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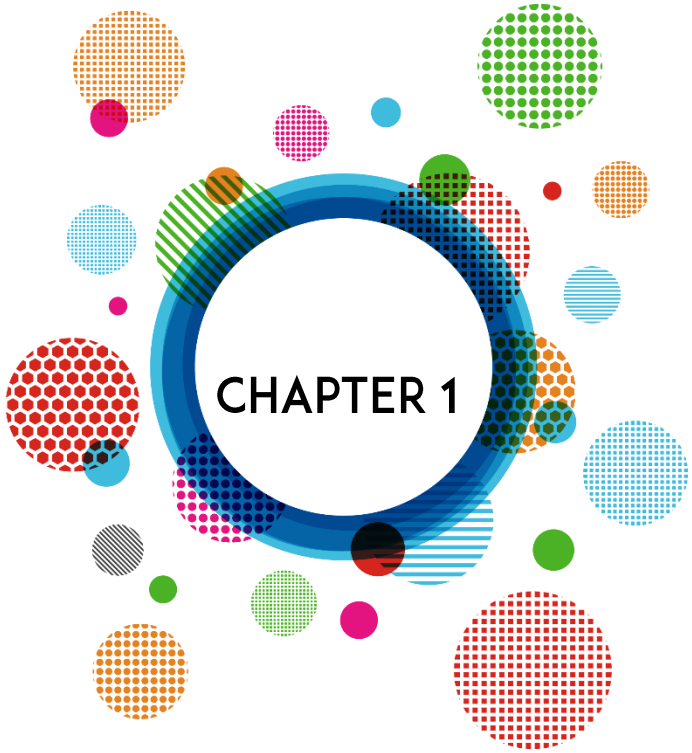

The Function of Sirtuins in Bone Biology and Osteoporosis

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1. Introduction

Bone is a dynamic material that continuously alters its structure in response to functional requirements, regulated by two main cell types: osteoblasts and osteoclasts (Harada and Rodan, 2003). The disproportionate activity of these two cell types interferes with bone development and resorption, resulting directly or indirectly in the emergence of numerous bone disorders (Rodan and Martin, 2000). Osteoporosis (OP) is a prevalent metabolic bone disorder characterised by diminished bone mass, reduced bone mineral density, and deterioration of bone tissue microarchitecture, resulting in increased bone fragility and an elevated risk of osteoporotic fractures (Kanis, 1994; Garnero and Delmas, 1997; Adami et al., 1995; Kupai et al., 2024; McCloskey et al., 2024). The pathophysiology of OP entails a disproportion between bone resorption and production, driven by hormonal alterations, aging, dietary inadequacies, and genetic predispositions (Harada and Rodan, 2003; Raisz, 2005). The interplay between osteoblasts and osteoclasts is crucial for maintaining bone homeostasis, ensuring a reasonably steady environment for bone. Upon activation, osteoblasts produce and release the receptor activator of nuclear factor-κB ligand (RANKL), facilitating the development of osteoclast forerunner into fully developed osteoclasts. Conversely, when bone resorption results in structural damage, osteoclasts may release insulin-like growth factor to facilitate distinction of osteogenesis (Gao et al., 2018). Abnormal bone remodelling affects bone mass. Osteoclasts, which break down the extracellular matrix, are limited active, resulting in increased bone resorption, while osteocytes, which produce mineralised organic extracellular matrix, are more active. However, a combination of these factors may be responsible for bone deterioration. The OP encompasses both main and secondary forms. Senile osteoporosis (SOP) and postmenopausal osteoporosis are two classifications for primary osteoporosis (Waykar et al., 2024). Secondary osteoporosis may result from pharmacological agents, extended immobilisation, hereditary factors, endocrine abnormalities, chronic renal illness, haematological conditions, inflammatory joint diseases, dietary and gastrointestinal issues, and connective tissue problems (North American Menopause Society, 2006; Favero et al., 2023; Sobh et al., 2022; Ebeling et al., 2022; El-Gazzar and Högler, 2021; Colangelo et al., 2019; Sheu and Diamond, 2016; Mirza and Canalis, 2015). Building effective methods for the prevention and treatment of osteoporosis requires a comprehension of the mechanisms of bone restructuring and the variables influencing bone mass.

Sirtuins represent a potential new therapeutic target among the several pathways implicated in the progression of OP (Li et al., 2021; Chang and Guarente, 2014). SIRT1 to SIRT7 are among the group of histone deacetylases known as sirtuins that are reliant on nicotinamide adenine dinucleotide (NAD+).

It has been shawn that sirtuins possess a multifaceted and all-encompassing defence mechanism against OP (Yamaguchi and Yoshino, 2017). In addition to regulating bone resorption, sirtuins also have an effect on intramembranous and endechondral osteogenesis, which in turn has an effect on bone production, mineral density, and the maintenance of bone strength. To protect the skeleton from teh effects of aging and bone loss caused by fixation, the activation of sirtuins is very necessary. (Almeida and Porter, 2019). This suggests that sirtuins might be a viable target for the treatment of OP. Moreover, it has been shown that sirtuins affect common elements linked to obesity and OP, including insulin, gastrointestinal microbiota, and mesenchymal stem cells, which in turn affect adipogenesis and osteogenesis.(Qiang et al., 2012; Jiang et al., 2015; Lu, 2023; Qu et al., 2020). To improve our understanding of the ways in which sirtuins interact with bone, it is necessary to do research on the biological functions of sirtuins in diseases that are associated with bone.

2. Bone Formation

Bone formation is a meticulously orchestrated process that entails the differentiation and interaction of various cell types within the bone marrow microenvironment, including bone marrow-derived stem cells (BMSCs), osteogenic precursor cells, adipocytes, osteoblasts, osteoclasts, bone marrow macrophages (BMMs), and osteocytes, each contributing significantly to the maintenance of bone homeostasis and the facilitation of bone remodelling (Harada and Rodan, 2003; Notelovitz, 1993; Rachner et al., 2011; Abdallah et al., 2015; Ohata and Ozono, 2014; Hu et al., 2024; Zhu et al., 2024; Li et al., 2024). As multipotent cells, BMSCs may develop into a variety of cell types, including chondrocytes, adipocytes, and osteoblasts. Osteogenic precursor cells are stored in BMSCs. When osteogenic signals are received, osteogenic precursor cells proliferate and develop into osteoblasts, aiding in the production and mineralisation of bone matrix. (Abdallah et al., 2015). Certain osteoblasts are entombed inside the mineralised matrix as a result of the bone formation process (Zou et al., 2020). Upon embedding, these osteoblasts develop into osteocytes. Osteocytes are essential since they function as mechanosensors, identifying mechanical strain inside the bone (Zou et al., 2020). In response to mechanical stress, this sensory capability ensures the maintenance of bone strength and integrity by faciliating the regulation of bone remodelling through communication with other bone cells. The overall osteogenic capacity of bone marrow adipocytes, which come from the same BMSCs, is influenced by substances released by these cells that impede osteoblasts development. This balance is particularly important in diseases like OP and aging. (Abdallah, 2017). Osteoclasts originate from BMMs and are tasked with bone resorption,

dismantling the mineralised bone matrix, and creating resorption voids that are subsequently filled with new bone matrix produced by osteoblasts.

The concerted activities of osteoclasts and osteoblasts facilitate ongoing bone repair and maintain bone integrity during the regulated process of bone remodelling (Cosman et al., 2014). Overall bone loss and increased osteoclasts activity are signs of an imbalance between these processes that leads to OP (Elango et al., 2018). Cytokines are crucial in regulating bone remodelling, with the RANK/RANKL/osteoprotegerin (OPG) signalling pathways being especially significant. RANKL, synthesised by osteoblasts, binds to the RANK receptor on osteoclasts, prompting their differentiation and activation (Harada and Rodan, 2003). By acting as a RANKL decoy receptor, OPG prevents this interaction and hence inhibits osteoclastogenesis. When elevated RANKL levels are combined with an imbalance in the RANK/RANKL/OPG pathway, osteoclast activity increases and bone loss follows (Harada and Rodan, 2003). Numerous cytokines and hormones, most notably estrogen and parathyroid hormone (PTH), modulate the release of RANKL. Whereas estrogen reduces osteoclast activity via the RANK/RANKL/OPG signalling pathway, PTH increases it (Li et al., 2020).

