

Review

Rhythms in your bones: how circadian rhythms impact bone cell function and skeletal health

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Circadian rhythms play a central role in how bone tissue is formed, resorbed, and remodelled during the 24-h cycle. Bone cells express core clock proteins, which coordinate the timing of these processes, each contributing differently to the balance between bone formation and resorption. These intrinsic bone-cell clocks are further influenced by external temporal cues, including hormonal effects and mechanical forces such as exercise. Taken together, these features indicate that skeletal bone tissue is significantly influenced by the peripheral clock. In this review, we summarise recent advances on how intrinsic and extrinsic timing cues interact to regulate skeletal physiology, and we discuss how emerging insights into bone circadian biology might inform therapeutic and regenerative strategies aimed at improving skeletal health.

Circadian rhythms in bone

Circadian rhythms are 24-h cycles generated by molecular clocks, which are a collection of proteins that together form an endogenous oscillator. These cellular clocks time the physiology and behaviour of the body with the external environment. These rhythms are organised in a hierarchical fashion, with the central clock residing in the suprachiasmatic nucleus (SCN) located in the brain's hypothalamus. The SCN receives light input from the retina via the retinal ganglion cells and, in turn, synchronises peripheral clocks through hormonal, metabolic, and autonomic pathways [1,2]. Most tissues, including bone, contain their own oscillators consisting of **transcriptional–translational feedback loops (TTFL)** (see [Glossary](#)). Clock genes such as *circadian locomotor output cycles Kaput (CLOCK)*, *brain and muscle ARNT-like protein 1 (BMAL1)*, *cryptochrome (CRY)*, *period (PER)*, nuclear receptor subfamily 1, group D (*REV-ERB*), and *retinoic acid receptor-related orphan receptors (ROR)* comprise the TTFL and together form a number of feedback loops. These genes, which oscillate over a 24-h period in their expression profile, control the timing of bone turnover and help match skeletal activity to behavioural demands. Bone tissue is not static but is continually remodelled through a process of **bone formation and resorption** performed by osteoblasts and osteoclasts, respectively. Indeed, all the main resident cells in bone tissue (osteoblasts, osteoclasts, and osteocytes; [Box 1](#)) express the clock genes that regulate their differentiation, activity, and timing.

Although this circadian profile in bone has been recognised for many years, the exact endogenous and exogenous mechanisms responsible remain unclear [18]. Peripheral clocks within osteoblasts, osteoclasts, and osteocytes are not only regulated by the SCN but also respond to local signals via systemic cues, feeding rhythms, sleep–wake patterns, and mechanical loading [2, 19–21]. Notably, osteoclast clocks appear to be controlled primarily by paracrine factors, whereas osteoblast clocks respond to both SCN-dependent and paracrine inputs [19,21]. Disruption of these rhythms weakens this coordination and shifts the balance towards reduced bone formation and increased resorption [22–26]

Highlights

Bone tissues show clear daily rhythms, coordinated by clock proteins in osteoblasts, osteoclasts, and osteocytes.

Core clock proteins such as brain and muscle ARNT-like protein 1, cryptochromes, periods, nuclear receptor subfamily 1 group D receptors, and retinoic acid receptor-related orphan receptors contribute differently to bone formation and resorption.

Endocrine signals, such as glucocorticoids and melatonin, and organ-derived signals, such as those from the gut and liver, reveal that the rhythms in bone are influenced by whole-body circadian coordination.

Mechanical loading, including exercise, interacts with circadian timing, leading to stronger or weaker bone adaptation depending on the time-of-day.

These insights further support the development of chronotherapeutic strategies that align bone-targeted treatments and regenerative approaches with the body's internal circadian timing.

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Box 1. Major bone cell types

Bone is a dynamic tissue that is continually renewed through the coordinated actions of osteoblasts, osteoclasts, and osteocytes. These cells regulate bone formation, removal, and adaptation to mechanical and metabolic demands. Osteoblasts are the cells responsible for forming new bone. They arise from mesenchymal stem cells and mature into bone-forming cells under the control of transcription factors such as runt-related transcription factor 2, osterix, and beta-catenin [3,4]. Mature osteoblasts synthesise the protein components that make up the bone matrix. Some osteoblasts remain on the bone surface as lining cells, while others become embedded within the tissue and differentiate into osteocytes [5]. Osteoclasts carry out bone resorption. They originate from monocyte-macrophage precursors and require signals such as macrophage colony stimulating factor and receptor activator of nuclear factor kappa-B (RANKL) for differentiation [6–9]. Osteoblasts regulate this process by producing RANKL and its decoy receptor osteoprotegerin, which together determine how many osteoclasts are formed and how active they become [10,11]. Mature osteoclasts attach to the bone surface and release acids and proteolytic enzymes, such as TRAP, which dissolve the mineral component of bone [12,13]. Osteocytes are the most abundant bone cells. They represent the terminal stage of the osteoblast lineage, formed when active osteoblasts become embedded in the matrix [5]. Osteocytes extend long processes that connect them to neighbouring cells, forming a communication network throughout the skeleton. They act as sensors of mechanical load and other environmental signals and adjust bone turnover accordingly by influencing both osteoblast and osteoclast activity [14–17]. Together, osteoblasts, osteoclasts, and osteocytes form an integrated system that maintains skeletal health. Osteoblasts build bone, osteoclasts remove bone, and osteocytes coordinate when and where these processes occur. Understanding how these cells interact is essential for interpreting how circadian rhythms influence bone remodelling across the 24-h cycle.

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(Figure 1, Key figure and Box 2). Understanding how circadian timing regulates these interactions is therefore important for identifying molecular pathways that link systemic timing to skeletal health and for developing therapeutic strategies that align treatments with endogenous bone rhythms. Given the growing recognition that circadian timing is a critical but underexplored dimension of skeletal health, this review provides a timely synthesis of current progress and key open questions in the field.

Core circadian regulators in bone cells

Daily variation in bone turnover reflects the activity of the core circadian proteins within bone cells. BMAL1, CRY1/CRY2, PER1/PER2/PER3, REV-ERB α /REV-ERB β , and ROR α /ROR β /ROR γ (Box 3) are amongst the best-characterised regulators and contribute in distinct ways to the organisation of osteoblast and osteoclast function during the 24-h cycle.

