Bone Turnover Markers After Sleep Restriction and Circadian Disruption: A Mechanism for Sleep-Related Bone Loss in Humans

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Context: Sleep abnormalities are associated with low bone mineral density. Underlying mechanisms are unknown.

Objective: Investigate the impact of sleep restriction with circadian disruption on bone biomarkers.

Design: Intervention study.

Participants and Methods: Four bone biomarkers [C-terminal cross-linked telopeptide of type I collagen (CTX) = bone resorption, *N*-terminal propeptide of type I procollagen (P1NP) = bone formation, sclerostin and fibroblast growth factor 23 = osteocyte function] were measured in bihourly serum samples over 24 hours at baseline and after ~3 weeks of sleep restriction (5.6 hours sleep/24 hours) with concurrent circadian disruption (recurring 28-hour "day" in dim light) in 10 men (age groups: 20 to 27 years, n = 6; 55 to 65 years, n = 4). The effects of sleep/circadian disruption and age on bone biomarker levels were evaluated using maximum likelihood estimation in a mixed model for repeated measures.

Results: P1NP levels were lower after intervention compared with baseline ($P < 0.001$); the decrease in P1NP was greater for younger compared with older men (28.0% vs 18.2%, $P < 0.001$). There was no change in CTX ($\Delta = 0.03 \pm 0.02$ ng/mL, $P = 0.10$). Sclerostin levels were higher postintervention in the younger men only (Δ = 22.9% or 5.64 ± 1.10 pmol/L, $P < 0.001$).

Conclusions: These data suggest that 3 weeks of circadian disruption with concurrent sleep restriction can lead to an uncoupling of bone turnover wherein bone formation is decreased but bone resorption is unchanged. Circadian disruption and sleep restriction may be most detrimental to bone in early adulthood. (***J Clin Endocrinol Metab*** **102: 3722–3730, 2017)**

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Abbreviations: BMD, bone mineral density; CTX, C-terminal cross-linked telopeptide of type I collagen; CV, coefficient of variation; FGF-23, fibroblast growth factor 23; P1NP, *N*-terminal propeptide of type I collagen.

Growing evidence suggests a link between sleep duration and bone metabolism (1), but the mechanisms underlying this relationship are poorly understood. Previous studies have shown that both short (2, 3) and long (4–7) sleep duration are associated with low bone mineral density (BMD) (8, 9). Sleep disturbance has been hypothesized to be a risk factor for fracture, in part, due to medication use (10), daytime sleepiness, and falls (11). In addition, rotating shift work with inherent sleep loss and circadian disruption (*i.e.*, a mismatch between the timing of the internal clock and daily behaviors, such as the sleep/wake cycle, fasting/feeding cycle, and light/dark cycle) has been linked to higher fracture risk (12). Evidence for a circadian rhythm in bone biomarkers, most notably serum C-terminal cross-linked telopeptide of type I collagen (CTX) (13), suggests that sleep is essential for normal bone function and that sleep or circadian disturbance could also directly affect bone physiology and metabolism. A better understanding of the sleep/circadian and bone relationship could alter how practitioners evaluate secondary causes of osteoporosis and could lead to additional treatment options for low bone mass.

In this study, we investigated the effects of a 3-week intervention akin to rotating shift work, including circadian disruption combined with cumulative sleep restriction common to most real-life circumstances with circadian disruption, on bone biomarkers to better understand how disturbances in sleep duration and circadian alignment alter bone metabolism. We hypothesized that circadian disruption with sleep restriction would negatively alter bone turnover.

Methods

Study design and participants

This intervention study used serum samples from a previously performed clinical research study (14). We recruited 11 healthy adult men, aged 20 to 65 years. To ensure circadian stability at the outset, participants reported no regular night shift work within 3 years and no travel across more than two time zones within 3 months (14). There were no specific exclusion criteria related to typical physical activity prior to study enrollment. Participants were admitted to individual suites in the Intensive Physiological Monitoring Unit of the Center for Clinical Investigation at Brigham and Women's Hospital between 2007 and 2010. While admitted to the inpatient unit, participants could ambulate but exercise was not permitted. To avoid resetting the circadian clock, the study was performed in dim light (<0.02 lux during sleep opportunity; <15 lux during wake). Participants received a eucaloric, controlled nutrient diet (55% to 60% carbohydrate, 15% to 20% protein, and 15% to 30% fat) and ≥2.5 L of fluid per 24 hours.