The bone remodelling process has four sequential phases: 1) The resorption phase, regulated by RANKL and macrophage colony-stimulating factor (M-CSF), facilitates the development of osteoclast precursors produced from haematopoietic stem cells into mature multinucleated osteoclasts. At these particular stage, mature osteoclasts, which have distinctive folding edges, are responsible fort he resorption of bone by the secretion of histone K, H^+, and Cl^- in the sealing zone. Subsequently, these osteoclasts detach from the surface of the bone and undergo apoptosis. (Harada and Rodan, 2003). 2) During the reversal phase, mesenchymal-derived osteoblasts proliferate anda re attracted to the resorption site when Wnt, bone morphogenetic proteins (BMPs), and transforming growth factor-β (TGF- β) are present. 3) Formative phase, during which osteoblasts synthesise a new organic bone matrix. 4) Ultimately undergo mineralisation. RANKL is secreted by additional immune cells in the absence of estrogen or in the presence of inflammation, which leads to increased osteoclast activity and augmented bone resoprtion, ultimately resulting in OP (Li et al., 2020).

3. Pathogenesis of Osteoporosis

OP primarily results from an imbalance between bone resorption and bone production, resulting in reduced bone mass and deterioration of the microstructure of bone tissue (Vasikaran and Chubb, 2016). This imbalance is affected by several variables, including as estrogen, aging, oxidative stress, mechanical stimulation, inflammation, obesity, and metabolic diseases (Giorgio et al., 2019; Stepan et al., 2019). Estrogen shortage, particularly in

postmenopausal women, significantly contributes to enhanced bone resorption by osteoclasts and diminished bone synthesis by osteoblasts (Notelovitz, 1993). In order to maintain bone density, estrogen regulates osteoclast apoptosis and the synthesis of RANKL and OPG, two crucial mediators of osteoclastogenesis (Manolagas, 2010). In the event of reduced estrogen levels, the up-regulation of RANKL and the down-regulation of OPG ocur, thereby increasing osteoclast activity and bone resorption, therefore resulting in postmenopausal OP (Rodan and Martin, 2000; Edwards and Mundy, 2011). SOP is defined by diminished bone growth (Golob and Laya, 2015). Research indicates that SOP-containing BMSCs experience senescence, linked to telomere destruction, oxidative stress, and genetic and epigenetic modulation (Li et al., 2017). Additionally, studies have shown that senior citizens who smoke for an extended period of time have a lower bone mineral density, especially in the hip region (Zou et al., 2020).

One common form of secondary OP is glucocorticoid-induced osteoporosis (GIO), which is marked by decreased bone formation and accelerated bone resorption (Herath et al., 2022). The Wnt signalling pathway, which is essantial for osteoblast function and viability, is inhibited by glucocoticoids, which subsequently impair osteoblast proliferation and differentiation (Ohnaka et al., 2005; Cheng et al., 2022). Furthermore, by decreasing OPG production and increasing RANKL expression, glucocoticoids promote osteoclastogenesis. (Herath et al., 2022). The combined impact on bone remodelling may lead to considerable bone loss and an elevated risk of fractures (Herath et al., 2022). Moreover, glucocorticoids elevate oxidative stress by increasing the formation of reactive oxygen species (ROS) (Callaway and Jiang, 2015). The elevated ROS levels lead to increased osteoclast activity by boosting the production of RANKL. Additionally, these levels impair osteoblast function by triggering apoptosis and inhibiting differentiation (Callaway and Jiang, 2015).

Oxidative stress and inflammation are pivotal in the aetiology of OP. ROS generated by external stresses and cellular metabolism may promote osteoclast differantiation and cause osteoblast apoptosis, leading to bone loss (Callaway and Jiang, 2015). Chronic inflammation is indicated by elevated levels of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, which exacerbate osteoclast activity and suppress osteoblast operations (Awasthi et al., 2018; Pouresmaeili et al., 2018). By establishing a detrimental loop that accelerates bone resorption and hinders bone production, the interaction between oxidative stress and inflammation contributes to the progression of OP (Zou et al., 2020).

Furthermore, new studies have emphasised how metabolic factors affect skeletal health. Research indicates that elevated blood glucose levels and advanced glycosylation end products (AGEs) promote apoptosis in osteoblasts and impede the production of RANKL and osteosclerostin, both critical for the

restructuring of bones (Tanaka et al., 2015; Cipriani et al., 2020). The proliferation of bone marrow adipose tissue (BMAT), which occurs with increasing age and obesity, has a negative impact on bone metabolism. It does this by releasing adipokines and inflammatory cytokines, which stimulate osteoclastogenesis and inhibit osteoblast activity (Li et al., 2020). In order to diagnose and treat OP, these findings highlight the need of comprehending the intricate connections between obesity, inflammation, oxidative stress, aging, hormones, and metabolism.

Comprehending and examining the pathophysiology of OP is crucial for therapeutic therapy. Despite the ability of current treatments (e.g., bisphosphonates, estrogen replacement therapy) to moderately decelerate bone loss, their adverse effects, safety issues regarding prolonged use, and limited effectiveness in specific patient demographics indicate a persistent clinical demand for improved prevention and management of OP (Reginster and Burlet, 2006). These medications often have a number of side effect. For example, biphosphonates might result in gastrointestinal irritation, jaw osteonecrosis, and atypical femur fractures, while estrogen replacement therapy can increase the risk of breast cancer and cardiovascular disease (Khosla and Hofbauer, 2017; Rossouw et al., 2002). Moreover, the safety of prolonged use of these medications has generated significant apprehension, particularly among patients with chronic conditions necessitating extended therapy (Black and Rosen, 2016). Consequently, novel therapeutic techniques and targets are critically required to enhance therapy effectiveness and reduce adverse effects. In order to achieve a superior balance between preventing bone resorption and promoting bone formation while lowering the possibility of side effects, optimal treatments must be safe and effective. Researching new therapeutic targets and developing new medications will address current clinical demands and provide new possibilities for personalised OP treatment, improving patient outcomes and quality of life.

4. Sirtuins Family

The sirtuins are a family of longevity proteins that were initially identified as lifespan regulators in yeast. They are conserved NAD^+-dependent deacetylases that modulate essential signalling pathways in both prokaryotes and eukaryotes, and they are involved in a variety of biological processes (Dai et al., 2018). The sirtuin family has seven members (SIRT1-SIRT7), each possessing distinct but overlapping activities, and may be categorised into four kinds based on their structural similarities (Jiao and Gong, 2020). SIRT1, SIRT2, and SIRT3 are classified as type I, SIRT4 as type II, SIRT5 as type III, and SIRT6 and SIRT7 as subtypes IVa and IVb of type IV, respectively (Yu and Auwerx, 2009; Yamamoto et al., 2007). A number of diseases, such as cardiovascular conditions, endocrine

disorders, neoplasms and respiratory disorders, are considered to be potential therapeutic targets fort he sirtuins family, which is comprised of NAD^+-dependent deacetylases and ADP ribosyltransferases. These enzymes play an important role in a number of processes, including apoptosis, inflammation, cellular metabolism, stress resilience and aging (Ivy et al., 1986; Kennedy et al., 1995; Kaeberlein et al., 1999). SIRT1 is the most extensively researched component and has been shown to modulate many pathways related to ageing and metabolic equilibrium, including glucose and lipid metabolism, mitochondrial function, and inflammation (Tissenbaum and Guarente, 2001; Vaziri et al., 2001; Yeung et al., 2004). SIRT1 exerts these effects via the deacetylation of critical transcription factors and co-regulators, including p53, NF-κB, and PGC-1α (Yamamoto et al., 2007; Rodgers et al., 2005; Haigis et al., 2006).