Rather than acting uniformly, core circadian proteins exert distinct effects on skeletal biology, including **osteogenesis**, resorption, and metabolic or inflammatory pathways. In the following sections, we outline the major regulators recently studied in bone and summarise how each contributes to the circadian control of skeletal biology.

BMAL1

BMAL1 is unique amongst clock proteins as it is the only protein to have a nonredundant role, as its loss cannot be compensated by any other clock factor [40]. BMAL1 is widely recognised as a positive regulator of skeleton development as demonstrated in both *in vitro* and *in vivo* studies [41]. *In vitro*, silencing *Bmal1* in bone marrow stromal cells (MSCs) reduces the expression of osteogenic differentiation markers such as runt-related transcription factor 2 (*Runx2*), a master transcription factor for osteoblast differentiation; alkaline phosphatase (*Alp*), which is involved in bone mineralization; and osteocalcin (*Ocn*), a late marker of osteogenesis, while simultaneously increasing apoptosis [42,43]. *In vivo*, global *Bmal1* knockout mice show reduced bone volume, thinner trabeculae, and impaired mineral apposition rates [44,45]. Moreover, there is evidence of calcification of the calcaneal tendons and costosternal joints [46], indicating that disrupted circadian regulation of bone and cartilage homeostasis is the likely cause of abnormal differentiation and extracellular matrix remodelling in these joints, as observed in clock disruption of intervertebral discs [47]. Similarly, pluripotent stem cell studies show that mechanically induced disruption of intracellular circadian oscillations during osteogenic induction alters cell fate through

Key figure

Circadian regulation of bone cell function and systemic inputs

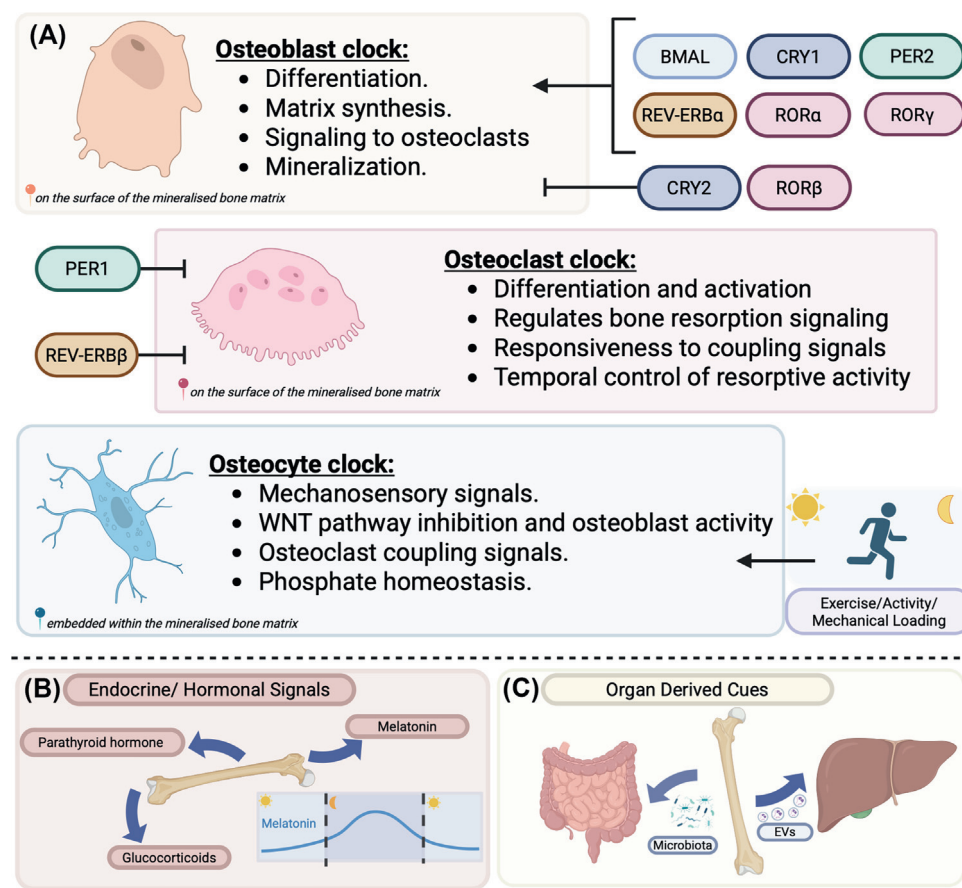


Figure 1. (A) Intrinsic circadian clocks operate within osteoblasts, osteoclasts, and osteocytes to coordinate bone remodelling. In osteoblasts, core clock components including BMAL1, CRY1, PER2, REV-ERBa, RORa, and RORγ regulate differentiation, matrix synthesis, mineralisation, and coupling signals to osteoclasts, whereas CRY2 and RORβ are associated with inhibitory effects on osteogenic activity. In osteoclasts, clock genes such as PER1 and the inhibitory nuclear receptor REV-ERBβ restrain differentiation and activation, influencing responsiveness to coupling signals, and contributing to the temporal control of bone resorption. The osteocyte clock integrates circadian timing with mechanosensory signalling, regulation of WNT pathway inhibition, and osteoclast coupling signals, allowing mechanical and metabolic cues to be coordinated across the day. (B) Endocrine signals with circadian rhythmicity, including parathyroid hormone, melatonin, and glucocorticoids, act systemically to modulate skeletal clock function and bone turnover. (C) Organ-derived cues from the gut and liver contribute to circadian regulation of bone through interorgan signalling pathways, highlighting that skeletal rhythms are shaped by systemic signals. Time-dependent exercise and physical activity provide additional external cues that interact with intrinsic bone clocks to influence skeletal adaptation. BMAL1: brain and muscle ARNT-like protein 1; CRY1: cryptochrome 1; CRY2: cryptochrome 2; EVs: extracellular vesicles; PER1: period 1; PER2: period 2; RORa: retinoic acid receptor-related orphan receptor alpha; RORβ: retinoic acid receptor-related orphan receptor beta; RORγ: retinoic acid receptor-related orphan receptor gamma; REV-ERBa: nuclear receptor subfamily 1 group D member 1; REV-ERBβ: nuclear receptor subfamily 1 group D member 2; WNT: Wingless-related integration site. The figure was created with BioRender (www.biorender.com).

the transcriptional enhanced associate domain (TEAD)–F-box only protein 13 (Fbxl3)–CRY axis, underscoring the importance of stem cell clocks in regulating bone differentiation [48]. BMAL1 also maintains osteogenesis via the Yes-associated protein (YAP) pathway, a transcriptional

Glossary

Bone formation and resorption: the two opposing processes of bone remodelling—osteoblast-driven deposition of new mineralised matrix and osteoclast-mediated degradation of existing bone tissue.

