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The importance of the circadian system & sleep for bone health☆,☆☆

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ABSTRACT

Summary. Adequate sleep timed appropriately during the circadian night is important for numerous biological processes and systems. New evidence suggests that both sleep timing and duration may be important for optimal bone health as well. This review examines the diurnal variation of bone turnover markers (BTMs) and the importance of circadian clock genes in regulating bone mass. In addition, this review explores the evidence for a link between shift work (and its associated disturbances in sleep duration/quality and circadian alignment) and alterations in bone metabolism and bone health. Finally, we review how

Abbreviations: BTMs, Bone turnover markers; CTX, C-terminal cross-linked telopeptide of type I collagen; P1NP, N-terminal propeptide of type I procollagen; FGF-23, Fibroblast growth factor-23; PTH, Parathyroid hormone; BMD, bone mineral density; vBMD, Volumetric bone mineral density; *PER*, Period; *CRY*, Cryptochrome; *BMAL1*, Brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1; *CLOCK*, circadian locomotor output cycles kaput; OTC, Over-the-counter; KO, knockout; RANKL, nuclear factor kappa B (NF-kB) ligand; OPG, osteoprotegerin; h, hour; SNS, sympathetic nervous system; OR, Relative Risk (RR), Odds Ratio.

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commonly used medications and over-the-counter substances (e.g. caffeine, melatonin) complicate the relationship between sleep and circadian disorders and bone health.

1. Introduction

Adequate amounts of appropriately timed sleep are necessary for optimal health and safety [1] while alterations in the timing and duration of sleep are associated with numerous metabolic, cardiovascular, endocrine, and neurological disorders [2–5]. Consistent with reports of increased fracture risk with shift work [6], sleep and circadian disruption can increase sleepiness and reduce vigilance to environmental hazards [7–9], and possibly adversely affect balance [10,11] which all can lead to an increased risk of falls and bone fracture [12,13]. Furthermore, the daily rhythm in bone turnover markers (BTMs) [14,15], the existence of clock genes in bone cells, the identification of altered skeletal phenotypes in clock gene knockout (KO) models [16–20], and the discovery that repeated sleep restriction arrests bone remodeling in laboratory rats [21], all indicate that disruptions in the physiology of sleep and circadian rhythmicity may also affect bone health.

Bone remodeling occurs throughout life as a tightly regulated process that balances bone resorption (performed by osteoclasts) and bone formation (performed by osteoblasts) (Fig. 1). Bone turnover, directed in part by the osteocyte, serves to regulate calcium balance, repair microscopic cracks sustained during normal activity, and heal fractures. An imbalance between bone resorption and formation, as occurs with aging, sex hormone deficiency, or use of medications that alter bone metabolism (e.g., glucocorticoids), results in fragile bones (osteoporosis), and an increased risk of fracture. Biochemical markers of bone resorption and to a lesser degree, bone formation peak overnight [14,22]. Conversely, some factors used for bone cell communication (e.g., nuclear factor kappa B (NF-kB) ligand [RANKL], osteoprotegerin [OPG], sclerostin) have not demonstrated consistent rhythmicity [22–25]. The increase in BTMs overnight suggests bone remodeling may be affected in the millions of individuals who experience sleep and circadian disturbances.

The timing and duration of sleep are influenced by environmental (light/dark cycles, work schedules, duration of prior wakefulness) and circadian (internal biological timing) factors. Circadian rhythmicity is maintained through a well-described molecular clock involving the transcription of circadian-related genes such as Period (*PER1*, *PER2*, *PER3*) and Cryptochrome (*CRY1*, *CRY2*) which are activated by the

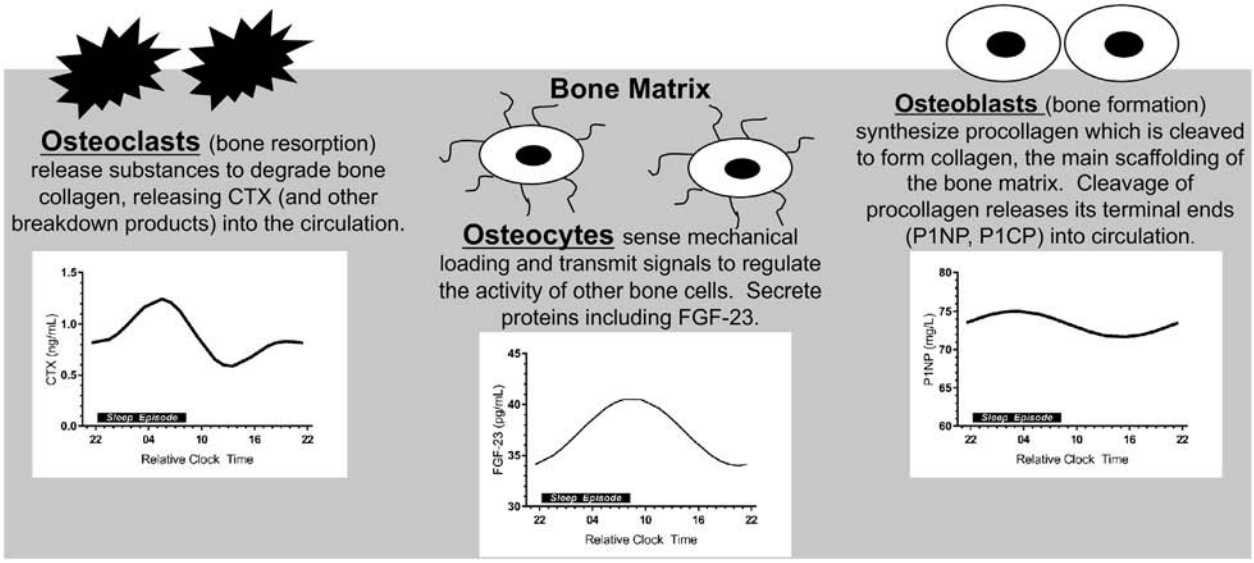

Fig. 1 – Bone matrix and cells with representative 24 h profiles of bone cell markers.Osteoclasts (bone resorption) attach to the bone surface and secrete factors that degrade bone collagen, releasing CTX (and other breakdown products). Osteoblasts form bone matrix to fill in the resorption cavity. An important element in bone formation involves procollagen synthesis and its cleavage to form collagen, the main scaffolding for the bone matrix. When procollagen is cleaved, its terminal ends are released (P1NP, P1CP). Osteoclast and osteoblast activity are coupled, and regulated, in part, by the osteocyte. The osteocyte is a terminally differentiated osteoblast imbedded in the bone matrix that secretes proteins (such as FGF-23). Osteocytes use dendritic processes to sense mechanical loading and transmit signals to regulate the activity of other bone cells. Markers of bone turnover, such as CTX and P1NP, and the osteocyte-derived protein FGF-23 display a 24 h diurnal variation (representative curves generated using data from Swanson et al. [22]).