SIRT1 is unable to perform its deacetylation function without the presence of NAD+-, which also plays a role as a cofactor in the process of repairing DNA damage. The cytoplasm contains a large amount of SIRT2, which is involved in the organisation of the cytoskeleton and the control of the cell cycle. It has also been shown to deacetylate histones and microtubule proteins, which impacts gene expression and cell division (Finkel and Deng, 2009; McCord et al., 2009). The mitochondrial logevity proteins SIRT3, SIRT4 and SIRT5 are responsible for modulating mitochondrial activiy and metabolism (Yamamoto et al. 2007). SIRT3 enhances ATP production and lowers oxidative stress by deacetylating and activating enzymes associated with the tricarboxylic acid cycle and oxidative phosphorylation (Haigis and Sinclair, 2010; Kincaid and Bossy-Wetzel, 2013). SIRT4 mitigates the impact of caloric restriction on insulin secretion by inhibiting glutamate dehydrogenase (Haigis et al., 2006). SIRT5 regulates the mitochondrial lysine succinylome, influencing metabolic networks and oxidative stress responses (Rardin et al., 2013). Mostly found in the nucleusi SIRT6 and SIRT7 have a role in transcriptional regulation, genomic stability and DNA repair (McCord et al., 2009; Li et al., 2016). SIRT6 stabilises DNA-dependent protein kinases on chromatin by deacetylating histone H3, which makes it easier to repair DNA double-strand breaks (McCord et al., 2009). It has an effect on glucose metabolism via modulating the expression of genes involved in glycolysis (Yu and Auwerx, 2009). The significance of long-lived proteins in preserving genome integrity is highlighted by the discovery of SIRT7 as a histone desuccinylase linked to chromatin compaction and chromosomal stability (Li et al., 2016).

The participation of the Sirtuins family in several cellular processes renders them crucial contributors to health and illness. Sirtuins play an important role for the control of osteoblasts, hence ensuring proper bone growth and homeostasis;

dysregulation of sirtuin expression may result in several bone disorders, including OP (Li et al., 2021).

4.1. SIRT 1 enhances osteogenesis and reduces bone resorption.

Numerous in vivo and in vitro studies have shown that SIRT1 may modulate bone mass and microarchitecture by directly influencing numerous cell types inside the bone microenvironment. SIRT1 activity diminishes with age, as seen by decreased SIRT1 mRNA levels in bone (Edwards et al., 2013) and diminished NAD levels necessary for SIRT1 deacetylase activity (Gomes et al., 2013). SIRT1 double deletion mice exhibited not only increased perinatal mortality but also a reduced rate of bone mineralisation (Cheng et al., 2003; McBurney et al., 2003). The activation of SIRT1 leads to the promotion of bone formation while simultaneously blocking bone resorption and bone marrow adipogenesis in SIRT1 over-expressing preosteoblasts, osteoblasts, osteoclasts, and bone marrow mesenchymal stem cells. This phenomenon is also seen in SIRT1 deficient murine models. (Iyer et al., 2014; Artsi et al., 2019; Artsi et al., 214).

The influence of SIRT1 on bone is mostly facilitated by osteoblast lineages. SIRT1 activators or SIRT1 overexpression alleviate age-related bone mass loss associated with diminished osteoblast synthesis (Iyer et al., 2014; Herranz et al., 2010; Mercken et al., 2014). Furthermore, a mouse model that lacked SIRT1 in mesenchymal cell lines indicated that SIRT1 in osteoblasts and osteocytes led to an increase in trabecular bone mass. On the other hand, SIRT1 in osteoblast progenitors led to an enhancement of cortical bone by stimulating bone formation on the inner surface of the cortex via the process of bone formation (Edwards et al., 2013; Simic et al., 2013). Multiple lines of evidence indicate that SIRT1 may enhance bone growth by activating the Wnt signalling pathway. The deacetylation of FoxO by SIRT1 inhibits its interaction with β-catenin and amplifies Wnt signalling, resulting in heightened osteoblast proliferation (Iyer et al., 2014). SIRT1 may enhance bone formation by reducing the expression of the Wnt signalling antagonist Sost (osteosclerosis protein) (Stegen et al., 2018; Cohen-Kfir et al., 2011).

Several recent studies have shown that iron excess is a new risk factor for OP. This is because iron overload may produce oxidative stress and raise levels of ROS, which can lead to damage to DNA, proteins, mitochondria, and other organelles of osteoblasts. Additionally, it can alter the homeostasis of bone tissue, which eventually leads to bone mass loss (Xiao et al., 2015; Zhang et al., 2021). When the SIRT1 activator niacin was given to iron-overloaded MC3T3-E1 cells and osteoporotic rats, SIRT1 expression was increased, which resulted in higher SOD2 levels and less ROS, as well as higher expression of alkaline phosphatase (ALP) and the development of calcified nodules. This implies that niacin inhibits bone loss in osteoporotic rodents that are iron-overloaded by activating SIRT1.

(Tao et al., 2024). Likewise, resveratrol and silymarin may safeguard against iron overload-induced OP by augmenting antioxidant capacity via the upregulation of SIRT1 (Tao et al., 2022; Zhao et al., 2015).

The skeletal effects of SIRT1 were shown to be variably influenced by sex, with negative outcomes mostly seen in females. Skeletal SIRT1 levels were considerably reduced in male wild-type mice compared to female wild-type mice, indicating a higher expression of SIRT1 in female bones (Artsi et al., 2022). Secondly, SIRT1+/− female mice (12 weeks) had a significant decrease in bone mass relative to SIRT1+/− male mice, shown by decreased bone formation and heightened bone marrow lipogenesis [101]. The skeletal consequences of targeted SIRT1 deficit in osteoblasts are evident in females but not in males, similar to the findings in SIRT1+/− animals (Edwards et al., 2013; Iyer et al., 2014).

To be more precise, it has been shown that sex hormone receptors have the ability to alter bone mass and microarchitecture in a manner that is distinct to each gender (Almeida et al., 2017). In adult females, oestrogen function in the bones depends on the estrogen receptor alpha (ERα). The cortical bone mass of female mice was reduced as a result of the induced deletion of ERα, while male mice were unaffected. (Doolittle et al., 2022; Maatta et al., 2013). Similar to the previous example, the androgen receptor (AR) is primarily responsible for regulating cancellous bone in male mice. The knockdown of AR resulted in a decrease in cancellous bone mass in male mice, but this was not the case in female mice (Notini et al., 2007). Through further study, the effect of SIRT1 on bone was investigated in both males and females, with particular attention paid to ERα and AR. According to Artsi et al. (Artsi et al., 2022), ERα was significantly lower in SIRT1+/− female mice's bones compared to wild type, while it was much higher in SIRT1+/− mice's MSCs. However, AR was not seen in the bones of SIRT1+/− male mice.

Comparable findings were reported by Yao et al. (Yao et al., 2010), indicating a reduction of ERα in murine embryonic fibroblast cells derived from SIRT1−/− animals. This indicates that SIRT1's function in bone may be somewhat facilitated by its modulation of ERα, positioning SIRT1 as a prospective curative target for enhancing OP in female. The method by which SIRT1 modulates bone mass via ERα remains unclear, and it is still to be determined if the upregulation of SIRT1 successfully enhances ERα to ameliorate female OP, as well as whether SIRT1 influences androgen receptor regulation of bone mass in men.

4.2. Deficiency of SIRT2 may impede osteoclast differentiation and diminish bone loss.

AGK2, a selective SIRT2 inhibitor, inhibited bone marrow-derived monocytes (BMDMs) osteoclast formation by downregulating c-Fos and NFATc1 expression, according to an in vitro study (Jing et al., 2019). Moreover, given the high expression of SIRT2 in the liver (Wang et al., 2019), a recent research examined the potential involvement of hepatic SIRT2 in the advancement of OP. Liver-specific SIRT2 deficit (SIRT2 kohep) was shown to decrease osteoclast production and reduce bone deficit in an osteoporotic mice model. The mechanism performs by increasing the levels of leucine-rich alpha-2 glycoprotein 1 (LRG1) in small extracellular vesicles (sEVs) derived from hepatocytes in SIRT2-deficient hepatocytes. This leads to an increase in the translocation of LRG1 to bone marrow-derived macrophages (BMDMs), which in turn reduces the nuclear translocation of NF-κB p65 and inhibits osteoclast differentiation (Lin et al., 2023). SIRT2 seems to have a role in the control of osteoclast development and activity; additional research is required to elucidate the mechanisms by which SIRT2 influences these processes and its impact on age-relevant bone loss. The administrative effects of SIRT2 on various bone cells during the progression of osteoporosis require further examination.