Circadian misalignment: misalignment between internal circadian rhythms and external or behavioural cycles that alters normal physiological regulation.

Hypercortisolism: a state of chronically elevated glucocorticoid levels, whether endogenous or pharmacological, associated with impaired bone formation, increased resorption, and osteoporosis-like phenotypes.

Odontoblastic differentiation: the process by which dental mesenchymal stem cells mature into odontoblasts, specialised cells that generate dentin and share molecular pathways with osteogenic differentiation.

Osseointegration: the biological process by which bone forms a direct, stable interface with an implant, requiring coordinated osteogenesis and vascularisation.

Osteoblastogenesis: the maturation of mesenchymal stem cells into functional osteoblasts, responsible for forming bone tissue.

Osteoclastogenesis: the differentiation of monocyte–macrophage precursors into mature osteoclasts capable of resorbing bone.

Osteogenesis: the differentiation and activity of osteoblast lineage cells that produce new mineralised bone matrix.

Oxidative phosphorylation (OXPHOS): the mitochondrial process in which electron transport drives ATP synthesis; essential for energy-intensive steps.

Parathyroid glands: small endocrine glands located behind the thyroid that regulate calcium homeostasis through the rhythmic secretion of PTH, which influences bone turnover.

Pineal gland: a neuroendocrine organ in the brain that synthesises and secretes melatonin in a rhythmic manner, providing a major hormonal cue for circadian timing.

Transcriptional–translational feedback loops (TTFLs): autoregulatory molecular cycles in which clock genes are transcribed and translated into proteins that later inhibit their own expression, generating ~24-h oscillations in cellular processes.

Box 2. Daily rhythms in bone turnover

In humans, circadian effects on bone metabolism are inferred from circulating bone turnover markers, hormones, and minerals. Bone resorption shows robust circadian rhythms, demonstrated by an overnight rise in serum CTX that peaks in the early morning and reaches its lowest levels in the afternoon, although the amplitude of this rhythm is significantly smaller in females than in males [27–29]. The timing of bone formation marker rhythmicity is similar to bone resorption but differs in amplitude, regulatory pathways, and peak timing. For example, osteocalcin, a surrogate marker of osteoblast activity during mineralisation, exhibits rhythmicity that is higher at night but less robust than CTX and is susceptible to life-style factors [30,31]. When these daily rhythms are disrupted, the consequences for skeletal health become apparent. In mice, repeated shifts of the light–dark cycle, used as a model of shift work or jet lag, reduce bone formation and cause structural changes consistent with bone loss [26]. In humans, sleep restriction lowers circulation markers for bone formation (e.g., P1NP and osteocalcin), while resorption markers remain stable or increase (CTX) [32,33]. Notably, these studies were conducted separately in women [32] and men [33], with young premenopausal women showing a more pronounced response, suggesting that sex and menopausal status may modify the skeletal impact of circadian disruption. These patterns indicate that **circadian misalignment** can shift bone turnover towards net bone loss.

coactivator involved in mechanotransduction and osteogenic differentiation, through a mechanism that appears independent of canonical Hippo signalling, linking circadian regulation to transcriptional control in bone cells [49].

More recent studies reveal a more complex picture, showing that the effects of *Bmal1* deletion depend largely on specific cell types. Postnatal conditional deletion of *Bmal1* in osteoblasts impairs endochondral ossification and causes skeletal anomalies, yet paradoxically increases trabecular bone mineral density and volume in young mice (8 weeks of age), accompanied by an increased number of osteoblasts and fewer osteoclasts [50]. Osteoblast *Bmal1* also influences resorption indirectly, as osteoblast-specific deletion reduces **osteoclastogenesis** and transiently increases trabecular bone mass [50]. This paradoxical phenotype was explained by reflecting on *Bmal1*'s role as a transcriptional repressor of bone morphogenetic protein 2 (BMP2), a key factor that drives bone formation. In the absence of *Bmal1*, BMP2/SMAD1 signalling becomes overactive, promoting osteoblast proliferation and trabecular bone [50]. This phenotype was observed in both male and female mice [50]. In contrast, in another study, osteoblast-specific *Bmal1* deletion led to reduced bone mass, caused instead by increased osteoclast activity and elevated *receptor activator of nuclear factor kappa-B* (*Rankl*) expression [51]. The differences between these studies likely reflect the distinct models used, which target different cell populations at different developmental stages. *Bmal1* deletion in a model targeting mature osteoblasts postnatally promoted BMP2 signalling and increased bone mass [50], whereas the

Box 3. Core circadian regulators

The mammalian molecular clock is controlled by cell-autonomous autoregulatory TTFLs. The core feedback loop involves the basic helix–loop–helix transcription factors BMAL1 and CLOCK, which heterodimerize to drive circadian gene expression. The BMAL1–CLOCK complex activates the transcription of target genes such as the period (*Per1*, *Per2*, *Per3*) and cryptochrome (*Cry1*, *Cry2*) families. Following translation, PER and CRY proteins accumulate in the cytoplasm, form multiprotein complexes, and translocate to the nucleus, where they inhibit BMAL1–CLOCK-mediated transcription, thereby generating near 24-h circadian oscillations [34] (Figure 1).