dimerization of brain and muscle aryl hydrocarbon receptor nuclear translocator-like protein-1 (*BMAL1*) and circadian locomotor output cycles kaput (*CLOCK*). PER and CRY proteins then inhibit the BMAL1-CLOCK complex, until they are degraded, a cycle that takes approximately 24 hours (h), at which time the cycle starts again [18,19]. Such clock genes have been identified in the central clock located in the hypothalamic suprachiasmatic nucleus (SCN), and in peripheral cells [26], including osteoclasts [16,27], osteoblasts [19,28], and osteocytes [19]. The central clock communicates with peripheral clocks through a variety of mechanisms including direct neural connections, hormonal signals (e.g. melatonin, cortisol), the sympathetic nervous system (SNS), and body temperature regulation to ensure synchronization across the organism [29]. In human osteoblast cell cultures, 2 h of exposure to dexamethasone or isoprenaline (a β–adrenergic agonist) induced expression of clock genes but variable expression of osteoblast markers (e.g. osteocalcin) [28]. Data from the same group suggests dexamethasone may also stimulate clock gene expression in murine osteoclast cultures [27]. These studies suggest that the SNS and glucocorticoids may play a role in synchronizing the SCN with peripheral bone clock genes. During entrainment to the light-dark cycle, the central clock and peripheral clocks located in cells outside of the SCN are synchronized. However, circadian disruption as can occur acutely with jet lag, or chronically with night shift work and "social jet lag" [30], can cause external/internal desynchrony resulting in numerous health consequences [2,31]. A 24 h rhythm can be driven by this endogenous circadian system that coordinates cellular processes in anticipation of expected daily behaviors. Physiological variability across the day and night can also occur acutely in response to (rather than in anticipation of) daily behavioral patterns (e.g. wake-sleep, fasting-feeding, light-dark). The anticipatory (endogenous) vs. reactive (behavioral) control of the daily patterns in biological processes has important implications for both how rhythms are affected by circadian disruption and for designing appropriate therapies. Disturbances in the timing and duration of sleep or in the biological processes normally served by sleep could potentially disrupt the rhythmicity of BTMs, the balance between bone resorption and formation, and consequently bone mass/quality and fracture risk.

Night shift work inherently alters sleep timing and duration and has been associated with low bone mineral density (BMD) [32] and an increased risk of fracture [6]. Night-shift work is unavoidable in today's society, and almost one in five of the American work-force performs some kind of shift work [33]. Therefore, it is important to understand how sleep and circadian disruption affect different biological systems, such as bone metabolism. That information may help identify and implement appropriate therapy to mitigate the risks associated with shift work. Here we review the physiology underlying the relationships between bone metabolism/health, the circadian system and sleep; examine the relationship between night-shift work (and its associated sleep/circadian disruptions) and bone health; and consider how medications and over-the-counter substances (e.g. caffeine, melatonin) commonly used to treat various sleep/circadian disturbances may influence the risk of osteoporosis and fracture.

2. Methods

PubMed was searched for the following terms: bone AND sleep; sleep AND fracture; (osteoblast OR osteoclast OR osteocyte) AND (circadian OR rhythm OR sleep); (shift work OR circadian misalignment OR sleep duration) AND (low BMD OR fracture OR osteoporosis); (sleep medication OR benzodiazepine OR Ambien OR Z-drugs) AND (bone OR fracture OR osteoporosis); (caffeine OR melatonin use) AND bone; and Suvorexant AND (falls OR fracture OR bone OR osteoporosis). Search results were reviewed for relevance based on title and abstract. Relevant articles were also identified from the authors' catalogs, if not already found in the above searches. Relevant articles in English were reviewed.

3. The Physiologic Link Between the Endogenous Circadian System and Bone Metabolism

Daily variations in normal bone physiology suggest there is a time-dependent component of bone turnover that is important for optimal bone health and that sleep or circadian disruptions could negatively affect the skeleton. Disturbances in circadian clock genes alter skeletal phenotype [16–20]. In addition, data suggest that circadian timing may play a role in fracture healing [34] and the optimal response to osteoporosis pharmacotherapy [35,36].

3.1. *BTMs Display a 24 h Rhythm*

BTMs display a diurnal rhythm in humans [14,15]. In vitro studies demonstrate clock gene expression in bone and suggest this peripheral tissue has endogenous circadian rhythmicity [37,38]. BTM levels increase overnight, with a peak in the early morning hours, and decrease across the day with a nadir in the late afternoon [14]. This rhythm is more robust in markers of bone resorption (such as C-terminal cross-linked telopeptide of type I collagen - CTX), than in markers of bone formation (such as N-terminal propeptide of type I procollagen - P1NP).