To ensure that participants had no sleep debt prior to initiation of the protocol [see Fig. 1 study protocol, adapted from Buxton et al. (14)], participants self-selected and maintained a continuous 10-hour sleep opportunity per night for at least 3 weeks prior to admission, verified by sleep diaries, time stamped call-ins, and wrist actigraphy. To further ensure participants were "sleep satiated," there was at least a 10-hour sleep opportunity per day for the first five inpatient nights. The subsequent protocol was a "forced desynchrony" in which the sleep-wake cycle is desynchronized from the internal circadian cycle (15). This was achieved by scheduling recurring 28-hour sleep-wake cycles with a 21.47-hour wake episode and a 6.53-hour sleep opportunity (equivalent to 5.6 hours of sleep opportunity per 24 hours) over an approximately 3-week interval (14). As a result, participants experienced circadian disruption (*i.e.*, a mismatch between the internal clock and external environment, similar to that seen during jet lag or shift work) with concurrent cumulative sleep restriction, which is common to most real-life circumstances with circadian disruption (*i.e.*, rotating shift work). Because individual bedtimes varied, laboratory sleep opportunities and sampling were performed relative to the midpoint of each individual's habitual sleep opportunity to align his data relative to sleep/wake and light/dark cycles at baseline and after circadian disruption with sleep restriction. Core body temperature minimum (used to estimate an individual's circadian phase) varied minimally across individuals at baseline (greatest difference = 40 minutes), and therefore no further alignment was deemed necessary.

Serum for this study was obtained hourly over a 24-hour sampling interval on days 5 to 6 (when sleep satiated and individuals had stable circadian alignment; Fig. 1), referred to as

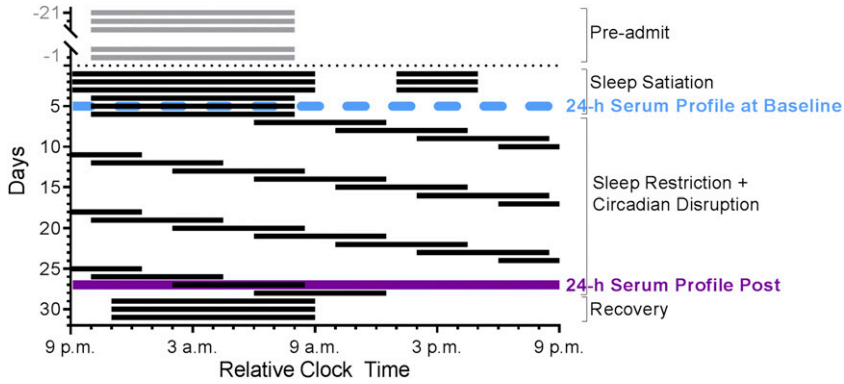

Figure 1. Study protocol. Gray and black horizontal bars represent sleep opportunities. Participants were admitted (horizontal dotted line) following a ≥21-day at-home preadmittance phase of a 10-hour sleep opportunity per night (gray horizontal bars). Initial in-laboratory sleep opportunities ("Sleep Satiation") were 16 h/d (12 h/night with 4-hour nap midday) followed by 10 h/d to ensure participants had no sleep debt at the time of baseline 24-hour sampling (blue dotted horizontal bar). Forced desynchrony protocol ("Sleep Restriction + Circadian Disruption") was then initiated in which participants lived on a 28-hour day for approximately 3 weeks with a short sleep opportunity (5.6-hour sleep opportunity per 24-hour). This produced circadian disruption and cumulative sleep restriction. The second 24-hour sampling profile (solid purple horizontal bar) performed after the forced desynchrony protocol was carefully selected to ensure participants were at the same circadian phase for both 24-hour profiles. Several days of "Recovery" sleep (10 h/night represented by black horizontal bars) were subsequently allowed. All clock times are relative to participants' biological clock time. Figure adapted from Buxton *et al.* (14).