4.3. SIRT3 enhances bone formation and suppresses bone resorption by mitigating oxidative stress.

SIRT3 is a NAD+-dependent deacetylase located in mitochondria, with various abilities to modulate mitochondrial form and function, including nutrient oxidation, ATP synthesis, and ROS generation (Lombart et al., 2007). Multiple lines of evidence indicate that SIRT3's influence on the osteogenic differentiation potential of osteoblasts, osteoclasts, and BMSCs is associated with oxidative stress levels. SIRT3 enhances ATP synthesis and osteoblast development via the deacetylation of SOD2 (Gao et al., 2018), while nicotine diminishes SIRT3 levels, ultimately resulting in OP (Li et al., 2015). SIRT3 deficiency in MC3T3-E1 cells, a murine preosteoblast cell line, may impair mitochondrial activity and biogenesis via the PGC-1α/SOD2 signalling pathway, resulting in osteogenic damage (Ding et al., 2017). Kim et al. (Kim et al., 2017) found that the knockdown of SIRT3 led to an increase in osteoclast development and RANKL-induced bone loss in 5-week-old female ICR mice. This occurs because SIRT3 inhibits osteoclast differentiation through the deacetylation of lysine 68, which enhances SOD2 activity and reduces intracellular ROS levels. Furthermore, as age advances, the adipogenic differentiation of BMSCs escalates while osteogenic differentiation diminishes, resulting in aberrant bone metabolism (Moerman et al., 2004; Jiang et al., 2008), with heightened oxidative stress being a significant contributing factor. By reducing ROS-induced deterioration and

elevating SOD2 expression and activity, SIRT3 supplementation may mitigate BM-MSCs senescence. SIRT3 expression is significantly reduced in the aged BM-MSCs model (Ma et al., 2020).SIRT3 mitigates oxidative stress-induced apoptosis in bone marrow stem cells by activating manganese superoxide dismutase (MnSOD) and catalase (Wang et al., 2014).

To conclude, SIRT3-mediated bone formation and resorption are intricately linked to oxidative stress levels. Nonetheless, the timing and methodology for transitioning the processes of osteogenic and lipogenic differentiation of BMSCs remain ambiguous. The osteogenic growth of bone marrow mesenchymal stem cells may be facilitated by SIRT3, which may also inhibit osteoclast differentiation and be responsible for maintaining bone mass stability. During the aging process, SIRT3 may play a role in the process of bone loss through facilitating the adipogenic differentiation of stem cells found in bone marrow, which in turn increases the development of osteoclasts and bone resorption. Furthermore, as SIRT3 knockdown impedes osteoclast differentiation with a little decrease in osteoblast synthesis throughout ageing (Liang et al., 2021), the mechanisms by which SIRT3 stabilises bone formation and resorption need further investigation.

4.4. SIRT6 regulates bone homeostasis by modulating the balance between bone formation and resorption.

The findings from SIRT6−/− mice demonstrated a significant reduction in bone mineral density (BMD), as well as in cancellous and cortical bone volume, compared to the control group. They also had lower blood levels of osteocalcin, which indicates a significant loss of bone mass, and higher concentrations of tartrate-resistant acid phosphatase 5b (TRAP5b), which indicates hyperactive bone resorption. (Sugatani et al., 2015; Zhang et al., 2016). This suggests that an imbalance between osteogenesis and bone resorption, characterised by decreased new bone formation and increased bone resorption, leads to bone loss in SIRT6−/−mice, ultimately resulting in OP.

The lack of SIRT6 in BMSCs resulted in compromised bone formation (Zhang et al., 2016; Sun et al., 2014). Both ALP and ColIa1-GFP levels in SIRT6-KO BMSCs were reduced in the latter stages, and there was a reduction in calcified nodules, indicating a substantial decline in bone formation capacity. Sugatani et al. (Sugatani et al., 2015) reported same findings, likely attributable to the high increase of Runx2 and Osterix (Osx), which might hinder osteoblast formation.

The elimination of SIRT6 enhances bone resorption, perhaps attributable to decreased levels of osteoprotegerin (OPG) and the stimulation of osteoclastogenesis (Kim et al., 2020). The stabilisation of ERα by SIRT6 is inhibited in myeloid SIRT6 mutant mice, which results in bone loss. Additionally,

the transcription of Fas ligands in preosteoclast cells is reduced, and the population of osteoclasts is elevated. The levels of SIRT6 in preosteoclasts showed a negative association with age, but a positive connection with BMD and ERα (Moon et al., 2019).

In conclusion, SIRT6 serves as a crucial regulator of bone homeostasis, and its loss may facilitate the onset and progression of OP via many mechanisms.

4.5. The bidirectional function of SIRT7 in the regulation of osteogenesis between osteoblasts and bone marrow mesenchymal stem cells

Mostly associated with ribosomal RNA, the histone demucinylation enzyme known as SIRT7 plays a vital role in the response to DNA damage and the survival of cells. It does this by aiding the repair of DNA double-strand breaks (Li et al., 2016). SIRT7 knockout mice were shown to have severe osteopenia, characterised by increased osteoclast numbers and decreased bone formation (Chen, 2019; Vazquez et al., 2016). The loss of interaction between SP7/Osx and SIRT7 has led to diminished transcription factor activity. SIRT7 favourably influences Osx reverse transcriptional activity and promotes osteoblast development by deacylating lysine K368 in the C-terminal region of Osx. Secondly, SIRT7-mediated K368 deacylation led to SIRT1-mediated Osx depropionylation, which increased Osx's retrotranscriptional activating activity. SIRT7 is crucial for bone production, and its loss may result in significant osteopenia owing to inadequate osteoblast differentiation.

On the other hand, even though SIRT7 promotes osteoblast formation, endogenous SIRT7 expression was significantly reduced when osteogenic differentiation was stimulated in human BMSCs (Chen, 2019). Human BMSCs' osteogenic differentiation is enhanced by downregulating SIRT7 expression without affecting their ability to proliferate; this may be related to the activation of the Wnt/β-catenin signalling pathway (Chen et al., 2017). It has been demonstrated that human BMSCs with SIRT7 knockdown may enhance bone regeneration in rat models of tibial bone lesions (Chen, 2019). According to the results, human bone marrow mesenchymal stem cells may differentiate more osteogenically in vitro if SIRT7 is deleted.

SIRT7 evidently influences osteoblasts and BMSCs to modulate bone metabolism in a bidirectional manner. Nevertheless, the existing evidence regarding the influence of SIRT7 on osteoclast function remains underexplored, suggesting that this domain may emerge as a forthcoming focal point for investigation.

In summary, the majority of sirtuin members have a protective function in bone homeostasis, facilitating bone formation and inhibiting bone resorption via many routes, including the Wnt/β-catenin and ROS signalling pathways, hence

preserving the equilibrium of bone homeostasis. Nonetheless, the overexpression of SIRT6 correlates with reduced expression of certain genes and diminished ALP activity in osteoblasts, resulting in compromised osteoblast development of human mesenchymal stem cells (Xiao et al., 2019). This indicates that both the lack and overexpression of sirtuins may have a bidirectional influence on the maintenance of bone homeostasis.

5. Conclusion

This review elucidates the significant functions of sirtuins in various bone diseases. Sirtuins influence several biological functions in bone cells, making them and their associated pathways attractive new targets for anti-osteoporotic therapy development. Present investigation suggests that SIRT1 is the most promising therapeutic target for preventing bone loss, since it decreases unbalanced bone remodelling via its effects on MSCs, osteoblasts, osteoclasts, and potentially hormone signalling pathways. The accumulated preclinical data supporting the anti-osteoporotic benefits of enhanced SIRT1 activity has established a basis for several planned or current clinical studies. Notwithstanding considerable advancements in recent years, several research enquiries remain unresolved. Furthermore, the biological roles of the less examined sirtuins, namely SIRT2, SIRT4, and SIRT5, in both physiological and pathological contexts remain little explored. Comprehensive mechanistic investigations are anticipated to further the knowledge of sirtuins' effects on various bone cell types by tissue-specific deletion and transgenic animal models. From a clinical standpoint, there is a need for more precise and selective sirtuin activators with well-defined pharmacokinetic and pharmacodynamic characteristics to achieve more reliable and definitive results.