Cryptochromes (CRY1 and CRY2) are core components of the circadian clock [35] and constitute the principal repressors of the negative arm of the feedback loop. CRYs suppress BMAL1–CLOCK transcriptional activity both independently and in complex with PER1, PER2, and PER3, thereby terminating circadian gene expression. Although all three PER proteins contribute to clock function, PER1 and PER2 play dominant roles in rhythm generation, whereas PER3 is primarily thought to modulate circadian amplitude and phase to a lesser extent than PER1 and PER2 [36,37]. A secondary regulatory loop involves the nuclear receptors REV-ERB α (NR1D1) and REV-ERB β (NR1D2), which act as heme-dependent transcriptional repressors of *Bmal1* (and *Npas2*) expression and are widely regarded as integral components of the mammalian circadian clockwork [38]. Conversely, the retinoic acid receptor-related orphan receptors (ROR α , ROR β , and ROR γ) form part of the positive limb of the clock, activating *Bmal1* transcription, thereby contributing to the stabilisation and robustness of rhythmic gene expression [35,39]. Together, this clockwork provides each bone cell type with an intrinsic circadian rhythm that contributes to the daily organisation of skeletal processes.

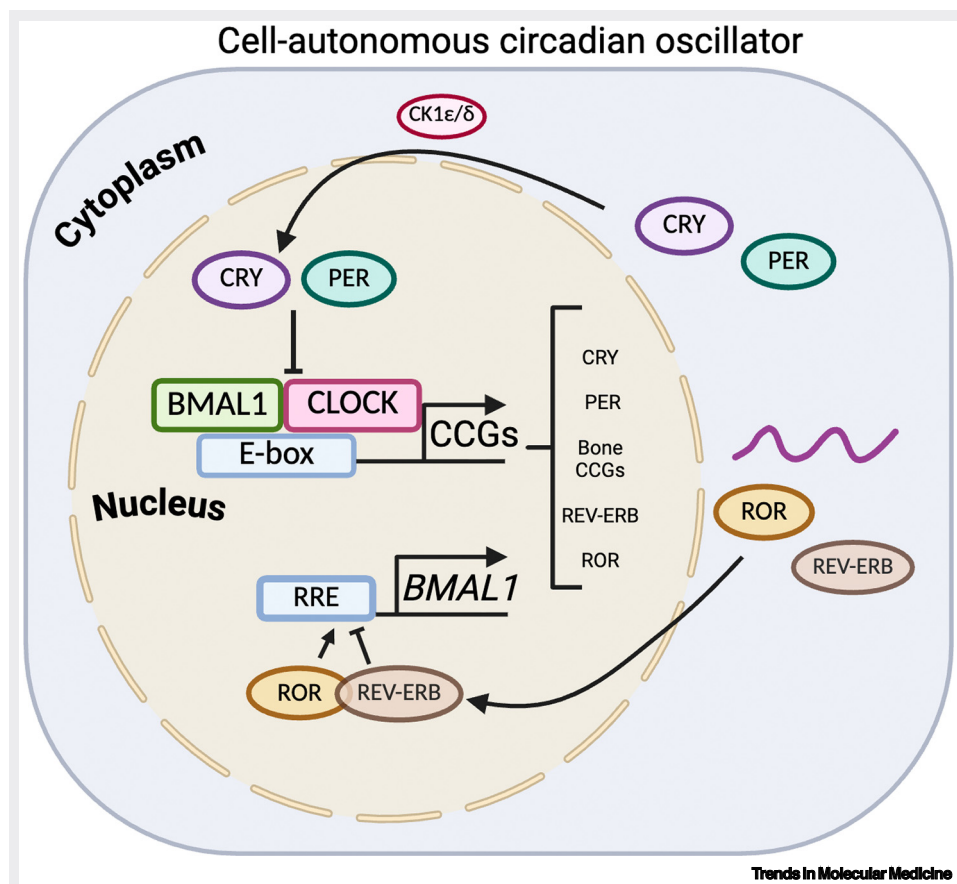


Figure 1. The mammalian circadian molecular clock responsible for temporal physiology, highlighting the transcriptional–translational feedback loops (TTFLs). The core transcriptional loop, driven by the BMAL1:CLOCK heterodimer, promotes the transcription of clock-controlled genes (CCGs), including its own repressors, period (PER) and cryptochrome (CRY). Accumulation of PER and CRY proteins over time, along with time-dependent nuclear translocation, leads to inhibition of BMAL1:CLOCK activity, completing the negative feedback loop. An ancillary loop involving ROR and REV-ERB nuclear receptors regulates and stabilises BMAL1 transcription via ROR/REV-ERB response elements within the promoter of *BMAL1*, thereby fine-tuning the amplitude and robustness of circadian oscillations. Collectively, these interlocked loops generate a near-24-h self-sustained circadian rhythm that is endogenous, temperature compensated, and synchronised to external environmental cues, known as zeitgebers (e.g., light, feeding, and activity). The figure was created with BioRender (www.biorender.com).

negative effects on bone mass were observed in models acting earlier in development, which also impacted osteoblast precursors and chondrocytes [51]. It is also worth noting that the latter findings were obtained in male mice only, and sex-specific effects may therefore also contribute to the divergent outcomes. Taken together, these findings indicate that BMAL1 can act as both an activator and a repressor of bone formation, depending on skeletal compartment, developmental stage, and systemic context. These results also highlight the complexity of circadian regulation in bone and underline the importance of distinguishing between global and cell-specific BMAL1 deletion when evaluating its effects on bone formation and resorption.

CRY1 and CRY2

In bone and elsewhere, the CRY family of genes primarily functions as repressors of osteogenic differentiation. Recent evidence shows that *Cry2* knockdown increases markers, including

Runx2, *Alpl*, collagen type I alpha 1 (*Col1a1*), *Ocn*, and osteopontin (*Opn*), while overexpression of *CRY2* abolishes these markers by binding the CLOCK/BMAL1 complex in human MSCs and suppressing the expression of coactivator P300 [52]. CRYs share structural features with light-sensitive photolyases, making CRY1 sensitive to low-intensity near-infrared (NIR) degradation [53]. Exposure to NIR light reduces CRY1 levels, and this activates BMP–SMAD signalling increasing *Runx2* and *osterix* (*Osx*) expression *in vitro* and, in a rat craniofacial bone defect model in male animals, significantly accelerating *in vivo* bone regeneration [54]. However, these proteins also help coordinate the day–night pattern of osteoblast gene expression. By adjusting when osteogenic genes become active and how much they are expressed, CRY proteins provide fine control over the daily rhythm of osteogenesis. This dual role allows CRY1 and CRY2 to influence both the overall level of osteoblast differentiation and the timing of these processes within the circadian cycle.