baseline, and again at the end of the forced desynchrony protocol, referred to as postintervention. Postintervention samples were obtained when participants were at a similar circadian phase compared with their baseline sample collection (as assessed by core body temperature minimum) (14). 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. Meal timing was controlled throughout the protocol relative to wake time, such that meals occurred at approximately the same times in both 24-hour sampling intervals (at hours 12, 17, 22, and 24, as depicted in Fig. 2).

Ten men with available serum were used for the current study (one man was excluded due to an incomplete 24-hour sample set). Of the 10 men included in this study, 7 self-reported their race/ethnicity as white/not Hispanic or Latino, 2 reported white/Hispanic, and 1 reported Asian and white/not Hispanic or Latino. Assays were performed on samples from every other hour for each participant. If a sample from the preferred hour was missing, a sample from the adjacent hour was used, which was necessary approximately 15% of the time. When available, each individual's actigraphically assessed wrist activity (Actiwatch_L; Mini Mitter, Bend, OR) in arbitrary counts/minute was averaged for each condition (*i.e.*, over all baseline days and over all forced desynchrony days). Percent change in wrist activity from baseline to postintervention was calculated for each individual who had data from both conditions (data were missing for two participants at baseline due to technical device issues). Average and standard deviation percent change in actigraphically assessed wrist activity were then calculated for the group (n = 8). Based on the screening process, all 10 men were healthy and reported performing some habitual exercise prior to admission, including walking, cardio (such as jogging, swimming), and resistance exercise, but none were competitive athletes.

All participants provided written informed consent (14). All procedures were approved by the Partners Human Research Committee and were conducted in accordance with the Declaration of Helsinki. The current analyses, performed at Oregon Health & Science University (OHSU) using de-identified samples, were deemed nonhuman subjects research by OHSU institutional review board (00010357), and study procedures were approved by Brigham and Women's Hospital institutional review board.

Assays

The Oregon Clinical and Translational Research Institute laboratory at OHSU performed all assays. To minimize interassay variability, each individual's samples were run in the same assay. As previously reported (16), serum sclerostin levels were measured on previously unthawed samples stored below −70°C until assay by enzyme-linked immunosorbent assay (ALPCO, Salem, NH). Duplicates of each sample were assessed, and values were averaged to obtain a final result. Interassay coefficient of variation (CV) was 7.5% at 60.9 pmol/L. Fibroblast growth factor 23 (FGF-23) was measured immunometrically (Kainos Laboratory, Tokyo, Japan) in serum that had undergone one freeze/thaw cycle. FGF-23 samples are stable up to 3 years at −80°C and up to six freeze/thaw cycles (17). Interassay CV was 4.9% at 48.4 pg/mL. The intact trimer of *N*-terminal propeptide of type I procollagen (P1NP) was measured by immunoassay (Orion Diagnostica, Espoo, Finland) in duplicate on serum that had undergone one freeze/thaw cycle. P1NP is stable for four or more freeze/thaw cycles (18). Duplicate values were averaged to obtain a final result. Interassay CV was 2.1% at 38.3 mcg/L and 3.0% at 118 mcg/L. CTX was measured by enzyme-linked immunosorbent assay (Immunodiagnostic Systems, United Kingdom) on serum that had undergone a single freeze/thaw cycle. CTX has been shown to be stable up to nine freeze/thaw cycles (19). Interassay CV was 15.7% at 0.18 ng/mL and 12.7% at 0.87 ng/mL.

Statistical analysis

The predetermined sample size (N = 10) had 80% power to detect a mean paired difference of 1.3 standard deviation in CTX and P1NP from baseline to postintervention at an α level of 0.0125. This was conservatively estimated using a two-sided paired t test to approximate the comparison of baseline and postintervention samples (assuming correlation between repeated measures on the same individuals to be at least 0.5). The level of significance was chosen to adjust for multiple comparisons using the Bonferroni correction (20).