Bone resorption markers, including serum and urinary CTX [14], serum and urinary N-terminal cross-linked telopeptide of type I collagen (NTX) [39], and urinary pyridinium crosslinks [40], have a clear sinusoidal rhythm across the 24 h day (Fig. 1). The amplitude of the rhythms is diminished with fasting [14,41,42] and with anti-resorptive therapy [39], however, the general 24 h sinusoidal curve persists. The 24 h profile of bone resorption markers is unrelated to sex [14,43], age [14], menopausal status [14], posture/bedrest [14], or parathyroid hormone (PTH) [44]. Two human studies have shown that the 24 h profile of bone resorption markers are not associated with serum cortisol [45,46]. These data conflict with two earlier reports [43,47]. There are challenges in studying the relationship between cortisol and the rhythmicity of bone resorption markers, including the inability to pharmacologically mimic the physiologic concentrations and pulsatility of cortisol. Although murine cell culture data suggest glucocorticoids may be important in central-peripheral osteoclast clock synchronization, human data (including the large, rigorous study designs

employed by Heshmati et al. [46] and Schlemmer et al. [45]) suggest cortisol does not have a dominant role in bone resorption marker rhythmicity. The persistence of CTX rhythmicity in blind individuals suggests independence from the light/dark cycle [14]. Oral intake of food and calcium can decrease levels of bone resorption markers, depending on the time of intake [48], with postprandial decreases likely mediated by glucagon-like-peptide-2 (GLP-2) [42,49]. Although circadian protocols in humans that are capable of separating endogenous (circadian) rhythms from exogenous (behavioral/environmental) diurnal profiles are lacking, the daily variation of bone resorption markers likely reflects an endogenous circadian rhythm that is important for normal bone metabolism but that can be influenced by exogenous effects (e.g. behavior or environmental changes).

The general shape and timing of bone formation marker rhythmicity is similar to that of bone resorption markers, however, the amplitudes and regulatory pathways differ. Osteocalcin, a protein produced by the osteoblast primarily during mineralization, is used to reflect osteoblast activity [50,51]. Osteocalcin displays a consistent rhythmicity, similar to that of bone resorption markers, peaking overnight in the early morning hours [14]. Its 24 h pattern is not related to that of growth hormone (GH) [50], but is related to serum cortisol [46]. Conversely, P1NP, the N-terminal portion of procollagen that is cleaved to form collagen for the bone matrix, has a smaller amplitude rhythm appreciated only in large studies (Fig. 1) [15,22,35]. It is possible that the rhythm robustness of bone cells is reflective of their respective functions or cell lineage –osteoclasts are derived from hematopoietic stem cells and osteoblasts are derived from mesenchymal stem cells. The clinical implications of the relatively larger overnight increase in bone resorption compared to bone formation markers are not yet understood. This balance may be important for normal bone metabolism and perturbations in circadian rhythmicity and timing of food intake (e.g., night-shift work) could alter bone metabolism and health.

The osteocyte is a terminally differentiated osteoblast that is imbedded in the bone matrix, represents over 95% of bone cells in the adult skeleton [52], and is responsible for sensing mechanical loading and transmitting that signal to regulate the activity of other bone cells [52]. *Bmal1* was identified in osteocytes [19] and some markers of osteocyte function display a similar 24 h profile to other BTMs. Fibroblast growth factor-23 (FGF-23) is a protein secreted by the osteocyte to regulate phosphate metabolism. FGF-23 levels also display a diurnal rhythm, peaking in the morning (Fig. 1) [22,53,54]. FGF-23 rhythmicity displays more inter-individual variability compared to the robust CTX rhythm [22]. This could be related to differences in sympathetic tone as FGF-23 rhythmicity appears to be regulated, at least in part, by β–adrenergic tone via *Bmal1* [54]. Sclerostin is an osteocyte-derived protein that suppresses bone formation and stimulates bone resorption during mechanical unloading. There have been conflicting reports regarding 24 h variation in levels of serum sclerostin [22,55]. It is possible that sclerostin levels are more heavily regulated by environmental/postural changes rather than inherent circadian rhythmicity. More research in this area is needed because it may impact the efficacy and recommended administration time of investigational pharmacological agents directed against sclerostin.

3.2. Clock Genes Alter Bone Health and Phenotype

In vitro data support the existence of endogenous circadian rhythmicity in BTMs [37,38]. Clock gene KO models demonstrate how alterations in clock gene and circadian physiology have the potential to alter bone turnover and skeletal phenotype [16–20]. Global and osteoblast-specific *Bmal1* KO mice have a low BMD phenotype due to higher levels of bone resorption (and formation) and decreased osteoblast differentiation [19,20]. Conversely, osteoclast-specific *Bmal1* KO mice have a high BMD phenotype due to decreased bone resorption [18]. *Peripheral* (not central) clock genes, specifically osteoblast *Bmal1*, are the proposed regulators of bone resorption by inhibition of osteoclastogenesis [18]. In the absence of *Bmal1* (globally or in the osteoblast), osteoclastogenesis is upregulated, in part, through osteoblastic *RANKL* expression [19]. Similarly, female mice lacking *Cry/Per* genes have a high bone volume phenotype [16,17]. *Cry2*-deficient female mice achieve high bone volume through reduced osteoclast activity and bone resorption. Conversely, the high bone volume phenotype in *Per2*-deficient female mice results from an increased bone formation rate in the absence of leptin [16,17]. These animal studies highlight the importance and complexity of the regulation of bone mass by peripheral clock genes in bone cells.

3.3. Circadian Medicine in Bone Health: Chronotherapy and the Role of the 24 h Clock in Fracture Healing

Chronotherapy utilizes time of medication administration to affect its pharmacokinetics, efficacy, and safety profile [56,57]. Classic examples of successful chronotherapy include treatment of hypertension and cancer. For example, evening administration of anti-hypertensive medications leads to better blood pressure control and a significantly decreased risk of cardiovascular events (RR 0.39, 95% CI 0.29, 0.51) compared to morning administration [58]. Similarly, the timing of chemotherapy drug administration affects the efficacy and patient tolerability of these agents [59]. There is likely a similar role for chronotherapy in osteoporosis.