PER1, PER2, and PER3

PER1, PER2, and PER3 are core components of the circadian negative feedback loop. Although often treated as functionally similar, PER proteins have distinct roles in bone. Recent work demonstrates that *Per1* functions as a cell-autonomous inhibitor of osteoclastogenesis. Conditional deletion of *Per1* in osteoclast-lineage cells lowers femoral bone mass and increases osteoclast number in male mice, while no changes were observed in female mice, indicating a sex-based bias. Further experiments focusing on male-derived *Per1* knockout cells show accelerated osteoclast differentiation. Mechanistically, *Per1* regulates a set of inflammatory genes. Knockdown of these inflammatory genes, such as *NLR family pyrin domain containing 3* (*Nlrp3*), promotes osteoclastogenesis in a manner similar to *Per1* depletion. Interestingly, these effects are specific to *Per1*, as *Per2* knockout does not alter bone mass or osteoclast formation, despite the high homology between the two proteins [55].

PER2 is best known for its role in systemically controlling the sleep–wake cycle, and mutations in this gene can lead to disrupted sleep timing [56,57]. *PER2* plays a different role and appears to influence bone primarily through mitochondrial activity and energy production rather than osteoclast regulation. Recent studies show that *PER2* supports the mitochondrial activity required for the osteogenic and **odontoblastic differentiation** of stem cells, linking circadian timing to the energy demands of lineage commitment [58]. These findings suggest that *PER2* influences bone formation primarily by increasing intercellular ATP levels and mitochondrial membrane potential. Together, these findings indicate that PER1 and PER2 contribute to skeletal physiology through distinct mechanisms, with PER1 acting as an inflammation-linked brake on osteoclastogenesis and PER2 supporting metabolic pathways that facilitate osteogenesis. While PER3 is a paralog of PER1 and PER2 and contributes to the circadian clock, it is considered less essential to the TTFL, and little is known about its role in bone [59].

REV-ERBs

The REV-ERBs comprise two isoforms, REV-ERB α and REV-ERB β . In bone, REV-ERB α is the dominant isoform and functions mainly as a brake on osteoclast differentiation. Its expression declines during RANKL-induced osteoclastogenesis, and knockdown markedly increases osteoclast formation. Using REV-ERB α agonists, such as SR9009, suppresses *nuclear factor of activated T-cells 1* (*Nfatc1*), *cathepsin K* (*Ctsk*), and tartrate-resistant *acid phosphatase* (*Acp5*; TRAP) expression, inhibits NF- κ B and the mitogen-activated protein kinase (MAPK) signalling, and reduces reactive oxygen species production, resulting in impaired osteoclast activity and reduced bone resorption in female mice [60].

REV-ERBs also regulate **osteoblastogenesis**, indicating that their effects extend beyond osteoclast control. Silencing both *Rev-erb α* and *Rev-erb β* *in vitro* increases osteoclastogenesis,

whereas knockdown of either alone produces a milder effect. In osteoblasts, REV-ERB α is the predominant regulator: its knockdown enhances differentiation, while its overexpression suppresses maturation and RUNX2 activity via the p38 MAPK pathway. In contrast, REV-ERB β downregulation has little effect on osteoblast differentiation. Together, these findings show that REV-ERB α integrates circadian timing with the regulation of both osteoclast and osteoblast development, and that its loss promotes osteoclastogenesis through p38 MAPK-related mechanisms [61,62]. Consistent with this, nuclear receptor co-repressor 1 (NCOR1), one of the corepressors recruited by REV-ERBs, also restrains osteoclast differentiation, and its deletion increases osteoclast number [63].

ROR nuclear receptors

Amongst the three members of the ROR subfamily (ROR α , ROR β , and ROR γ), ROR α is the most studied in bone. In a mouse model of jetlag combined with periodontitis, activation of *Rora* restored circadian rhythmicity, reduced osteoclastic activity, and limited alveolar bone resorption, indicating a protective role for *Rora* under both rhythm-disrupted and normal periodontal conditions [64]. In human MSCs, *ROR α* is upregulated during osteogenic differentiation, while under non-differentiating conditions, only *ROR α* and *ROR γ* are expressed, with no detectable *ROR β* expression [65]. ROR α also appears to support bone formation and maintenance, as demonstrated in the osteopenic ROR α -deficient staggerer mice that exhibit reduced bone mineral content [65]. Conversely, *Ror β* expression is decreased during osteoblastic differentiation *in vitro*, and ROR β levels in bone samples in mice and humans increase during ageing, supporting the view that ROR β inhibits osteogenesis [66]. However, in a different study, in a *Ror β* mutant mouse model, early-onset osteoporosis was observed at 4 weeks of age, with impaired osteogenic differentiation of the underlying trabecular bone [67], indicating that ROR β may play context-dependent, stage- or cell-type-specific roles in osteogenesis.

The effect of extrinsic cues on bone circadian rhythms

While the clock described previously operates intrinsically in bone cells, their rhythms are also strongly influenced by external cues [68]. Endocrine factors such as melatonin, parathyroid hormone (PTH) and glucocorticoids (GCs), organ-derived cues from the gut and liver, and mechanical inputs such as the timing of exercise contribute to the timing and organisation of skeletal function. Together, these signals help synchronise or modulate circadian rhythms in bone tissue throughout the skeleton.

Endocrine and hormonal signals

Systemic hormonal signals interact with circadian regulation in bone tissue. Amongst these, melatonin plays a central role as the major endocrine mediator of the day–night cycle, secreted rhythmically by the **pineal gland** in the brain during the dark phase [69]. Beyond its canonical role in sleep–wake regulation, melatonin enhances osteogenesis while reducing oxidative stress, and its supplementation has been shown to improve bone mass in both normal and osteoporotic models [70,71]. The melatonin pathway also contributes to the circadian regulation of resorption. When this pathway is disrupted, the melatonin-related gene casein kinase 1 delta (CSNK1D) is upregulated, and recent evidence shows that CSNK1D contributes to osteoporosis by promoting osteoclast differentiation and reducing osteoblast function. These findings position CSNK1D as a key link between circadian biology, melatonin signalling, and bone metabolism [72]. Conversely, melatonin supplementation directly suppresses osteoclast activity while promoting osteogenesis [71]. Notably, some *in vivo* rodent models may not be suitable to determine the role of melatonin in bone health, for example, C57BL/6J mice that have negligible pineal melatonin production [73]. This is due to mutations in the *aralkylamine N-acetyltransferase* (*Aanat*) and *acetylserotonin O-methyltransferase* (*Asmt*) genes responsible for the enzymatic conversion of serotonin to melatonin.