Maximum likelihood estimates for a mixed model with repeated measures were obtained to assess the effects of the protocol and age on bone biomarker levels across a 24-hour interval using the following cosinor model:

$$Y = M + \beta_1 c24 + \gamma_1 s24 + \beta_2 c12 + \gamma_2 s12 + AGE + Treatment,$$

where

$$c24 = \cos((h*\mathbf{2}*\boldsymbol{\pi})/\mathbf{24})\text{and}$$
$$s24 = \sin((h*\mathbf{2}*\boldsymbol{\pi})/\mathbf{24})\text{estimate the first harmonic,}$$
$$c12 = \cos((h*\mathbf{2}*\boldsymbol{\pi})/\mathbf{12})\text{and}$$
$$s12 = \sin((h*\mathbf{2}*\boldsymbol{\pi})/\mathbf{12})\text{estimate the second harmonic,}$$

where h is the hour, AGE is an indicator variable for older vs younger men, M is the mesor (midline statistic of rhythm), and β and γ are the parameters that estimate the amplitude $[(\beta^2 + \gamma^2)^{1/2}]$ and acrophase $[\arctan(\gamma/\beta)]$ for each harmonic; treatment indicates study condition (baseline vs postintervention); subject was specified as a random effect. We also tested for an interaction between age and treatment. The second (12-hour) harmonic was statistically significant for CTX only and was dropped from the other models.

Trapezoidal area under the curve was computed for each biomarker by participant and treatment condition. Spearman correlation coefficients were used to test the association between area under the curve values for each biomarker at baseline and postintervention.

All analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC). All figures were generated using GraphPad Prism 7.02 (GraphPad Software, La Jolla, CA).

Results

The 10 participants included 6 younger (20 to 27 years, mean 23.5 years) and 4 older (55 to 65 years, mean 58.75 years) healthy men. All were free of sleep disorders as verified by questionnaires and polysomnography (14). Core body temperature minimum (a robust measure of circadian phase) indicated excellent alignment of