Kunimoto et al. identified an endogenous 24 h interval of *Per2* expression at femur fracture healing sites using bioluminescence in mice [34]. The addition of PTH resets this rhythmicity and shortens the time to the next peak. These findings highlight the role of clocks in fracture repair and may have additional relevance for the optimal administration time of PTH-related medications (e.g. teriparatide, abaloparatide) used to treat osteoporosis. Luchavova et al. found that mean [CTX] was higher in women who received evening compared to morning teriparatide [35]. Furthermore, after 12 months of teriparatide treatment, lumbar spine BMD increased more in women who received morning teriparatide injections (9.1%) compared to evening injections (4.8%) [36]. Administration time is likely less important for bisphosphonates or denosumab, which have longer half-lives. However, timing of administration may augment the therapeutic effect for shorter-acting agents, particularly PTH and PTH-rp analogs.

4. Associations Among Shift Work, the Associated Sleep/Circadian Disturbances, and the Risk of Osteoporosis, Falls, and Fractures

Circadian rhythmicity of BTMs are likely important for maintaining optimal bone health across the lifetime. Sleep and circadian disturbances, as occur in night-shift work, could potentially disrupt bone physiology and impair bone health. Night-shift work inherently alters the sleep/wake cycle and often results in less restful and shortened sleep duration, thereby disrupting circadian rhythmicity and/or functions served by sleep [60–62]. Human and other animal studies suggest that shift work and its associated sleep and circadian disruptions may be detrimental to bone health and increase fall risk.

4.1. *Night-Shift Work is Associated With Low BMD and Increased Fracture Risk*

Quevedo et al. identified lower BMD at the lumbar spine and femoral neck in Chilean postmenopausal female nurses who reported working a rotating shift (N = 39) compared to those who worked daytime shifts (N = 31) [32]. However, this study did not adjust for the higher prevalence of smoking and coffee intake in the women working rotating shifts [32]. A subsequent larger (N = 3005) study of young (average age 36.4 years) Korean men and women also found lower BMD at the lumbar spine and total hip in people who worked shifts outside daytime hours, particularly in those who worked the night shift [63]. Conversely, there was no difference in BMD in middle-aged male and female shift workers (N = 225) in the NHANES cohort compared to regular workers (N = 738) [64].

Two studies observed an increased fracture risk in shift workers. The Nurses Health Study identified a higher risk of hip and wrist fractures after 8 years of follow-up in women who reported 20+ years of night-shift work compared to women who had never worked night shifts [6]. In addition, Kim et al. reported a nearly 2-fold increase in the percentage of individuals who experienced self-reported fractures (femur, wrist, or spine) in non-daytime workers compared to daytime workers in the Korea National Health and Nutrition Examination Survey (2.1% vs. 1.2%) [63]. Although this difference was not statistically significant at the traditional statistical cutoff ($p = 0.06$), the magnitude of the difference may be clinically significant [63].

These investigators speculated that increases in cortisol and/or inflammation or differences in light exposure, vitamin D status, and/or physical activity could explain the increased risk of low BMD and fracture in night-shift workers. However, cross-sectional and epidemiological designs preclude identification of cause and effect relationships and mechanisms. An interventional study that exposed 10 healthy men to a forced desynchrony protocol (cumulative sleep restriction with concurrent circadian misalignment), akin to the stresses endured during rotating shift work, identified significantly lower levels of a bone formation marker (P1NP) with no change in a bone resorption marker (CTX) [65]. These changes were more pronounced in the younger men who had higher BTM concentrations at baseline [65]. It is unclear if sleep restriction or the history of circadian misalignment caused the changes in bone formation, however, these findings are consistent with those from a prior chronic sleep restriction study in rats [21]. Taken together, these studies indicate that night-shift work is likely detrimental to bone health, potentially by altering the balance between bone resorption and formation.

Animal data support the link among sleep and circadian disturbances and altered bone health. In a study by Everson et al. [21], repeated 10-day periods of sleep restriction during 10–15% of the expected rat lifespan resulted in decreased osteoblast number and activity and an increased marker of osteoclast activity (TRACP 5b). These cellular changes were thought to be the result of the sleep restriction itself rather than an independent effect of alterations in circadian rhythm, and were reflected in BMDs averaging nearly three standard deviations below control values. Similar findings were subsequently found by Xu et al., using different methodologies [66]. Furthermore, mice exposed to continuous light exposure for 24 weeks had decreased trabecular bone volume compared to mice that experienced normal light-dark cycles associated with decreased behavioral and SCN rhythmicity, and an increase in inflammatory markers (TNF-α) [67]. No changes were appreciated in cortical bone but trabeculae in the mice exposed to continuous light were fewer in number, thinner, and had more porosity compared with control mice. These changes reversed rapidly after restoration of normal light-dark cycles [67]. These animal data are consistent with negative molecular and structural skeletal effects of sleep and circadian disruption that could predispose individuals to lower bone strength and increased fracture risk.

4.2. *Long and Short Sleep Duration Have Been Associated With Low BMD*

Disrupted and insufficient sleep are major complaints of shift workers. Both long [13,68–75] and short [70,71,74–80] self-reported sleep duration have been associated with low BMD/osteoporosis or fracture in previous, mostly cross-sectional, studies (Table 1). In addition, one study found that long (≥8 h) compared to short (<6 h) sleep duration was associated with an increased risk of osteoporosis [81] and two studies reported no association between sleep duration and BMD [82,83]. A recent meta-analysis [84] determined that long sleep duration (defined as ≥8 h/day) was associated with a 22% higher risk of osteoporosis in middle-aged and elderly women (OR 1.22, 95% CI 1.06–1.38), while no association was identified for women with short sleep duration (defined as ≤7 h/day). Although this analysis highlighted the significant heterogeneity in past studies, it did not include two studies that found a significant association between short sleep duration and BMD, including one that was published after the meta-analysis was performed [76,77].

Studies investigating the association between sleep duration and BMD differed significantly from each other in the following ways: (i) study population including age, race, gender, sex-hormone status, and the presence/absence of sleep comorbidities; (ii) the method/anatomical site used for BMD assessment thereby diminishing the ability to consistently detect a difference in effect on cortical vs. trabecular bone; (iii) the cutoffs used to define short/normal/long sleep durations; and (iv) whether or not naps were included (Table 1). In addition, the

Table 1 – Summary of epidemiological studies examining associations between self-reported sleep duration and bone outcomes in humans.