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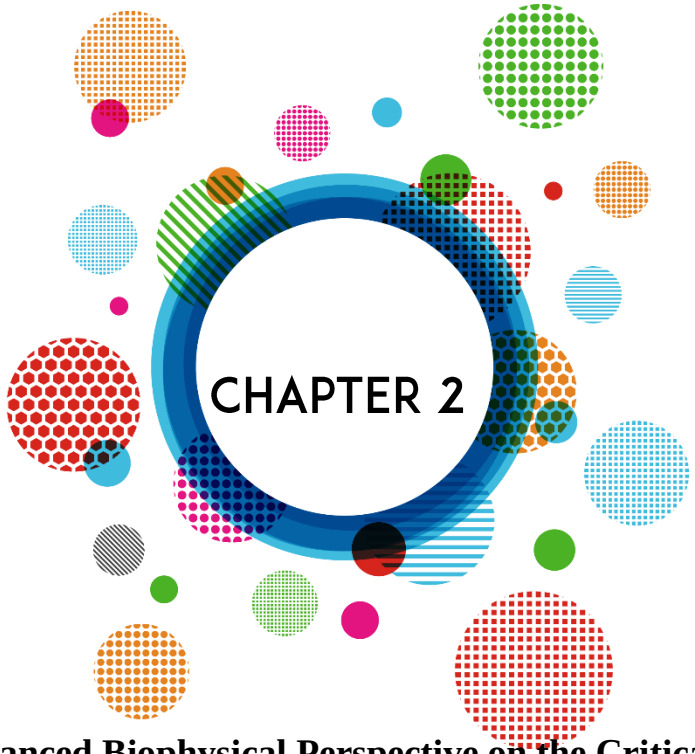

An Advanced Biophysical Perspective on the Critical Role of Sirtuins in Maintaining Bone Remodeling Balance and Combating Osteoporosis

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INTRODUCTION

From a biophysical perspective, bone tissue is not merely a static support structure but a dynamic system that continuously regulates itself through mechanisms of energy transfer, electrical signaling, and mechanical feedback. Owing to its multiscale hierarchical organization, this tissue exhibits both high mechanical strength and remarkable biological adaptability. At the nanoscale, hydroxyapatite (HA) crystals are aligned along type I collagen fibrils, forming a structure that optimizes energy absorption and load distribution during mechanical stress. This nanocomposite configuration defines the bone-specific elastic modulus (~18–20 GPa), viscoelastic behavior, and fracture toughness (Gc) (1).

An important feature of this nanoscale organization is the piezoelectric behavior of collagen fibrils. The generation of electrical potential by collagen under mechanical loading represents a process that converts mechanical information into electrical signals within bone. This phenomenon is described by the equation $V=d\cdot\sigma$, where d is the piezoelectric coefficient and σ is the stress (2). Osteocytes sense this electrical potential and initiate the mechanotransduction process. The potential generated at the osteocyte membrane triggers the opening of mechanosensitive channels such as TRPV4 and PIEZO1, allowing Ca^{2+} influx into the cell. This ion flow results in the activation of YAP/TAZ transcription factors and the Wnt1 signaling pathway in the nucleus. Genetic deletion or reduced expression of PIEZO1 leads to loss of mechanosensitivity and the development of an osteoporotic phenotype, highlighting that the biophysical homeostasis of bone tissue is dependent on mechanical loading (3, 4).

This mechanical-electrical feedback loop is directly linked to cellular energy biophysics. Energy-redox homeostasis is a key parameter that determines the functional response capacity of bone cells, and this process is regulated by the sirtuin family (SIRT1–SIRT7). As NAD^+-dependent deacetylases, sirtuins play a critical role in cellular energy metabolism, oxidative stress management, and epigenetic regulation. The intracellular NAD^+/NADH ratio ($R=[NAD^+]/[NADH]$) is the main determinant of sirtuin activity and shapes the biophysical responses of bone cells.

SIRT1 is activated in osteoblasts under conditions of elevated NAD^+, promoting osteoblast differentiation through the deacetylation of FoxO and Wnt/β-catenin pathways while reducing oxidative stress. This mechanism enhances osteoblastogenesis and increases bone anabolism (5, 6). SIRT3 plays a central role in maintaining mitochondrial energy production. It stabilizes the mitochondrial membrane potential ($\Delta\Psi m$), suppresses ROS generation, and

enhances antioxidant defense via SOD2 activation. This biophysical regulation ensures the energy sustainability of osteoblast functions; however, with aging, the effect of SIRT3 becomes biphasic, ROS accumulation increases, and osteoclastic resorption activity predominates (7). SIRT6 and SIRT7 are involved in chromatin integrity and DNA repair mechanisms. In the absence of SIRT7, the deacetylation of the Osx (SP7) transcription factor is impaired, leading to weakened osteoblast differentiation and the development of an osteopenic phenotype (6).

The biophysical pathogenesis of osteoporosis is not limited to the reduction in bone mineral density (BMD). A decrease in the elastic modulus, reduction in fracture energy (G_c), weakening of piezoelectric signal generation, and accumulation of microcracks explain the mechanical dimension of the disease. Mitochondrial dysfunction, decreased NAD^+ levels, and ROS accumulation further exacerbate this impaired biophysical environment. ROS negatively affects both energy metabolism and DNA repair, suppresses sirtuin activity, and shifts the remodeling process in favor of osteoclasts. This interaction can be modeled using differential equations:

$$\frac{dB}{dt} = k_1 SIRT1 - k_2 ROS, \qquad \frac{dC}{dt} = k_3 . ROS - k_4 SIRT1$$

In this equation, B represents osteoblast activity, C represents osteoclast activity, and the k_i coefficients define the mutual regulation between ROS and sirtuins (8, 9).

Mechanical loading (F) emerges as a powerful biophysical regulator capable of disrupting this pathological cycle. Loading enhances NAD^+ synthesis, triggers SIRT1 activation, and suppresses oxidative stress. This effect can be described using a logarithmic response model:

$$S=S_0+\alpha ln(1+F)$$

In this equation, S_0 represents the basal sirtuin activity, while α denotes the mechanical load-sensitivity coefficient. This formulation illustrates how mechanical signals are integrated into sirtuin activation and bone remodeling through energy biophysics.

In recent years, systems biology-based models have incorporated network analyses that encompass the multiple feedback loops among osteocyte mechanosensory functions, NAD^+ metabolism, mitochondrial energy flow, and ROS regulation. These models reveal correlations between biophysical parameters such as the BV/TV ratio, elastic modulus, NAD^+/NADH balance, and PIEZO1 expression, contributing to a comprehensive understanding of osteoporosis pathophysiology (1, 10). Consequently, bone tissue is redefined not only as a biochemical entity but also as a system integrating electrical,

mechanical, and energy-based signals. This perspective underscores the significance of sirtuin-focused biophysical strategies in the future treatment of osteoporosis.

BONE AS A BIOPHYSICAL TISSUE

Biophysical Significance of the Hierarchical Structure

Bone tissue is a hierarchical biocomposite with a fractal organization rarely found in nature. This structure exhibits multiscale integration, ranging from nanoscale mineral-organic interactions to macroscopic load-bearing capacity. Each level of the hierarchy serves specific functions essential for the mechanical strength, energy dissipation capacity, and self-renewal potential of bone. Therefore, bone is not only a biomechanical structure but also a central component of biophysical processes such as energy transformation and signal transmission.

At the nanoscale, type I collagen fibrils (~100 nm in diameter) are aligned with hydroxyapatite (HA) crystals (~50 nm in length) deposited along their orientation. Collagen provides flexibility and energy absorption under load, whereas HA crystals confer stiffness and rigidity. The synergy between these two components enables bone to be both rigid and resistant to fracture (11). The ordered arrangement of HA crystals optimizes load transfer depending on their orientation, and this anisotropic property forms the basis for mechanical responses that vary with load direction. The biophysical foundation of anisotropy is determined by the crystal orientation at the nanoscale and the interaction between the collagen fibril network and the elastic modulus.