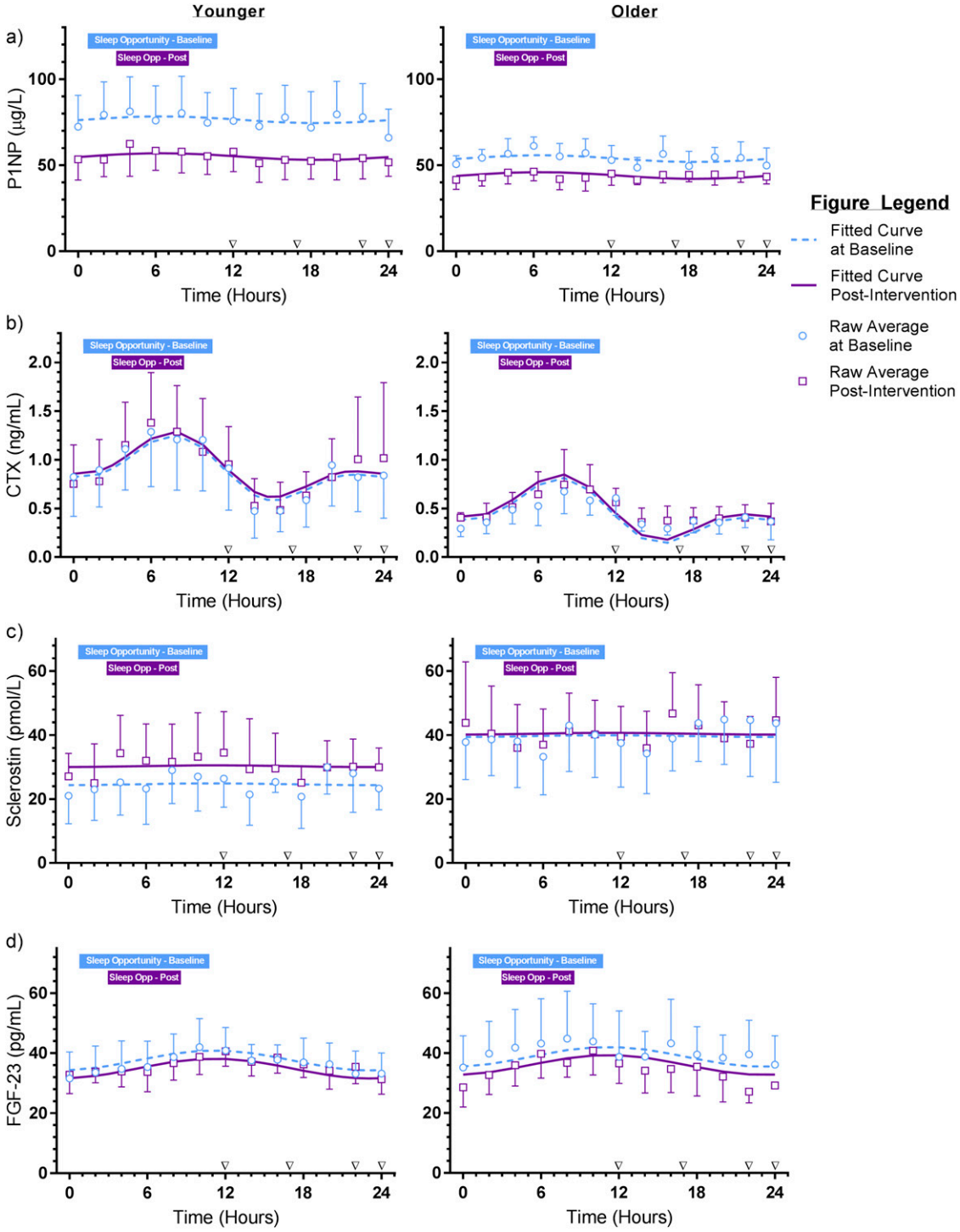

Figure 2. The 24-hour serum profiles of bone biomarkers at baseline and postintervention (by age group). The 24-hour fitted profiles from baseline (dotted light blue) and after circadian disruption with sleep restriction (solid purple) presented for (a) P1NP, (b) CTX, (c) sclerostin, and (d) FGF-23. Simple averages of data from baseline (light blue open circles) and after circadian disruption + sleep restriction (purple open squares) are shown every 2 hours with error bars reflecting standard deviation. Sleep opportunity is presented as a solid horizontal bar at the top of each graph (light blue = baseline—10 hours; purple = postintervention—approximately 5.6 hours). Mealtimes for both 24-hour sampling intervals are represented along the x-axis by upside-down triangles (hour 12 = breakfast, hour 17 = lunch, hour 22 = dinner, hour 24 = snack). Data are presented by age group with younger men (age 20 to 27 years) displayed on the left and older men (age 55 to 65 years) displayed on the right. The x-axis represents number of hours into the 24-hour sampling profile, as two-digit military time. The two 24-hour serum profiles (baseline and after circadian disruption with sleep restriction) were carefully selected so each participant was at the same circadian phase for both. (a) Levels of the bone formation marker, P1NP, were statistically significantly lower after sleep restriction with a history of circadian disruption compared with baseline. The magnitude of these changes was statistically significantly larger for the younger men (−28.0% or −21.43 ± 1.05 mcg/L, $P <$ 0.001), with higher levels of bone turnover at baseline, compared with older men (−18.2% or −9.80 ± 1.32 mcg/L, $P < 0.001$). (b) Levels of the bone resorption marker, CTX, were higher for younger compared with older men but did not change with the imposed circadian disruption

Table 1. Effect of Circadian Disruption With Concurrent Sleep Restriction on Biomarkers of Bone Metabolism