Study	Study population	Definition of short sleep duration	Definition of normal sleep duration	Definition of long sleep duration	Outcome measure	Findings
Sleep duration and bone mineral density (BMD) or osteoporosis						
Kobayashi et al. 2012 [81]	N = 19,321 (only 12,589 included in analyses) Age: ≥50 years (avg 60.9 years) 48% female (Japan)	<6 h/day	N/A (comparator group: <6 h/day)	≥8 h/day	Radial BMD by DXA	• Long sleep (≥8 h) associated with increased risk of osteoporosis vs. short sleep (<6 h)OR 1.35; CI 1.06–1.73
Niu et al. 2015 [68]	N = 750 Age: 47–79 years 72% female (Puerto Ricans in Boston)	≤5, 6 and 7 h/day	8 h/day	≥9 h/day	L-spine, total hip, femoral neck BMD by DXA	• In men, long sleep (≥9 h/day) associated with lower femoral neck BMD vs. 8 h/day • No associations seen at other skeletal sites, in women, or for shorter sleep durations vs. 8 h/day
Tian et al. 2015 [69]	N = 31,769 Age: 45–86 years 53% female (China)	<7 h/day	7–8 h/day	8–9 h/day ≥9 h/day	BMD by calcaneal quantitative ultrasonography (QUS)	• Long sleep (≥9 h/day) associated with increased risk of osteoporosis in men (OR 1.40; CI 1.22–1.62) and women (OR 1.20; CI 1.07–1.33) vs. 7–8 h/day • 8–9 h/day sleep associated with increased risk of osteoporosis in men only (vs. 7–8 h/day) • Increased risk of osteoporosis with early sleep timing (before 21:00 h) vs. normal sleep timing (21:00–23:00 h) in men (OR 1.43; CI 1.16–1.78) • Potentially underpowered for short sleep duration due to small numbers

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Table 1 *(continued)*

Study	Study population	Definition of short sleep duration	Definition of normal sleep duration	Definition of long sleep duration	Outcome measure	Findings
Chen et al. 2014 [70]	N = 8688 Age: ≥40 years (avg 54 years) 55% female (China)	<7 h/day 7–8 h/day	8–9 h/day	9–10 h/day ≥10 h/day	BMD by Calcaneal QUS	• Short sleep (7–8 h/day) and long sleep (9–10 h/day and ≥10 h/day) associated with increased risk of osteoporosis in postmenopausal women only (not in men or premenopausal women)
Wang et al. 2015 [71]	N = 6510 Age: ≥40 years (avg 58 years) 100% female (China)	≤7 h/day >7 to ≤8 h/day	>8 to ≤9 h/day	>9 to ≤10 h/day >10 h/day	BMD by Calcaneal QUS	• Short sleep (≤7 h/day) and long sleep (>10 h/day) associated with increased risk of osteopenia and osteoporosis vs. normal sleep (>8 to ≤9 h/day) in postmenopausal women only
Cunningham et al. 2015 [74]	N = 5288 Age: ≥50 years (avg 66 years) 50% female (NHANES – U.S.)	<6 h/day	6–8 h/day	≥9 h/day	Self-report or femoral neck BMD by DXA	• Short sleep (OR 1.59; CI 1.07–2.37) and long sleep (OR 1.51; CI 1.01–2.24) associated with increased risk of osteoporosis
Moradi et al. 2017 (Systematic Review & Meta-Analysis) [a] [84]	N = 31,625 Age: 40–86 years (China, USA, Japan)	≤7 h/day	7–8 h/day	≥8 h/day	N/A	• Inverse relationship between sleep duration and osteoporosis (OR 1.07; CI 1.00–1.15) • In middle aged and elderly women long sleep duration associated with osteoporosis (OR 1.22; CI 1.06–1.38) but not short sleep duration (OR 0.98; CI 0.90–1.05)
Specker et al. 2007 [79]	N = 1146 Age: 20–66 years 57% female (South Dakota)	<6.5 h of sleep on average on weekdays	6.5–10 h of sleep on average on weekdays	N/A	Distal radius by pQCT Spine & hip BMD by DXA	• Lower cortical vBMD in women with short vs. adequate sleep at 20% distal radius • Higher vBMD in men with short vs. adequate sleep at 20% distal radius • Lower estimate of torsional bending strength in men with short vs. adequate sleep
Fu et al. 2011 [76]			8 h/day	≥9 h/day	BMD by DXA	

	N = 602 Age: 18–80 years 100% female (China)	≤5 h/day 6 h/day 7 h/day				• Short sleep (≤ 5 h/day and 6 h/day) associated with increased risk of lower BMD at the spine, particularly in the ≥45 years group
Lima et al. 2012 [75]	N = 2637 Age: ≥18 years (avg 42 years) 52% female (Brazil)	≤6 h/day	7–8 h/day	≥9 h/day	Self-reported osteoporosis	• Short sleep (OR 1.66; CI 1.01–2.73) and long sleep (OR 1.90; CI 1.05–3.44) duration associated with an increased risk of osteoporosis
Kim et al. 2014 [73]	N = 2679 Age: ≥60 years 48% female (Korea)	≤5 h/day <6 h/day	7 h/day	≥8 h/day	L-spine, total hip and femoral neck BMD by DXA	• Longer sleep associated with lower BMD at the total hip and femoral neck in women
Saint Martin et al. 2016 [72]	N = 500 (avg 66 years) 58% female (France)	<6 h/day	6–8 h/day	≥8 h/day	L-spine and femur BMD by DXA	• Long sleep associated with increased risk of osteopenia and osteoporosis vs. 6–8 h/day
Kuriyama et al. 2017 [77]	N = 221 (avg 55 years) 51% female (Japan)	<6 h/day	≥6 h/day	N/A	BMD by ultrasonic bone densitometer at distal radius	• Short sleep associated with decreased cortical bone thickness and increased marker of bone resorption (TRACP-5b)
Marques et al. 2017 [82]	N = 5764 Age: 66–96 years (avg 77 years) 58% female (Reykjavik, Iceland)	<6 h/day	6–8 h/day	>8 h/day	Proximal femur integral vBMD by CT	• No association between short or long sleep duration and vBMD in men or women in fully adjusted models
Lucassen et al. 2017 [83]	N = 915 Age 45–65 years (avg 59 years) 56% female (Netherlands)	N/A	N/A	N/A	L-spine and hip BMD by DXA	• No overall association between sleep duration (analyzed as a continuous variable) and BMD or osteopenia at the spine or hip. - Men with shorter sleep duration had lower L-spine BMD • Pittsburgh Sleep Quality Index (PSQI) score, self-rated poor sleep quality, sleep latency and later sleep timing associated with increased risk of osteopenia.