At the microscale, the lamellar structure, osteonal rings, and trabecular network shape the adaptive responses of bone to loading. Lamellar bone can absorb varying stresses under load due to the alternating orientations of mineral crystals. Osteons exhibit an increase in rigidity under torsional loads, a phenomenon known as “torsional stiffening,” which is associated with the circular arrangement of osteonal lamellae. The trabecular bone represents a dynamic network capable of adapting to load direction, being remodeled along lines of mechanical stress. Micromechanical tests have demonstrated that trabecular structures undergo load-oriented reshaping, during which the propagation of microcracks is guided (1,12).

At the macroscale, cortical bone absorbs loads with its high load-bearing capacity, while trabecular bone minimizes impacts through its energy-dissipating capability. Cortical bone, due to its compact structure, can withstand substantial mechanical loads, whereas the flexible and porous trabecular network absorbs energy during fracture, preventing catastrophic failure. The combination of these two components endows bone with a unique fracture toughness. This property is

further supported by its piezoelectric behavior, which distributes energy not only mechanically but also through electrical signals; the electrical potentials generated at the nanoscale during loading are sensed by osteocytes, thereby guiding the remodeling process.

This multiscale hierarchical organization can be described using fractal geometry. The fractal approach demonstrates that the behavior of bone under load cannot be fully explained by linear elasticity theories alone. The fractal dimension (*Df*) quantitatively characterizes the complexity of the trabecular network and its capacity for mechanical response. The reorganization of the fractal structure under load ensures optimal performance in energy dissipation and load transfer (13). Therefore, bone is among the first biological structures analyzed through fractal geometry in modern biomechanics, with its load-dependent adaptive behavior modeled using fractal parameters.

The hierarchical structure of bone tissue is a multiscale system that can be characterized by biophysical parameters. At the nanoscale, collagen-HA interactions; at the microscale, osteon-trabecular organization; and at the macroscale, cortical-trabecular synergy collectively explain the unique mechanical properties of bone. The fractal organization approach provides a powerful theoretical framework for understanding the adaptive responses of this multilayered structure under load and opens new research avenues at the intersection of bone biomechanics and biophysics.

Piezoelectric and Electromechanical Behavior

Bone tissue is one of the rare biomaterials that naturally exhibits piezoelectric properties, enabling it to convert mechanical forces into electrical signals and thereby playing a critical role in initiating cellular responses. The triple-helix structure of collagen fibrils and the ordered arrangement between fibrils allow for the redistribution of loads and the generation of piezoelectric potentials during mechanical deformation. This piezoelectric behavior is one of the fundamental mechanisms underlying the biophysical adaptation of bone to mechanical loading and provides a molecular and electromechanical explanation of Wolff's Law.

The piezoelectric phenomenon is defined by the direct relationship between the electrical potential (V) and the applied mechanical stress (σ):

$$V = d \cdot \sigma$$

In this equation, d is the piezoelectric coefficient specific to collagen fibrils. This coefficient can vary depending on fibril orientation, the degree of mineralization, and the rate of loading. For example, increased mineralization reduces the value of d, while fibril orientation determines the direction of the generated potential. This electrical potential can be measured in the millivolt

range within local microenvironments during loading and is sensed by osteocytes through ion channels such as PIEZO1 and TRPV4 (2).

Piezoelectric signals not only generate electrical potentials but also trigger multilayered biophysical responses within the cell. The mechanosensory functions of osteocytes are activated through these potentials. Electrical stimulation opens Ca^{2+} channels in the cell membrane, initiating a rapid influx of calcium ions. This Ca^{2+} oscillation produces signals that influence energy metabolism via the mitochondria and endoplasmic reticulum. At the same time, ATP release increases, and this extracellular ATP activates paracrine signaling through purinergic receptors. Moreover, piezoelectric stimulation enhances nitric oxide (NO) production, a potent biochemical regulator involved in vasodilation and remodeling processes. These signals serve as critical primary biophysical triggers in initiating the bone remodeling cycle.

The piezoelectric effect manifests at different intensities in trabecular and cortical bone regions. Due to its higher surface area and porosity, trabecular bone can generate broader distributions of electrical potential under load. In contrast, cortical bone, with its more compact structure, transmits load more linearly, resulting in electrical potentials concentrated in more localized areas. This distinction also alters the electrical stimulation profiles of osteocytes during mechanical loading.

Modern biomechanics and biophysics studies have shown that piezoelectric signals not only initiate the mechanotransduction cascade but also guide the long-term adaptation of remodeling processes. Piezoelectric stimuli can indirectly influence the activity of energy-redox regulatory proteins such as sirtuins, thereby reprogramming cellular energy metabolism. This demonstrates that electrical signals are integrated with cellular networks at both biochemical and energy levels.

Furthermore, studies suggest that piezoelectric potentials in bone tissue can be clinically modulated. Biophysical approaches such as low-intensity pulsed ultrasound (LIPUS) and electromechanical stimulation can enhance piezoelectric effects, thereby accelerating bone healing. These methods are being experimentally and clinically tested in the treatment of fractures and osteoporosis (14).

The piezoelectric property of bone functions as a natural sensor system that converts mechanical forces into biophysical signals and initiates cellular responses. This electromechanical behavior forms the biophysical basis of Wolff's Law and clearly demonstrates that the bone remodeling process is regulated through the integration of energy, electrical, and mechanical signals.

Osteocytes: The Primary Mechanosensors in Bone

Osteocytes are the most abundant cell type in bone tissue and serve as the primary sensors of mechanical loads due to their embedded position within the lacuno-canalicular network. These cells detect microscopic fluid movements within the bone matrix during loading and convert them into biophysical signals that regulate the bone remodeling process. The lacuno-canalicular system facilitates the flow of interstitial fluid around the thin cytoplasmic extensions of osteocytes; this fluid flow varies with loading and generates fluid shear stress (τ) on the osteocyte membrane.

Fluid shear stress is a critical parameter in transmitting mechanical loads to the cell surface and is defined as:

$$\tau = \mu \frac{\partial_V}{\partial_x}$$

In this equation, μ\muμ represents the viscosity of the fluid, v the flow velocity, and x the positional parameter. This expression quantitatively describes how fluid flow under loading generates shear forces on the surface of osteocytes. An increase in τ triggers the activation of mechanosensitive ion channels located in the osteocyte membrane, particularly PIEZO1 and TRPV4 (15, 16). PIEZO1 is an ion channel sensitive to mechanical deformations that opens in response to tension, while TRPV4 responds to both mechanical and osmotic changes. The activation of these channels leads to a rapid increase in Ca^{2+} influx into the cell.

Increased intracellular Ca^{2+} initiates a series of biochemical and biophysical responses. Among these responses, ATP release, prostaglandin E_2 (PGE_2) production, and nitric oxide (NO) synthesis are particularly significant. ATP communicates with neighboring cells via purinergic signaling, ensuring coordination during remodeling processes. PGE_2 acts as a mediator that activates osteoblasts and enhances matrix synthesis. NO production, on the other hand, suppresses osteoclast activity, thereby limiting bone resorption (15, 16). These signals play a critical role in initiating the anabolic response within the bone remodeling cycle.

Mechanical signals are not confined to the membrane level; they are transmitted to the nucleus through the cytoskeleton. In osteocytes, mechanical stimulation begins with the stretching of actin filaments, and this tension is conveyed to the nuclear membrane via lamin A/C proteins. The response of lamin A/C to mechanical deformation leads to changes in chromatin organization and the reprogramming of gene expression (3). Thus, mechanical loading directly regulates genes at the epigenetic level, exerting long-term effects on bone homeostasis.

This mechanotransduction cascade clearly highlights the biophysical sensitivity of osteocytes under mechanical load. The opening of ion channels in

the cell membrane, the reorganization of the cytoskeleton, and the activation of the nuclear response constitute a multistep process that links loading to remodeling. Moreover, this mechanism is also interconnected with previously described energy-redox networks, such as energy metabolism and sirtuin activity. Osteocytes function as central hubs that integrate mechanical signals with energy production, ROS regulation, and gene expression.

In conclusion, osteocytes are the primary mechanosensors in bone, and the biophysical process initiated by the detection of fluid shear stress regulates bone remodeling through the integration of ion channels, the cytoskeletal network, and nuclear mechanotransduction mechanisms. These cells are fundamental components of biophysical signaling networks that integrate mechanical loads with energy, redox, and genetic responses.