PTH, secreted rhythmically by the **parathyroid glands**, is also an important circadian regulator since it influences osteoblast activity, and osteoblast responsiveness to PTH varies across the day due to circadian shifts in downstream cAMP- and calcium-dependent signalling [74]. Beyond its established use in osteoporosis therapy, PTH also stimulates osteoblast differentiation and proliferation, and alterations in its circadian rhythm have been observed in postmenopausal osteoporosis [75]. GCs are another major systemic cue with strong circadian rhythmicity. Experimental disruption of the normal GC rhythm, even in the absence of **hypercortisolism**, produces an osteoporotic phenotype in mice that resembles bone loss seen during pharmacological GC treatment [76]. GC rhythms dampen with ageing, becoming progressively misaligned with the light–dark cycle and thereby losing their capacity as an effective temporal signal for bone. This disruption to GC rhythms impairs circadian coordination of bone turnover and contributes to age-related bone loss [77]. Similar disruptions occur in shift work and jet lag conditions. Computational modelling studies show irregular light schedules disturb the central clock in the SCN, which in turn destabilises the normal GC rhythm [78]. These findings indicate that loss of the normal GC rhythm disrupts circadian control in bone and may help explain the increased fracture risk associated with GC treatment, shift work, and ageing.

Systemic circadian signals originating from peripheral organs also influence skeletal homeostasis. Rhythmic cues generated outside the skeleton contribute to osteoclast regulation, highlighting that bone is not an isolated tissue but part of a broader body-wide circadian network. A clear example of this interorgan regulation is provided by the gut. Deletion of BMAL1 in colon epithelial cells impairs trabecular bone mass acquisition in males, while females remain protected, revealing a sex-dependent circadian gut–bone axis [79]. This sex-dependence aligns with the broader circadian biology, where sexual dimorphism has been shown to influence metabolism and homeostasis in other tissues [80]. The liver provides another route of communication through cell-derived secretomes, most notably extracellular vesicles (EVs). EVs are nanosized particles that transport bioactive molecules between cells and organs. In sirtuin 2 (SIRT2)-deficient livers, hepatocytes release EVs enriched in leucine-rich alpha-2 glycoprotein 1 (LRG1). These vesicles are taken up by bone marrow monocytes, where LRG1 blocks NF- κ B nuclear translocation and prevents osteoclast differentiation. Both preclinical and clinical data show that hepatocyte-derived LRG1-small EVs protect against osteoporosis [81]. Immune mechanisms also contribute to circadian disruption by reducing IL-10 coming from regulatory T cell IL-10 expression, diminishing Treg ability to restrain osteoclasts, which leads to bone loss. Restoring IL-10 rescues skeletal integrity [82].

Thus, bone rhythms are not only shaped by the intrinsic clock within bone cells but also by extrinsic signals. Building on this, mechanical loading represents another powerful extrinsic signal that can further fine-tune skeletal rhythms.

Circadian control of mechanoadaptation

Osteocytes embedded within the bone matrix act as key mechanosensors and regulators of skeletal homeostasis. Unlike osteoblasts and osteoclasts, osteocytes exhibit limited direct involvement in bone matrix turnover; instead, they coordinate this process indirectly by sensing mechanical load and releasing regulatory factors that modulate osteoblast and osteoclast activity. Additionally, osteocytes have been implicated in linking circadian regulation with bone mechanosensing. A major circadian-controlled pathway in osteocytes is the wingless-related integration site (WNT) signalling system. The rhythmic expression of WNT signalling components, particularly the WNT inhibitors sclerostin (SOST) and Dickkopf-1 (DKK1), directly influences bone formation. Tibial loading performed during the early active phase elicits a stronger osteogenic response than loading during the rest phase, a pattern that corresponds with reduced SOST and DKK1

expression [2]. Notably, *Sost* and *Dkk1* were found to be diurnally expressed at the mRNA level in bone, identifying them as clock-controlled genes; however, this rhythmicity was not observed at the protein level, and importantly, mechanical loading itself did not affect clock gene expression [2]. This finding illustrates a ‘time-of-day-dependent mechanoadaptation’, ensuring that bone formation is optimized when mechanical activity and circadian signals are appropriately aligned.

Perhaps the most striking demonstration of circadian–bone interplay in mechanoadaptation arises from the timing of exercise. Mice that exercise during the early active phase exhibit increased bone length and trabecular mass compared with those exercising later in the circadian cycle. Early active-phase exercise enhances bone adaptation through increased **oxidative phosphorylation (OXPHOS)**, ATP production, and rhythmic activation of WNT and AMP-activated protein kinase pathway (AMPK) signalling in growth plate chondrocytes. Inhibition of OXPHOS abolishes these benefits, whereas pharmacological activation of OXPHOS enhances them [83]. These findings may also reflect time-of-day-dependent systemic signals that influence skeletal adaptation. However, studies in aged rats show that exercise improves tibial bone mass and structure regardless of timing, suggesting that aging reduces the circadian sensitivity of the skeleton [84]. In humans, deconsolidation of circadian rhythms is observed with aging, but this has largely been unexplored with regards to the skeletal system [80,85]. Circadian regulation also contributes to skeletal development, where growth plate chondrocytes display daily rhythms in proliferation that support normal ossification in male mice [86]. Although not tested in bone specifically, mechanical loading and hyperosmolarity do affect the phase and amplitude of circadian clocks in cartilage and in intervertebral discs in the spine through the PLD2–mTORC2–AKT–GSK3 β axis [87]. This implies similar potential in load-bearing skeletal tissues such as bone, which requires further study. BMAL1 also supports **osseointegration**, the process by which bone forms a direct interface with a surgical implant. This process requires rhythmic regulation for proper implant integration with bone [42]. In addition, endothelial BMAL1 regulates angiogenesis–osteogenesis coupling, suggesting that circadian disruption within the vasculature can compromise bone growth [88].