Characteristic	Intercept at Baseline for Group, Older, or Younger, Estimate ± SEE	Effect of Sleep Intervention on Group, Older, or Younger, Estimate ± SEE (*P* Value)
P1NP, mcg/L	55.04 ± 4.99	
Older	53.88 ± 7.89	−9.80 ± 1.32 (<0.001)
Younger	76.47 ± 1.04	−21.43 ± 1.05 (<0.001)
CTX, ng/mL	0.92 ± 0.13	0.03 ± 0.02 (0.10)
Older	0.45 ± 0.15	
Younger	0.89 ± 0.13	
Sclerostin, pmol/L	30.26 ± 3.49	
Older	39.68 ± 4.28	0.76 ± 1.33 (0.57)
Younger	24.62 ± 3.49	5.64 ± 1.10 (<0.001)
FGF-23, pg/mL	38.06 ± 2.04	−2.70 ± 0.74 (<0.001)
Older	38.76 ± 3.37	
Younger	37.59 ± 2.76	

N = 10.

Abbreviation: SEE, standard error of estimate.

specimen sampling times across all 10 individuals at baseline (all 10 men varied within 40 minutes; older men varied within 16 minutes). The second 24-hour sampling interval was carefully selected so that participants were at a similar circadian phase compared with their baseline sampling interval, and meal timing and composition were standardized throughout the protocol. Actigraphically assessed wrist activity increased 49.04% ± 28.41% from baseline to postintervention (n = 8). Table 1 includes all results of the effect of cumulative sleep restriction and a history of circadian disruption on markers of bone metabolism.

Except FGF-23, bone biomarker levels varied significantly by age at baseline (CTX and P1NP were higher in younger men (P = 0.01 and 0.03, respectively; Fig. 2); sclerostin levels were higher in older men [P = 0.005; Fig. 2(c)]. There was no difference in FGF-23 levels by age (P = 0.37); therefore, FGF-23 analyses were performed for the whole group (N = 10). P1NP levels were statistically significantly lower postintervention compared with baseline [Fig. 2(a)]; the decrease in P1NP occurred at every time point, and this decline was statistically significantly greater ($P < 0.001$) for the younger men with higher bone turnover at baseline (−28.0% or −21.43 ± 1.05 mcg/L, $P < 0.001$) compared with older men (−18.2% or −9.80 ± 1.32 mcg/L, $P < 0.001$). The intervention resulted in no change in CTX (Δ = 0.03 ± 0.02 ng/mL, P = 0.10 for both older and younger). Sclerostin levels were statistically significantly higher postintervention in the younger men (Δ = 22.9% or 5.64 ± 1.10 pmol/L, $P < 0.001$) but not in older men (Δ = 0.76 ± 1.33 pmol/L, P = 0.57). After the circadian disruption with sleep restriction, FGF-23 levels were 7.1% lower [Δ = −2.70 ± 0.74 pg/mL; $P < 0.001$; Fig. 2(d)].

CTX and P1NP were moderately correlated both before and after the intervention (r = 0.64, P = 0.05; Tables 2 and 3). No other statistically significant correlations were identified (Tables 2 and 3).

Discussion

To our knowledge, this is the first description of lower bone formation markers after sleep/circadian disturbance in humans. In healthy men, we found evidence of lower P1NP, a marker of bone formation, after approximately 3 weeks of circadian disruption and sleep restriction with no change in the bone resorption marker CTX, creating a potential "bone loss window." Furthermore, these data suggest that the changes may be magnified in early adult life during bone consolidation—a critical time for attainment of optimal peak bone mass. If sustained, these alterations in bone metabolism induced by sleep/circadian disruption could result in suboptimal peak bone mass, bone loss, osteoporosis, and fracture.

Our data are consistent with prior findings in rats that show chronically inadequate sleep impairs bone formation and subsequently reduced BMD (21, 22). The decrease in P1NP we noted with circadian disruption and sleep restriction is substantial. For instance, it is of similar magnitude to the expected increase in P1NP seen with responders to teriparatide treatment. An increase in

Figure 2. (Continued). and sleep restriction. (c) Sclerostin levels were statistically significantly higher after sleep restriction with a history of circadian disruption for younger men only (Δ = 22.9% or 5.64 ± 1.10 pmol/L, $P < 0.001$), who had lower levels at baseline compared with older men. (d) Levels of FGF-23 did not differ by age (P = 0.37) and were statistically significantly lower after sleep restriction with a history of circadian disruption (Δ = −7.1% or −2.70 ± 0.74 pg/mL; $P < 0.001$).