Biophysical Model of Mechanotransduction

Mechanotransduction is one of the most fundamental biophysical processes through which bone tissue responds to biomechanical loading, involving the conversion of mechanical signals into biochemical responses at the cellular level. This process occurs through the integration of multiple interconnected networks, including energy metabolism, ion flux, redox state, and gene expression. Osteocytes lie at the center of this process, functioning as the primary cellular sensors that detect mechanical loads. The biophysical model of mechanotransduction is defined by three sequential stages:

Sensing of Mechanical Stimulus: During mechanical loading, fluid shear stress is generated within the lacuno-canalicular system of osteocytes, triggering the opening of mechanosensitive ion channels such as PIEZO1 and TRPV4 in the cell membrane. The activation of these channels represents the first step in converting mechanical energy into electrical and biochemical signals. Through PIEZO1, mechanical deformation is transmitted into the cell via Ca^{2+} influx, while TRPV4 responds to both mechanical and osmotic stimuli, amplifying the signal (3).

Signal Transmission: Following the initial sensing, the intracellular Ca^{2+} oscillation is integrated with energy metabolism through the mitochondria and endoplasmic reticulum. During this process, ROS production is maintained at controlled levels, while excessive ROS formation is suppressed by antioxidant mechanisms. At the same time, secondary messengers such as NO and PGE_2 are released; NO suppresses osteoclast activity, thereby reducing bone resorption, whereas PGE_2 supports osteoblast activity and matrix synthesis (15, 16).

Biochemical Response: In the final stage, the RANKL/OPG balance is reorganized in response to mechanical signals. Increased OPG levels limit osteoclast formation, while decreased RANKL activity suppresses bone

resorption. The shift of this balance toward an anabolic state results in osteoblast stimulation and the strengthening of bone tissue. At the same time, sirtuin activation reprograms gene expression and cellular energy status in a manner dependent on NAD^+ levels. Thus, the mechanical signal generates a biochemical response that is fully integrated with the energy-redox network (17, 18).

The mathematical representation of this process can be expressed through a model that incorporates both logarithmic and linear components related to mechanical load (F) and NAD^+ level (N).

$$R=S_0+\alpha ln(F)+\beta N$$

Here, R represents the osteocyte response, S_0 denotes the basal cellular activity, α is the coefficient of mechanical load sensitivity, and β is the coefficient of NAD^+-mediated energy sensitivity. This model reveals that mechanical signals are logarithmically amplified and reinforced through sirtuin-mediated genetic and metabolic responses driven by NAD^+ levels. The integration of energy metabolism with mechanical signaling ensures that osteocytes operate with high precision in regulating the bone remodeling process.

This biophysical model emphasizes that mechanotransduction is not merely the sensing of mechanical forces but a multiscale process achieved through the integration of energy-redox networks. The central position of osteocytes within this integrated signaling network is critically important for maintaining bone homeostasis and preventing pathological conditions such as osteoporosis.

Energy Biophysics: Mitochondrial Response and ROS Regulation

The osteocyte response to mechanical loading cannot be explained solely by classical parameters of energy production and consumption. This response involves the dynamic integration of energy biophysics, redox control, and sirtuin-mediated epigenetic regulation. The mitochondria of osteocytes rapidly adapt to energy demands under load by increasing their OXPHOS capacity. This enhancement strengthens the proton motive force across the electron transport chain and elevates the rate of ATP synthesis. However, the increased metabolic rate inevitably raises the production of reactive oxygen species (ROS). Since ROS can disrupt energy balance and damage DNA, the cell tightly regulates ROS levels through sirtuin-mediated mechanisms.

The energy flow in osteocytes can be described using a differential model as follows:

$$\frac{dE}{dt} = P_{ATP}(1-\delta S) - C_{ROS}(1-\lambda S) - L_{DNA}(1-\theta S) + \eta N$$

Here:

P_{ATP} is the mitochondrial ATP production rate,

C_{ROS} epresents energy loss due to oxidative stress,

L_{DNA} is the energy load devoted to DNA repair,

S is sirtuin activity,

δ, λ, ϕ are regulatory coefficients describing the effects of sirtuins on energy production, ROS mitigation, and DNA repair efficiency, respectively

N is the NAD^+ level, an additional parameter supporting energy flow,

η is the coefficient representing the contribution of NAD^+ to energy dynamics.

This extended model clearly expresses the enhancing effect of sirtuins on ATP production (δS), their suppressive influence on ROS-induced energy loss (λS), and their optimizing role in reducing the energy cost of DNA repair (ϕS). NAD^+ levels are integrated into the model as the primary determinant of sirtuin activity, emphasizing their critical role in maintaining energy homeostasis.

This biophysical framework provides a more realistic representation of cellular responses compared to classical energy models. Mechanical loading enhances NAD^+ synthesis, triggering sirtuin-mediated energy optimization. SIRT3 deacetylates mitochondrial ETC complexes, reducing proton leakage and increasing the efficiency of energy conversion. SIRT1 and SIRT6 minimize energy consumption during DNA repair while limiting ROS-induced energy loss. Elevated NAD^+ levels not only support energy production capacity but also strengthen redox balance (6, 19).

The energy response of osteocytes is governed by a multilayered biophysical network that optimizes both energy production capacity and oxidative stress control during mechanical loading. This model mathematically demonstrates that osteocytes function not only as energy producers but also as integrated regulators of energy and redox homeostasis.

OSTEOPOROSIS: MECHANISMS AND BIOPHYSICAL FACTORS

Osteoporosis has long been considered a clinical disease defined solely by the reduction of bone mineral density (BMD). However, findings over the past decade at the biophysical, cellular, and molecular levels have demonstrated that this condition is directly linked to energy metabolism, mechanotransduction, and epigenetic signaling networks (20). The multiscale structure of bone tissue (nano → cellular → tissue → organ) reveals that osteoporosis is not merely a loss of mineral content but rather a complex pathophysiological state characterized by disruptions in energy management, redox homeostasis, mechanical signal sensing, and genetic regulation (1,12).

Energy Biophysics Perspective: In osteoporosis, disturbances in cellular energy metabolism are associated with decreased NAD^+ levels, mitochondrial dysfunction, and reduced sirtuin activity (19). The decline in energy efficiency of osteoblasts limits matrix synthesis and mineralization. At the same time, increased oxidative stress stimulates osteoclast activity, accelerating bone resorption. Therefore, osteoporosis should be regarded not only as a condition of mineral loss but also as a biophysical disease characterized by impaired energy flow and disrupted redox balance.

The Role of Mechanotransduction: Osteocytes integrate mechanical signals with electrical and biochemical responses by sensing fluid shear stresses within the lacuno-canalicular network. In osteoporotic bone, this mechanosensory function is impaired; decreased activity of PIEZO1/TRPV4 channels and increased expression of sclerostin weaken the response to mechanical loading (15, 16). This disruption in mechanical signal detection leads to a reduction in the anabolic response during the remodeling process.

Molecular Signaling Networks and Epigenetic Regulation: At the molecular level of osteoporosis, suppression of the Wnt/β-catenin pathway, a shift in the RANKL/OPG balance toward osteoclastogenesis, and dysregulation of transcription factors such as FoxO and RUNX2 play central roles (6). The sirtuin family (particularly SIRT1, SIRT3, SIRT6, and SIRT7) emerges as key regulators coordinating energy-redox balance and epigenetic programming. Reduced activity of these regulators results in decreased DNA repair capacity, telomere shortening, and accelerated cellular aging (6, 21).

The Importance of a Biophysical Approach: To fully understand osteoporosis, it is necessary to move beyond classical clinical parameters (e.g., BMD). Biophysical parameters such as elastic modulus, fracture toughness, microcrack accumulation, and energy absorption capacity are becoming increasingly important in determining disease prognosis and treatment response (1, 12). Moreover, the combination of biophysical and pharmacological interventions (e.g., sirtuin activators and mechanical loading therapies) is opening new horizons in the management of osteoporosis (13).

In light of current knowledge, osteoporosis should not be regarded solely as a clinical disease of mineral loss but as a multidimensional biophysical pathology characterized by disruptions in the interactions among energy biophysics, mechanotransduction, and molecular signaling networks. This perspective enables not only a deeper understanding of the disease but also the development of personalized therapeutic strategies.