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Table 1 *(continued)*

Study	Study population	Definition of short sleep duration	Definition of normal sleep duration	Definition of long sleep duration	Outcome measure	Findings
Sleep duration and fracture						
Stone et al. 2006 [13]	N = 8101 Age: ≥69 years (avg 77 years) 100% female (USA)	<6 h/day 6 to <8 h/day	8 to <9 h/day	9 to <10 h/day ≥10 h/day	Self-reported falls and adjudicated fractures	• Increased risk of falls and non-spine fractures with long sleep (≥10 h/day) vs. 8 to <9 h sleep in age-adjusted but not multivariate analyses • Included naps
Chen et al. 2017 [80]	N = 512,187 (avg 48 years) 49% female (China)	<7 h/day (average sleep time per day)	≥7 h/day (average sleep time per day)		Self-reported fractures, verified by research team	• <7 h sleep/day identified as a risk for traumatic fractures (OR 2.70; CI 1.28–5.70)
Osteoporosis and sleep problems						
Foley et al. 2004 [78]	N = 1506 Age: 55–84 years 58% female (National Sleep Foundation - USA)	<6 h/night	N/A	N/A	Self-reported osteoporosis diagnosis	• Those with osteoporosis more likely to report sleeping <6 h/night (OR 1.67; CI 1.04–2.68)

Abbreviations: average (avg); bone mineral density (BMD), volumetric BMD (vBMD), quantitative ultrasonography (QUS), dual-energy x-ray absorptiometry (DXA); hours (h); Sample size (N); Not applicable (N/A); United States of America (USA); peripheral quantitative computed tomography (pQCT); computed tomography (CT); Tartrate-resistant acid phosphatase 5b (TRACP-5b); 95% confidence interval (CI); odds ratio (OR), Pittsburgh Sleep Quality Index (PSQI).

[a] Studies listed under "Sleep Duration and Bone Mineral Density (BMD) or Osteoporosis" below Moradi et al. 2017 were not included in that meta-analysis.

small sample sizes for the osteoporosis/very low BMD groups [83] and analyses using categorical designations for sleep duration and BMD (e.g. "6–7 h" or "osteoporosis" instead of continuous BMD variables) may limit the ability to accurately detect the relationship between sleep duration and BMD. Arguably the most important limitation of these studies is utilization of subjectively measured sleep duration, often assessed at one time point and without regard to the amount of sleep needed by an individual to feel rested. Future research should determine whether subjective or objective sleep parameters are more strongly and consistently correlated with skeletal outcomes. Furthermore, prospective studies with sleep duration monitored periodically over months or years may give more insight into the stability of sleep duration over time and how that correlates with bone parameters (BTMs, BMD), which change slowly over time.

The mechanisms by which sleep duration affects bone metabolism and bone density are unknown. Possible mechanisms include alterations in the normal rhythmicity of bone cells, hormone levels (e.g. sex steroids, cortisol), increases in sympathetic tone [29,77], inflammation [85,86], metabolic derangements, or fatigue/physical inactivity (Fig. 2). The role of the central nervous system in regulating bone metabolism may be particularly important in the skeletal effects of disrupted sleep because of the SNS activation associated with sleep and circadian disruption. The SNS can influence bone cell clock genes and negatively impact bone metabolism through a complex network involving leptin, serotonin, neuropeptide Y, balance with the parasympathetic nervous system, and direct stimulation of bone cells via β–adrenergic receptors on osteoblasts (*readers are referred to* Fig. 1 *in a recent review by Dimitri* et al. [87]. Although the relationship between sleep stages and bone metabolism has not been evaluated, rapid eye movement (REM) sleep, which is associated with higher levels of sympathetic tone [88], predominates in the latter half of the night when BTMs peak [14], whereas non-REM (NREM) sleep predominates in the early part of the night. The individual effects of sleep duration and circadian alignment on bone health need further investigation to determine if these disturbances produce similar, additive, or synergistic impairments in bone health [71,83].

4.3. *Obstructive Sleep Apnea (OSA) and Bone Health*

Data are mixed regarding the association between OSA and bone density [29], however, it is likely that OSA with its associated co-morbidities and underlying physiologic/metabolic derangements (increased sympathetic drive, inflammation, insulin resistance, nocturnal hypoxia etc.) are associated with increased bone resorption [89] and subsequently lower BMD [90].

4.4. *Sleep and Circadian Disturbances Increase Fall Risk*

Sleep and circadian disturbances cause sleepiness and decreased vigilance to environmental hazards [7–9], which may result in an increased risk of falls and fractures. In addition, the acute impairment in performance sometimes seen upon wakening ("sleep inertia") and prolonged wakefulness both decrease postural stability and balance, increasing the risk for falls [10,11,91]. Moreover, insomnia has been associated with an increased risk of falls [12] and the greater the burden of insomnia symptoms predicts 2-year fall risk in older adults [92]. Circadian regulation of the cardiovascular response to postural stress in humans increases the risk of pre-syncope during the biological night, potentially increasing the risk for syncope and falls in night-shift workers [93].