Table 2. Correlations Between Bone Biomarkers at Baseline Using Analyses of 24-Hour Integrated Areas Under the Curve

	CTX	P1NP	FGF-23	Sclerostin
CTX	1.0	0.64 (0.05)	−0.07 (0.85)	−0.16 (0.65)
P1NP		1.0	−0.04 (0.91)	−0.02 (0.96)
FGF-23			1.0	0.31 (0.38)

Data are presented as correlation coefficient, *r* (*P* value).

P1NP of at least 10 mcg/L (similar to the degree of change seen in this study) at 1 or 3 months after starting treatment is considered an indication of positive response to teriparatide (23, 24). Furthermore, change in P1NP with teriparatide treatment predicts positive changes in BMD and finite element analysis strength (23, 25, 26). Therefore, it is likely the decrease seen in P1NP during this study is meaningful and may be particularly detrimental if sustained. Conversely, the change in CTX was minimal and not statistically significantly different, suggesting that bone formation may be preferentially affected by these sleep and circadian disturbances.

We found that circadian disruption with sleep restriction was associated with a greater reduction in P1NP and increases in sclerostin in younger men. The younger men had higher levels of bone turnover markers at baseline, a finding consistent with higher bone (re)modeling rates during the peak bone mass consolidation that occurs at this age (27) and is critical for lifetime skeletal health. That circadian disruption and sleep restriction had a greater effect on P1NP in younger men suggests that the adverse effects of this type of sleep/circadian disturbance may be magnified during skeletally vulnerable times when bone turnover is higher, such as bone modeling/consolidation and possibly menopause. Future studies should focus on these processes in women.

Furthermore, these findings may have additional important implications for specific populations, such as military recruits and astronauts, who are subjected to sleep/circadian disruption in early adulthood during this critical window of bone development/consolidation. The increased incidence of stress fractures during basic training is likely multifactorial. However, these data may explain part of the pathophysiology underlying these injuries, and the incidence of stress fractures during basic training may be partially mitigated by sleep extension or at least minimizing the interval during which recruits are exposed to sleep/circadian disturbance (28, 29).

Table 3. Correlations Between Bone Biomarkers After Circadian Disruption With Sleep Restriction Using Analyses of 24-Hour Integrated Areas Under the Curve

	CTX	P1NP	FGF-23	Sclerostin
CTX	1.0	0.64 (0.05)	0.18 (0.63)	−0.16 (0.65)
P1NP		1.0	0.07 (0.85)	0.01 (0.99)
FGF-23			1.0	0.43 (0.21)

Data are presented as correlation coefficient, *r* (*P* value).

The mechanisms for impaired bone formation with sleep disturbance and circadian disruption need to be investigated. They may include direct effects on diurnal bone remodeling (either related to sleep restriction or prior circadian disruption) or indirect effects via changes in sex hormone status, inflammation, cortisol, insulin/glucose metabolism, and/or sympathetic tone induced by sleep disruption. Our participants were ambulatory, and thus major effects of bed rest are unlikely to have occurred; in fact, a bed rest phenotype was not seen in measures of glucose metabolism obtained from the same protocol, as previously published (14). However, postural changes and the decreased physical activity compared with free living inherent in our study conditions may have contributed to our findings. In our participants, there was some reduction in movement compared with free living, although actigraphically measured movement actually increased from baseline to postintervention, presumably because participants spent more time out of bed. Although it is feasible that a change in activity could have affected levels of biochemical markers of remodeling, it is unlikely that the modest change in activity would induce the reduction in bone formation marker that we observed. Most studies of complete bed rest have not observed reductions in markers of bone formation (30, 31). Moreover, such studies have consistently observed an increase in markers of resorption (32), which was not present in our evaluation of sleep/circadian disruption. Hence, we do not believe that a change in activity contributed substantially to our results. Sclerostin levels were higher after the sleep/circadian intervention in the younger men, perhaps suggesting a partial mechanism for the decrease in bone formation. Meal timing and composition were standardized throughout the protocol; therefore, it is unlikely that the changes seen were a result of food intake. Increased inflammation could be a potential mechanism linking sleep restriction and alterations in bone health. Levels of C-reactive protein are increased after 10 days of partial sleep deprivation (33) and higher inflammation has been linked to increased risk of fracture (34). The significance of the lower FGF-23 levels with circadian disruption and sleep restriction is unknown.

