechanisms by which sleep and circadian disruptions unfavorably alter bone metabolism are unknown. Sclerostin increases in response to bone unloading and inhibits bone formation. It is possible that sclerostin mediates the observed changes in BTMs in response to sleep/circadian disruption because of decreases in activity levels and skeletal loading compared to free-living conditions. Similar to our previous findings in men (5), the effect of SRCD on sclerostin levels in women differed by age. However, in the prior analysis, *young* men had higher sclerostin after SRCD with no change in sclerostin levels in older men. In women, sclerostin was higher after SRCD in *older* women, although this was of borderline statistical significance. If sclerostin mediated the observed changes in bone formation markers, we would expect the magnitude of sclerostin increase to inversely correlate with declines in bone formation markers. In this study, bone formation marker declines were greatest in young women who reported less physical activity prior to study entry, and had lower wrist activity counts at baseline and FD than older women. Further, actigraphically assessed wrist activity increased from baseline to after intervention, suggesting sclerostin is not the main mediator of the observed changes. However, some data suggest that serum sclerostin levels do not correlate with levels in the bone microenvironment (17, 18). Therefore, the role of sclerostin in mediating changes in bone metabolism in response to SRCD remains unclear.

Bone loss is often observed with moderate to large amounts of weight loss, particularly in older adults (19). Bone loss is often reflected by *increases* both in resorption and, to a lesser extent, formation. Therefore, it was notable that P1NP *decreased* in the present study in which women lost a very small amount of weight. It is unclear how the small amount of weight loss in the present study (–1.4%) would mediate changes in P1NP unless it was a finding due to chance or reflects underlying changes in nutritional status, metabolism, or sympathetic nervous system activation. In fact, P1NP results were unchanged when adjusted for change in weight. Other potential mechanisms that could be investigated in future studies include inducement of a senescent cell population in bone and alterations in cortisol (although not captured in the present analysis given that we examined only fasted morning cortisol). Furthermore, given the possible link between osteocalcin and glucose metabolism (20), it is possible that the decline in osteocalcin in response to SRCD contributes to impaired glucose metabolism observed with similar interventions (21).

These data were obtained from a highly controlled inpatient study of healthy individuals. However, the study had some limitations. First, we reported data obtained from 9 women. Sample size is often limited in intensive, rigorous sleep studies of this kind, but it limits our ability to draw firm conclusions based on sex and/or age differences and therefore we regard these results as preliminary data. Despite this, the magnitude and direction of our results were fairly consistent across the present and prior studies (5) for sex and age, bolstering confidence in the reproducibility of our findings. Second, data collection did not account for menstrual cycle phase in premenopausal women or variability in estradiol levels. Similar bone formation marker declines in postmenopausal women (and previously in men [5]) make it unlikely that the observed changes are due to potential differences in menstrual phase between the two 24-hour collections. Furthermore, data suggest that menstrual phase has no impact on the 24-hour levels or peak timing of urinary melatonin or bone resorption (as evidenced by urinary N-telopeptide of type I collagen) (22). However, additional studies are needed to understand what role menstrual phase had in the observed increase in CTX in premenopausal women. Third, environmental differences compared to free-living conditions (eg, diet, activity) and heparinized intravenous tubing used for overnight blood draws may have contributed to the observed changes. The controlled meal composition/timing and increase in actigraphically assessed wrist activity from baseline to FD diminished this concern. Moreover, because heparin was used during both collections it is unlikely to explain the changes seen from baseline to postintervention. Fourth, the highly controlled protocol that prohibited caffeine use and included a consistent calorie intake may not reflect habitual dietary patterns or typical adjustments to sleep restriction or rotating shift work. For example, other studies have shown increased food intake, particularly carbohydrates, with sleep restriction when food is provided ad libitum (23-25). Therefore, extrapolations

to real-world sleep/circadian disruption may be limited. However, this also means that the observed changes were not due to increased caffeine intake with sleep restriction, which can also negatively affect bone in large quantities (26). Finally, the study lacked a control group exposed to the same inpatient environment but without SRCD. However, we did use within-subject comparisons from 2 well-matched 24-hour profiles that showed a consistent magnitude of P1NP decline and, for young women, CTX increase across all time points.