5. The Risk of Falls/Fractures With Caffeine and Medications Used for Sleep/Circadian Disruption

Medications taken for sleep and circadian disturbances can alter sleep architecture [94], sleep inertia [95,96], and fall risk. Individuals with sleep/circadian disorders, including night-shift workers, frequently use over-the-counter (OTC) substances (e.g. caffeine) to compensate for daytime fatigue due, in part, to inadequate sleep duration [97,98]. They also use OTC and prescription medications (e.g., melatonin, benzodiazepines, zolpidem) to induce, prolong, and re-entrain their sleep [97–100]. Of rehabilitation patients who sustained a femoral neck fracture after a fall, 51% reported using a hypnotic or other tranquilizing medication for sleep [101]. These OTC and prescription agents likely influence the sleep-bone relationship either by mechanistically altering bone metabolism and/or calcium balance (caffeine), or by increasing the risk of falls and fracture through impairments in muscle tone, balance, and cognition.

5.1. *Benzodiazepines and Z-drugs are Associated With an Increased Risk of Falls and Fractures*

Untreated insomnia and other sleep disturbances are associated with falls [12,102–104], but physician-prescribed sleep medications increase fall risk further [92]. Epidemiological studies have consistently shown an increased risk of falls and fractures with use of hypnotics such as benzodiazepines [105,106]. More recent systematic reviews and meta-analyses have confirmed these findings with a reported Relative Risk (RR) of hip fracture of 1.52 (95% CI 1.37–1.68 $p < 0.001$) [103] with the highest risk seen at time of initiation (RR 2.40, 95% CI 1.88–3.05 $p < 0.001$ with short term use vs. RR 1.20, 95% CI 1.08–1.34 $p < 0.001$ with long term use) [103]. The risk of fracture increases with the duration of action of the specific benzodiazepine. The highest risk is seen with diazepam (long half-life), followed by lorazepam [107]. Like benzodiazepines, newer agents such as zolpidem and zaleplon (often called "Z-drugs") induce central nervous system sedation by binding to the GABA-benzodiazepine receptor complex to enhance the effects of GABA [103]. Although Z-drugs were initially thought to be safer than traditional benzodiazepines because of their shorter half-life and lower risk of subsequent daytime sedation and dependency [103,106], zolpidem appears to have a similarly increased risk of hip fracture (RR 1.90, 95% CI 1.68–2.13 $p < 0.001$) [103]. A recent meta-analysis found that zolpidem was associated with a 92% higher risk of fracture (RR 1.92, 95% CI 1.65–2.24; $I^2 = 50.9\%$) and that this risk was highest for hip fractures compared to any other site [108]. The limited evidence on Z-drugs suggests that the risk of falls and fractures are greatest with higher doses [108] and

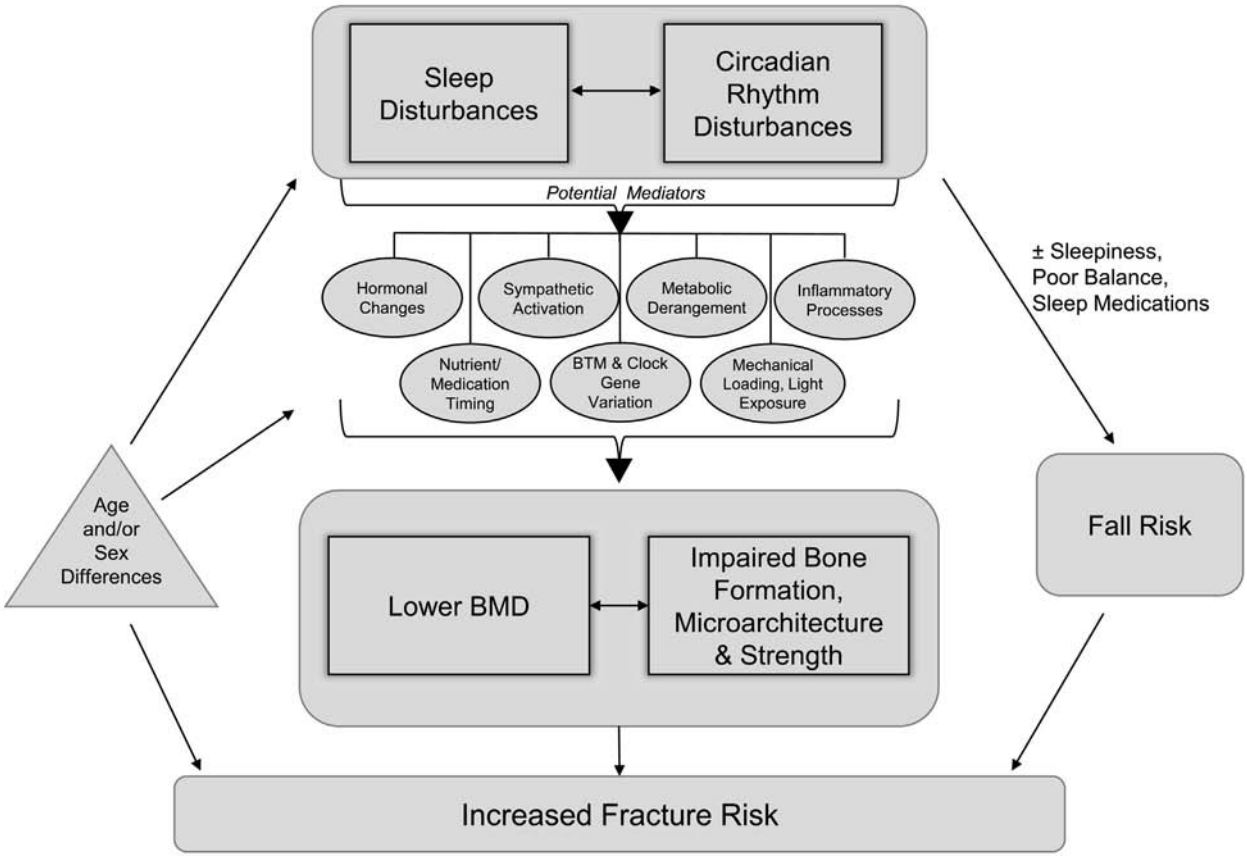

Fig. 2 – Conceptual framework of how circadian rhythm and sleep disturbances alter bone health.Sleep and circadian disturbances, in the form of night-shift work and/or altered sleep duration, impair bone formation, microarchitecture and strength and are associated with lower BMD through several potential mediators (ovals). Together, these changes in bone density and quality lead to an increased fracture risk. Circadian rhythm and sleep disturbances can also increase fracture risk by increasing the risk for falls, with or without sleep medications used to treat these disorders (e.g. benzodiazepines, Z-drugs like zolpidem). Age and/or sex differences may influence the circadian rhythm and sleep disturbances, mediators, and fracture risk.

in the initial weeks of use [103,108]. Tom et al. confirmed the increased risk of hip fracture with zolpidem, however, no relationship was identified for a different Z-drug, eszopiclone [109]. Therefore, the association between zolpidem and increased fracture risk may not indicate a drug class effect for Z-drugs. It is presumed that these medications increase fracture risk through sedation, subsequent impairments in balance, cognition, and reaction times, and an increased risk of falls [10,103,110].