Together, these findings indicate that bone biology is shaped by circadian timing at multiple levels, opening the possibility that treatments could benefit from being matched to the body’s rhythms. This concept underlies the current interest in chronotherapeutic strategies for skeletal health.

Sex differences in circadian bone regulation

Prevailing research trends indicate that women may have more robust daily rhythms and greater resilience to circadian disruption than men, and the internal body clock runs slightly shorter in women [89,90]. Bone is strongly influenced by sex, as women have less dense bones and face a much higher risk of osteoporosis and fracture than men [91], making sex an important variable when studying circadian bone regulation. Both older men and women show daily rhythms in bone turnover markers, but these differ between sexes: the nighttime peak in bone resorption is higher in women, while PTH is higher in men—differences that may help explain divergent rates of age-related bone loss [92]. In healthy adults aged 52–76, the C-terminal telopeptide of type I collagen (CTX) shows a larger amplitude in women than men, while procollagen type 1 B-terminal propeptide (P1NP) shows a nighttime rise in men but not in women [93]. In young adults, however, the CTX is lower in females than males, possibly reflecting hormonal contraceptive use or reproductive stage [27]. Removal of ovarian hormones by ovariectomy in mice dampens clock gene oscillations in the femur and spine, suggesting the bone clock may become less well-coordinated after menopause [94]. Together, these findings show that sex differences in circadian bone rhythms change with age, hormone levels, and reproductive status, and that the circadian clock does not regulate bone in the same way in men and women.

Potential for chronotherapeutics in skeletal health

Chronotherapeutics uses endogenous circadian rhythms to guide the timing or targeting of therapeutic interventions [95]. In skeletal health, chronotherapeutics represents a promising strategy, as bone remodelling is regulated by molecular clocks within bone cells, as well as in endothelial and immune cells [96]. Moreover, the effectiveness of established anabolic stimuli, such as mechanical loading, is strongly influenced by the time-of-day. Disruption of this circadian rhythm has been associated with negative effects on skeletal health; however, the underlying mechanisms remain unclear. Understanding how therapeutic pathways align with biological timing offers an opportunity to optimise both pharmacological and regenerative interventions, while also minimising potential side effects (Figure 2). Furthermore, less research has been conducted on manipulating circadian rhythms by drugging the clock itself through antagonising or agonising individual components of the molecular machinery, which represents a promising avenue for discovery in bone research [80]. A major implication of these discoveries is that pharmacological

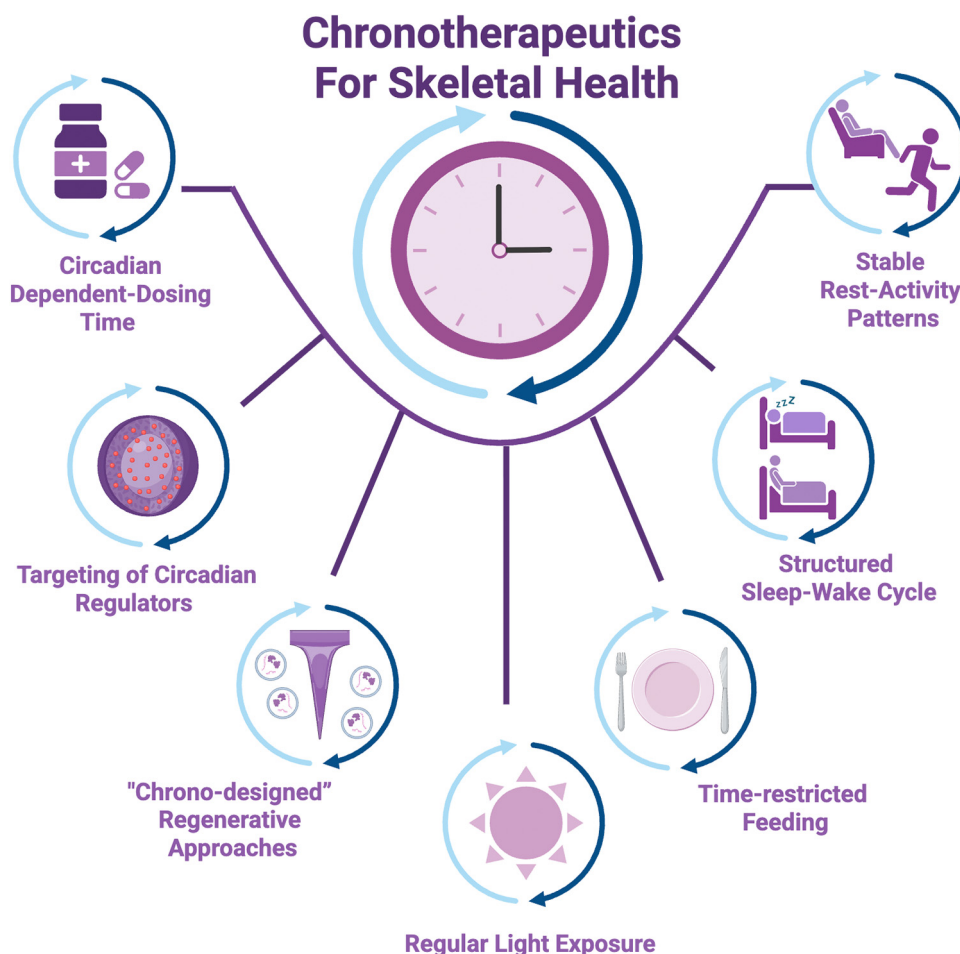
Clinician's corner

Bone responds to both biological timing and mechanical cues; however, the influence of time-of-day on treatment or rehabilitation is not yet widely recognised in clinical practice.

Evidence shows that rhythms in bone activity change across the day, which may affect responses to treatment or rehabilitation. While treatment timing is not routinely considered, future time-based approaches would need to balance potential benefits with feasibility and patient adherence.

A growing number of studies show that time-of-day effects are currently stronger for mechanical loading and exercise, suggesting that the timing of exercise may support bone adaptation and recovery.

As the understanding of circadian regulation in bone improves, timing may become an additional, noninvasive component of skeletal therapy.