In conclusion, these preliminary data from a small group of women indicate that cumulative sleep restriction with a history of recurrent circadian disruption uncouples BTMs, with significant declines in markers of bone formation (P1NP, osteocalcin), despite an increase (young women) or no change (older women) in a marker of bone resorption (CTX). Young, premenopausal women may be most vulnerable to the adverse skeletal effects of sleep and circadian disruption, which may limit attainment of optimal peak bone mass and increase future fracture risk.

Acknowledgments

Financial Support: This work was supported by National Institutes of Health (NIH) awards K23 AR070275 (to C.M.S.), UL1 TR000128, UL1 TR002535, and a grant from the Center for Women's Health Research located at the University of Colorado Anschutz Medical Campus. Data collection was supported by grants P01 AG009975, K24 HL76446, and the National Space Biomedical Research Institute through the National Aeronautics and Space Administration NCC 9-58 (HFP01601), and was conducted in the Brigham and Women's Hospital's General Clinical Research Center supported by M01 RR02635, and the Center for Clinical Investigation of the Harvard Clinical and Translational Science Center UL1 RR025758-01. S.A.S. received support from NIH grants R01 HL142064, R01 HL125893, HL125893-03A1, and R01 HL140577, Department of Defense (DoD) grant PT150133 (to L. Hammer) and Centers for Disease Control and Prevention (CDC) grant U19 OH010154 (to W.K. Anger) and The Oregon Institute of Occupational Health Sciences at Oregon Health & Science University via funds from the Division of Consumer and Business Services of the State of Oregon. N.V. was supported by the following NIH grants: F32AG051325, R01DK099512, R01HL118601, and R01DK105072. O.M.B. was supported in part by the National Institute on Aging (NIA) (P01 AG009975). E.S.O. as overall principal investigator for the Osteoporotic Fractures in Men Study is supported by NIH funding via the following institutes: the National Institute on Aging, the National Institute of Arthritis and Musculoskeletal and Skin Diseases, the National Center for Advancing Translational Sciences, and NIH Roadmap for Medical Research, under the following grant numbers: U01AG027810, U01AG042124, U01AG042139, U01AG042140, U01 AG042143, U01 AG042145, U01 AG042168, and U01 AR066160. K.P.W. received support from NIH grants R01HL132150, R01HL131458, U01HL111478, R01DK115502, R01DK114272, R01HD087707, R01HL135598, and from a Pac-12 Grant Application and N00014-15-1-2809, Office of Naval Research during the time of this research. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Clinical Trial Information: ClinicalTrials.gov no. NCT00506428 (registered April 27, 2007).

Author Contributions: Study concept and design: C.M.S., S.A.S., E.S.O., O.M.B.; data collection and study performance: S.W.C., M.M., N.V.; data analysis: C.M.S.; data interpretation: C.M.S., S.A.S., W.M.K., K.P.W., E.S.O., O.M.B.; drafting of manuscript: C.M.S.; manuscript revisions and approval of final manuscript: C.M.S., S.A.S., W.M.K., K.P.W., S.W.C., M.M., N.V., C.A.C., E.S.O., O.M.B.; responsibility for integrity of data analysis: C.M.S.

Additional Information

Correspondence and Reprint Requests: Christine M. Swanson, MD, Division of Endocrinology, Metabolism and Diabetes, University of Colorado Anschutz Medical Campus, 12801 E. 17th Ave., Mail Stop 8106, Aurora, CO 80045. E-mail: Christine.Swanson@CUAnschutz.edu.