Although change in bone formation is classically thought to lag behind resorption, it is possible that this study was not long enough to appreciate changes in the bone resorption marker CTX. Xu *et al.* (22) found that

chronic sleep deprivation in rats decreased both bone formation and resorption, but it took 3 months to see the decrease in bone resorption marker N-terminal cross-linked telopeptide of type I collagen but only 1 month to see the decrease in serum P1NP. In that study, there was also a decrease in serum 25-hydroxyvitamin D and BMD (22), consistent with our proposed hypothesis that insufficient sleep negatively affects bone health and may be a risk factor for osteoporosis and fracture.

These data suggest that sleep disturbance, including both inadequate sleep and circadian disruption, such as occurs with shift work, jet lag, or social jet lag (35), could be additional risk factors for low BMD, bone loss, and fracture. In addition, due to the co-occurrence of sleep disruption and accelerated bone turnover across the menopausal transition, it is possible that women who experience menopausal sleep disturbance are at greater risk for excess bone loss compared with women who get adequate sleep. If sleep disturbance is found to be an important risk factor for osteoporosis, then evaluation for sleep disturbance should be included in the evaluation of the approximately 50% of patients with idiopathic or primary osteoporosis. Screening could lead to diagnosis and intervention for sleep disturbance, and pharmacological interventions for low bone mass may be avoided and/or their efficacy improved by prompt amelioration of the sleep abnormality.

This study has several strengths, including the rigorously designed forced desynchrony protocol with a highly controlled environment, as well as behavioral schedule and meals across a prolonged interval in the laboratory. Thus, 10 participants represents a relatively large sample size for such a complex, prolonged, and highly controlled protocol. However, there are some potential limitations. The samples used for this analysis were from a study designed to examine the metabolic effects of sleep disturbance rather than bone-related issues. However, the study design was also appropriate to evaluate bone biomarkers. This study did not include a direct measure of bone turnover, such as bone biopsy. However, bone biomarkers usually correlate with direct measures of bone remodeling (*i.e.,* histomorphometry obtained from bone biopsy) (36). There was no control group that experienced the same in-laboratory conditions without the sleep/circadian disturbance, and therefore it is possible that any changes seen are the result of the study setting (*e.g.,* lack of exercise). However, we feel this is less likely because patients were ambulatory, meals were identical at baseline and during intervention for each participant, and mealtimes were standardized to occur at the same times for each sampling interval (as depicted in Fig. 2). Nevertheless, future studies should include a control population to investigate this possibility. Serum sclerostin levels may not accurately reflect sclerostin in the bone microenvironment (37, 38). However, we identified a change in serum sclerostin with intervention that may be mechanistically important and should prompt further investigation. It is unclear if the changes seen in this study were due to the induced sleep restriction, history of circadian misalignment, or desynchrony between central and peripheral clocks. Future studies are needed to investigate the individual effects of sleep restriction and circadian misalignment on bone turnover. Last, this study included only men, and therefore results many not extend to women. Thus, future studies should explore sex differences in the circadian-sleep-bone relationship.

Conclusion

These results suggest that 3 weeks of exposure to circadian disruption with concurrent sleep restriction can lead to an uncoupling of bone turnover and a potential "catabolic" or "bone loss window," wherein bone formation is decreased but bone resorption is unchanged. Such changes may possibly explain the increased incidence of fractures reported in women who worked rotating nightshifts (12). These data further suggest that circadian disruption and/or sleep restriction may be more detrimental to bone health in early adulthood (*i.e.,* bone consolidation). Further studies are needed to confirm these data and to explore sex differences and mechanisms.

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