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Figure 2. Schematic overview of proposed chronotherapeutic approaches for skeletal health. Circadian rhythm can be targeted through multiple strategies to improve skeletal outcomes. These include circadian-dependent dosing time of pharmacological therapies, direct targeting of molecular clock components such as REV-ERBs and chrono-designed regenerative approaches, such as microneedle delivery of circadian-conditioned cell-free products. In parallel, lifestyle interventions like regular light exposure, time-restricted feeding, structured sleep–wake cycles, and stable rest–activity patterns support circadian alignment and skeletal homeostasis. The figure was created with BioRender (www.biorender.com).

efficacy depends strongly on dosing time [97]. Beyond timing alone, recent studies show that drugs can act directly on circadian regulatory pathways to enhance skeletal outcomes. For example, the vitamin D analogue eldecacitol (ED-71) increases bone mass in diabetic mice by upregulating BMAL1, reducing oxidative stress, and activating sirtuin 1 (SIRT1) while suppressing glycogen synthase kinase 3 beta (GSK3 β). These effects are attenuated by SIRT1 inhibition and fully reversed by REV-ERB agonists, demonstrating that circadian repressors can counteract pharmacological stimulation of osteogenesis [98]. Natural compounds point in the same direction: icariin, a plant-derived flavonoid, promotes osteogenic differentiation through BMAL1–BMP2 signalling, illustrating how nutraceuticals may also modulate circadian pathways that govern bone formation [99].

Recent advances in biomaterial-based delivery of chronotherapeutics further extend strategies into regenerative medicine. Rather than acting systemically, these approaches directly target circadian regulators within the local bone microenvironment. Porous microspheres loaded with a REV-ERB modulating compound accelerate alveolar bone repair by reshaping inflammatory dynamics, demonstrating that REV-ERBs can be effectively targeted using small-molecule approaches [100]. These findings show that drug-delivery platforms can be engineered to deliver circadian-compatible release profiles, effectively incorporating ‘chrono-design’ principles into regenerative biomaterials and offering a new route for targeting bone rhythms to enhance healing efficiency.

Melatonin-based therapies are also relevant for chronotherapeutic approaches. Melatonin reinforces daily biological rhythms and has direct effects on oxidative stress, inflammation, and osteogenesis [27]. These properties can be applied in cell-free therapies; when adipose-derived stem cells are preconditioned with melatonin, they produce EVs enriched with factors such as platelet factor-4, which more effectively reduce inflammation and support skeletal repair. A microneedle system has also been developed to provide sustained release of these EVs at the injury site. This demonstrates that circadian regulation can be embedded directly into the therapeutic product, with melatonin-regulated EVs and supporting biomaterials serving as practical examples of using circadian-regulated components for regenerative approaches [101].

Finally, chronotherapy also extends to lifestyle and behavioural interventions. Time-restricted feeding, structured sleep–wake cycles, regular light exposure, and stable rest-activity patterns all influence bone turnover rhythms and may help restore circadian organisation in individuals experiencing chronic disruption, such as shift workers. Maintaining consistent timing of sleep, rest, and daily activities supports circadian stability, and these nonpharmacological strategies may help preserve bone health and reduce fracture risk [25,70,102].

Altogether, these approaches position chronotherapeutics as a promising strategy for improving skeletal health. By integrating circadian timing into pharmacological treatments, biomaterial design, and regenerative approaches, it is possible to enhance therapeutic precision and better support the rhythms that regulate bone physiology.

Concluding remarks

Research on circadian biology and skeletal physiology is now advancing in parallel, revealing that bone tissue is far more rhythmic than previously appreciated. Yet this progress also raises several unresolved questions (see [Outstanding questions](#)), particularly concerning the roles of individual core clock proteins. Many circadian regulators, including PER3 and ROR γ , remain only partially understood in bone. A further challenge comes from the distinction between global and tissue-specific circadian clocks: while the central clock sets systemic rhythms, peripheral clocks in

Outstanding questions

Are there distinct patterns of circadian regulation across bone cell types that explain why the manipulation of the same clock gene produces different outcomes in osteoblasts, osteoclasts, and osteocytes?

Is it more effective to improve bone health by directly targeting core clock proteins, or by timing existing bone-active treatments to align with daily rhythms?

How does circadian timing influence bone responses to mechanical loading and exercise, and can this be used to optimise therapeutic approaches?

How can insights into circadian regulation of bone be translated into therapeutic strategies that improve treatment efficacy without compromising bone quality or long-term skeletal function?

osteoblasts, osteoclasts, osteocytes, and stem cells can generate cell-specific outcomes. This likely contributes to the differing phenotypes seen in global versus cell-specific knockout/knock-down studies. Clear reporting and careful interpretation of genetic models will, therefore, be essential for understanding clock-dependent effects on bone biology.

Beyond the molecular complexity of the clock itself, sex represents an important but under-explored biological variable in circadian bone regulation. Dedicated studies are needed to understand how sex modifies circadian clock function within osteoblasts, osteoclasts, and osteocytes, and sex should also be treated as a primary variable when evaluating chronotherapeutic strategies for osteoporosis, including the optimal timing of anabolic and antiresorptive treatments. The skeletal consequences of circadian disruption through shift work or sleep restriction may also differ between men and women, and across age groups, which need further investigation.

There is also growing recognition that bone is part of a wider rhythmic network. Studies on the gut–bone, liver–bone, and immune–bone axes show that extrinsic cues outside the skeleton shape bone turnover, and further exploration of these interorgan interactions remains an important direction. Mechanical loading adds a behavioural and systems layer to this regulation, but how mechanorhythms vary with age, disease, or sex remains largely unknown.

Finally, these insights provide a strong rationale for chronotherapeutic strategies (see [Clinician's corner](#)). Although time-dependent effects on bone are increasingly observed, we still lack a framework for determining the most effective timing for treatment or for aligning interventions to an individual's internal clock, which would encompass a new form of personalised therapy [103]. Future work should clarify how intrinsic clocks, systemic cues, and mechanical rhythms interact *in vivo*, and how these relationships can be harnessed to improve bone regeneration, reduce fracture risk, and enhance responses to existing and emerging therapies. These insights further support the development of chronotherapeutic strategies that align bone-targeted treatments and regenerative approaches with the body's internal circadian timing.

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Declaration of interests

A.M.C. is a member of the *Trends in Molecular Medicine* Scientific Advisory Board.

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