Disclosure Summary: C.M.S., W.M.K., S.W.C., M.M., and N.V. have nothing to disclose. In the interest of full disclosure, we report the following; however, we do not believe any of these pertain to the current work. S.A.S. has received support from The Oregon Institute of Occupational Health Sciences at Oregon Health & Science University via funds from the Division of Consumer and Business Services of the State of Oregon, and NIH grants R01 HL142064, R01 HL125893, HL125893-03A1, and R01 HL140577, DoD grant PT150133 (to L. Hammer) and CDC grant U19 OH010154 (to W.K. Anger). K.P.W. reports research support from the NIH, Office of Naval Research, Pac-12, Philips Inc, CurAegis Technologies (formerly known as Torvec Inc), and Somalogics; financial relationships, consulting fees from, or served as a paid member of scientific advisory boards for the NIH (Sleep Disorders Research Advisory Board–National Heart, Lung and Blood Institute), CurAegis Technologies, Circadian Therapeutics, LTD, Kellogg Company; board of directors: Sleep Research Society; speaker/educational honorarium fees: American Academy of Sleep Medicine, American College of Chest Physicians, American Diabetes Association. C.A.C. is or was a paid consultant to Bose, Boston Celtics, Boston Red Sox, Cephalon, Institute of Digital Media and Child Development, Jazz Pharma, Merck, Purdue Pharma, Samsung, Teva, and Vanda Pharmaceuticals, in which he also holds an equity interest. He also receives or has received research/education support from Cephalon, Mary Ann & Stanley Snider via Combined Jewish Philanthropies, NFL Charities, Jazz Pharma, Optum, ResMed, San Francisco Bar Pilots, Sanofi, Schneider, Simmons, Sysco, Philips, and Vanda Pharmaceuticals. In addition, since 1985

he has served as an expert witness in a number of legal cases, including those involving Amtrak, Bombardier, C&J Energy, Casper Sleep, Inc, Columbia River Bar Pilots, Dallas Police Association, Enterprise Rent-A-Car, FedEx, Greyhound, Purdue Pharma, UPS, and Vanda Pharmaceuticals. He currently serves as the incumbent of a professorship endowed by Cephalon and receives royalties from McGraw-Hill and from Philips Respironics (for the Actiwatch-2 and Actiwatch Spectrum devices). His interests have been reviewed and are managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies. E.S.O. has received research support from or consulting for Amgen, Mereo, and Bayer. O.M.B. reports research support from the NIH, the National Science Foundation (NSF), CDC/National Institute for Occupational Safety and Health, the Air Force Office of Scientific Research, Academy of Architecture for Health, the Facilities Guidelines Institute, and The Center for Health Design, and the Robert Wood Johnson Foundation; outside the submitted work, prior investigator-initiated research grant support from Sepracor (now Sunovion) and Cephalon (now Teva), and received 2 subcontract grants to Pennsylvania State University from Mobile Sleep Technologies /Proactive Life (National Science Foundation/ Small Business Technology Transfer Program number 1622766, NIH/NIA Small Business Innovation Research R43AG056250); previously served as consultant to Takeda Pharmaceuticals North America (speakers bureau), Dinsmore LLC (expert witness testimony), Matsutani America (scientific advisory board), Wake Forest University (consultant), and Chevron and Allstate (speaking fees); and received speaking fees/travel support from Tufts School of Dental Medicine, Oklahoma State University, Boston University, Boston College, and the Harvard T.H. Chan School of Public Health, and honoraria for his role as editor in chief of *Sleep Health* (sleephealthjournal.org).