Studies investigating the association between benzodiazepines and Z-drugs with falls and fractures have limitations that potentially underestimate fracture risk. Prescription records were often used to ascertain exposure status rather than an actual account of medication administration. In addition, many of the studies of Z-drugs used prescription databases to ascertain medication exposure after identifying cases that had a fracture requiring hospitalization. These surveys ignore fractures that do not require hospitalization/surgery, making it difficult to ascertain how drug dose and timing truly affect the medication risks [111]. Therefore, both the indication (e.g., insomnia, short sleep duration) and medication increase the risk of falls and fractures [104].

5.2. *Caffeine and Bone Health*

There are several mechanisms by which caffeine is potentially deleterious to bone. Caffeine has been shown to negatively alter calcium homeostasis through hypercalciuria [112] and decreased gastrointestinal calcium absorption [112]. Caffeine could induce bone loss through direct effects on bone cells that favor osteoclast differentiation and osteoblast apoptosis, as indicated by rat models [113,114]. It is possible that consumption of caffeine-containing drinks limit intake of other calcium-rich (e.g. milk) or more bone-neutral (e.g. water) beverages or because it contains other substances that might impact bone health (e.g. phosphorous, polyphenols, acid, sugar) [115,116]. There have been some epidemiological studies of the role caffeine has in bone health, with mixed results [105,116–118]. The conflicting data may be due, in part, to differences in study population (age, sex, menopausal status) [117], follow-up time [112,119], fracture definition/ascertainment [116], availability of information on confounders (such as calcium intake) [112] and subsequent appropriate statistical adjustments, and/or several aspects of caffeine intake that are difficult to capture accurately [112]. These include the source of caffeine (coffee, soda, tea), geographic

variation in coffee strength and preparation that can affect caffeine levels, and assumptions regarding average serving size [112,116]. For example, differing results for caffeine-associated fracture risk were found in the Swedish Mammography Cohort study in 2006 compared with 2013 [112,119], possibly reflecting longer follow-up time and refinements in questionnaires. In addition, two recent meta-analyses that used slightly different inclusion/exclusion criteria concluded that coffee intake was associated with an increased fracture risk in women but not men [116,117]. The increased fracture risk in women was seen with as little as 2 cups of coffee per day (RR 1.02, 95% CI: 1.01–1.04) and increased with greater intake (RR of 8 cups/day 1.54, 95% CI: 1.19–1.99) [116]. Since the protective effect in men was of greater magnitude (RR 0.76, 95% CI 0.62–0.94) for all but the highest levels of female coffee consumption, the overall effect of caffeine is unclear. A long-term, prospective intervention trial is needed to clarify if these associations translate into a clinically significant cause-and-effect relationship at typical levels of coffee/caffeine consumption.

5.3. *Melatonin and Bone Health*

Melatonin is low in night-shift workers [120] and is commonly used as an OTC supplement for jet lag and insomnia and therefore bears special mention. Melatonin is thought to be beneficial to bone by promoting osteoblast formation and decreasing bone resorption through decreased synthesis of RANKL and increased OPG synthesis [121,122]. In addition, animals lacking melatonin have lower BMD compared to controls [121,123,124]. A small, randomized controlled trial in humans identified an increase in femoral neck BMD (an anatomical site with a larger precision error than other anatomical sites) with daily melatonin (1 mg/day or 3 mg/day) for a year and an increase in lumbar spine volumetric BMD (vBMD) by QCT with high dose melatonin only (3 mg/day) [125]. However, no significant change was identified in BTMs or in areal BMD at other skeletal sites [125], or in other studies [126]. Larger human studies of longer duration are needed to determine the pharmacological role (and dose) for melatonin in the treatment of postmenopausal osteoporosis.

6. Summary & Future Directions

The diurnal variation in BTMs and animal clock gene KO models suggest that circadian rhythmicity is important for bone health [14,15,17,20]. Some epidemiological studies support this inference as night-shift work, which causes both sleep disruption and circadian misalignment, has been associated with lower BMD and increased fracture risk [6,32]. Experimental studies of rats and healthy men reveal that sleep and circadian disruption impair bone formation [21,65]. Use of OTC and prescription medications for sleep/circadian disorders further increase the risk of falls and fractures [103,116,117]. Moreover, it seems plausible that these factors interact, such that sleep deficiency and/or circadian disruption may increase the risk of falls due to reduced vigilance/balance, and may make a fracture more likely to occur due to suboptimal bone health. Further investigations are needed to clarify:

i. If and how communication among bone cells is affected by disturbances in sleep and circadian rhythms.
ii. Cause and effect relationships between sleep duration and sleep stages with bone health using objective sleep measures over time with standardized bone mass and quality assessments.
iii. If BTM rhythmicity uses the *anticipated* rest/activity cycle (via endogenous circadian control) to regulate bone metabolism or if this rhythm is *a response* to behavioral changes.
iv. The specific effects of sleep disorders and circadian disruption on bone modeling, and remodeling and if these disturbances differentially affect trabecular and cortical bone.
v. The mechanisms by which sleep and circadian disorders affect BMD and fall/fracture risk.
vi. The effects of age, sex, and body composition on the sleep/circadian-bone relationship.

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