Data Availability: The data sets generated during and/or analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Feskanich D, Hankinson SE, Schernhammer ES. Nightshift work and fracture risk: the Nurses' Health Study. *Osteoporos Int*. 2009;**20**(4):537-542.
2. Swanson C, Shea SA, Wolfe P, et al. 24-hour profile of serum sclerostin and its association with bone biomarkers in men. *Osteoporos Int*. 2017;**28**(11):3205-3213.
3. Qvist P, Christgau S, Pedersen BJ, Schlemmer A, Christiansen C. Circadian variation in the serum concentration of C-terminal telopeptide of type I collagen (serum CTx): effects of gender, age, menopausal status, posture, daylight, serum cortisol, and fasting. *Bone*. 2002;**31**(1):57-61.
4. Redmond J, Fulford AJ, Jarjou L, Zhou B, Prentice A, Schoenmakers I. Diurnal rhythms of bone turnover markers in three ethnic groups. *J Clin Endocrinol. Metab*. 2016;**101**(8):3222-3230.
5. Swanson CM, Shea SA, Wolfe P, et al. Bone turnover markers after sleep restriction and circadian disruption: a mechanism for sleep-related bone loss in humans. *J Clin Endocrinol Metab*. 2017;**102**(10):3722-3730.
6. Swanson CM, Kohrt WM, Wolfe P, et al. Rapid suppression of bone formation marker in response to sleep restriction and circadian disruption in men. *Osteoporos Int*. 2019;**30**(12):2485-2493.
7. Buxton OM, Cain SW, O'Connor SP, et al. Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci Transl Med*. 2012;**4**(129):129ra43.
8. Czeisler CA, Buxton OM. Human circadian timing system and sleep-wake regulation. In: Kryger M, Roth T, Dement WC, eds. *Principles and Practice of Sleep Medicine Sixth Edition. 6th ed*. Philadelphia, PA: Elsevier; 2015:362-376.
9. Shanahan TL, Czeisler CA. Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *J Clin Endocrinol Metab*. 1991;**73**(2):227-235.
10. Everson CA, Folley AE, Toth JM. Chronically inadequate sleep results in abnormal bone formation and abnormal bone marrow in rats. *Exp Biol Med (Maywood)*. 2012;**237**(9):1101-1109.
11. Xu X, Wang L, Chen L, et al. Effects of chronic sleep deprivation on bone mass and bone metabolism in rats. *J Orthop Surg Res*. 2016;**11**(1):87.
12. Cornelissen G. Cosinor-based rhythmometry. *Theor Biol Med Model*. 2014;**11**:16.
13. Glover SJ, Eastell R, McCloskey EV, et al. Rapid and robust response of biochemical markers of bone formation to teriparatide therapy. *Bone*. 2009;**45**(6):1053-1058.
14. Lucassen EA, Coomans CP, van Putten M, et al. Environmental 24-hr cycles are essential for health. *Curr Biol*. 2016;**26**(14):1843-1853.
15. Staab JS, Smith TJ, Wilson M, Montain SJ, Gaffney-Stomberg E. Bone turnover is altered during 72 h of sleep restriction: a controlled laboratory study. *Endocrine*. 2019;**65**(1):192-199.
16. Hughes JM, Smith MA, Henning PC, et al. Bone formation is suppressed with multi-stressor military training. *Eur J Appl Physiol*. 2014;**114**(11):2251-2259.
17. Clarke BL, Drake MT. Clinical utility of serum sclerostin measurements. *Bonekey Rep*. 2013;**2**:361.
18. Roforth MM, Fujita K, McGregor UI, et al. Effects of age on bone mRNA levels of sclerostin and other genes relevant to bone metabolism in humans. *Bone*. 2014;**59**:1-6.
19. Ensrud KE, Ewing SK, Stone KL, Cauley JA, Bowman PJ, Cummings SR; Study of Osteoporotic Fractures Research Group. Intentional and unintentional weight loss increase bone loss and hip fracture risk in older women. *J Am Geriatr Soc*. 2003;**51**(12):1740-1747.
20. Liu DM, Mosialou I, Liu JM. Bone: another potential target to treat, prevent and predict diabetes. *Diabetes Obes Metab*. 2018;**20**(8):1817-1828.
21. Arble DM, Bass J, Behn CD, et al. Impact of sleep and circadian disruption on energy balance and diabetes: a summary of workshop discussions. *Sleep*. 2015;**38**(12):1849-1860.
22. St Hilaire MA, Rahman SA, Gooley JJ, Witt-Enderby PA, Lockley SW. Relationship between melatonin and bone resorption rhythms in premenopausal women. *J Bone Miner Metab*. 2019;**37**(1):60-71.
23. Brondel L, Romer MA, Nougues PM, Touyarou P, Davenne D. Acute partial sleep deprivation increases food intake in healthy men. *Am J Clin Nutr*. 2010;**91**(6):1550-1559.
24. Markwald RR, Melanson EL, Smith MR, et al. Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. *Proc Natl Acad Sci U S A*. 2013;**110**(14):5695-5700.
25. Broussard JL, Kilkus JM, Delebecque F, et al. Elevated ghrelin predicts food intake during experimental sleep restriction. *Obesity (Silver Spring)*. 2016;**24**(1):132-138.
26. Hallström H, Wolk A, Glynn A, Michaëlsson K. Coffee, tea and caffeine consumption in relation to osteoporotic fracture risk in a cohort of Swedish women. *Osteoporos Int*. 2006;**17**(7):1055-1064.