Disruptions at the Nanoscale

Osteoporosis is characterized not only by a reduction in bone mineral content but also by significant disruptions in its nanoscale structural organization. Healthy bone exhibits high mechanical strength and energy absorption capacity through the orderly hierarchical interaction of type I collagen fibrils with hydroxyapatite (HA) crystals (11). In osteoporotic bone, this nanoscale architecture becomes compromised; the bonds between collagen fibrils weaken, and irregularities appear in the alignment of HA crystals (1, 12). These structural losses lead to inefficient absorption of strain energy during load-bearing and result in heterogeneity in load distribution.

Reduction in Elastic Modulus and Mechanical Strength: Disruption of the orientation of HA crystals reduces the elastic modulus of bone tissue. Under normal conditions, the elastic modulus ranges between approximately 18–20 GPa (11), but in osteoporosis, this order is lost, leading to lower values. This mechanical weakening particularly facilitates the propagation of microcracks, resulting in decreased fracture toughness (1, 12). Consequently, osteoporotic bone exhibits more brittle behavior under load.

Weakening of Piezoelectric Properties: The piezoelectric properties of collagen fibrils play a critical role in converting mechanical loads into electrical signals. This feature enables osteocytes to sense mechanical stimuli and regulate the remodeling process (15, 16). Disruption of the nanoscale structure reduces the piezoelectric coefficient (d) of collagen, which in turn lowers the electrical potential generated during mechanical loading ($V=d\cdot\sigma V$). The decrease in electrical signal production weakens the mechanotransduction cascade, resulting in a diminished response of the remodeling process to mechanical stimuli.

Energy Biophysics Perspective: Nanoscale disorganization is not limited to mechanical weakening; it also affects the energy-sensing capacity of bone cells. The reduction in piezoelectric signals can negatively influence ion currents that trigger NAD^+ biosynthesis and sirtuin activation. This impairment further diminishes the anabolic capacity of osteoblasts and the mechanosensory function of osteocytes. Consequently, nanoscale structural disruptions compromise bone homeostasis at both mechanical and biochemical levels.

Biophysical Significance: The nanoscale disruptions observed in bone are considered a fundamental step in the progression to the fragile bone phenotype characteristic of osteoporosis. Understanding the biophysical effects of nanoscale disorganization highlights the growing importance of employing nanomechanical tests (e.g., nanoindentation) and advanced imaging techniques (e.g., AFM, SAXS) in the early diagnosis of osteoporosis (1, 12).

Disruptions at the Microscale

In osteoporosis, not only the nanoscale structure but also the microscale bone architecture is profoundly disrupted. In healthy bone, the trabecular network has a three-dimensional structure capable of adapting to load orientation. This network absorbs energy during loading and ensures homogeneous stress distribution (1,12). In osteoporotic bone, the trabecular network undergoes perforation; trabeculae become thinner, connections become sparse, and the ability to adapt to load orientation is lost (20). This condition leads to uncontrolled accumulation of stress within the bone.

Trabecular Thinning and Perforation: The reduction in trabecular thickness and the increase in perforations severely weaken the load-bearing capacity of bone. Under normal conditions, the trabecular network reshapes according to load orientation, but in osteoporosis, it loses this adaptive capability (1, 12). The decrease in mechanical strength facilitates the formation and propagation of microcracks, which over time coalesce, significantly increasing the risk of macroscopic fractures.

Weakening of Inter-Osteonal Connections in Lamellar Bone: In cortical bone, osteons are surrounded by lamellar structures that facilitate load distribution. In osteoporosis, the connections between osteons weaken, creating discontinuities in load transfer (22). Disruptions in the mineral and collagen organization around the Haversian canals make energy dissipation heterogeneous. This reduction in energy absorption capacity leads to a decrease in fracture toughness.

Disruption of Energy Distribution and Microcrack Accumulation: These microscale structural weaknesses lead to localized stress concentrations in energy distribution. In the transition zones between osteons, energy transmission is reduced, creating a critical environment for crack propagation (1, 12). The accumulation of microcracks accelerates the process of irreversible structural weakening in bone.

Biophysical Significance: The deterioration of the microstructure is one of the primary reasons for the increased fragility in osteoporotic bone. Structural changes at this level have dramatic effects on fracture energy (G_c) and the elastic modulus. Furthermore, microstructural parameters-such as trabecular thickness, connectivity density, and the number of inter-osteonal connections-play a critical role in advanced biomechanical models for predicting fracture risk (1,12).

Effects on the Macroscale Structure

Osteoporosis leads to significant alterations in the macroscale architecture of bone. In cortical bone, thinning, increased porosity, and reduced load-bearing capacity are among the most prominent structural changes observed in advanced stages of the disease (20). Healthy cortical bone, due to its dense structure, exhibits high load-bearing capacity and fracture resistance. In osteoporotic bone,

however, this density is lost, pore size and number increase, and stress distribution during loading becomes heterogeneous. This heterogeneity raises the likelihood of fractures in regions where stresses are concentrated (1,12).

Cortical Bone Thinning and Increased Porosity: The thinning of cortical bone reduces the cross-sectional area, thereby decreasing the maximum load the structure can bear. The increase in porosity not only weakens mechanical strength but also lowers the elastic modulus and fracture toughness (1, 12). These changes form the biophysical basis for the increased risk of fractures in elderly individuals, even under low-energy trauma (20).

Reduction in Mechanical Strength and Increased Fracture Risk: These macrostructural changes cause osteoporotic bone to fracture under lower loads. While healthy bone exhibits a certain degree of plastic deformation during load-bearing, the plastic deformation capacity is significantly reduced in osteoporotic bone (22). This indicates a decreased energy absorption capacity of the bone tissue and an increased risk of fracture.

Weakening of Mechanical Signals and Impairment of the Remodeling Response: Macrostructural deterioration also affects the cellular perception of the mechanotransduction process. Increased porosity in cortical bone creates discontinuities in load transmission pathways, reducing the fluid shear stress sensed by osteocytes through the lacuno-canalicular system (2). The weakening of mechanical signals limits NAD^+ production and sirtuin activation, thereby suppressing the remodeling response (15, 16). As a result, cells fail to initiate an anabolic response under mechanical load, and bone resorption surpasses anabolism.

Integration of Multiscale Deterioration: Disruptions occurring at the nano-, micro-, and macro-scales form a complementary chain. The loss of collagen–hydroxyapatite interactions at the nanoscale (11) combines with trabecular thinning and weakened inter-osteonal connections at the microscale (1, 12, 22), ultimately resulting in cortical thinning and increased porosity at the macroscale (20). This multiscale deterioration holistically reveals the mechanotransduction and energy biophysics dimensions of osteoporosis: mechanical signals are weakened, osteocytes fail to initiate the remodeling response, and bone homeostasis is disrupted (2, 15, 16).

Cellular Mechanisms in Osteoporosis

Osteoporosis is a complex pathology in which bone homeostasis is disrupted at the cellular level, and the processes of energy biophysics and mechanotransduction are impaired. Under normal conditions, the bone remodeling process is maintained by a dynamic balance between resorption carried out by osteoclasts and bone formation mediated by osteoblasts, with

osteocytes functioning as mechanosensors and regulatory cells in this process (20). In osteoporosis, however, the functioning of this tri-cellular network is severely compromised:

- Osteoclasts become overactivated, leading to the dominance of the resorption phase.
- Osteoblasts lose their function under conditions of energy deficiency and oxidative stress, resulting in reduced anabolic capacity.
- Osteocytes not only decrease in number but also lose their mechanosensory properties, thereby failing to coordinate the remodeling process.

This cellular imbalance arises not only from alterations in molecular signaling pathways but also from disturbances in energy metabolism (particularly NAD^+ homeostasis) and redox balance. The reduction in NAD^+ levels suppresses the activity of the sirtuin family (SIRT1, SIRT3, SIRT6, SIRT7), leading to weakened DNA repair, increased oxidative stress, and impaired epigenetic regulation (6, 19, 21)

From an energy biophysics perspective, the resorptive processes of osteoclasts require ATP-intensive mechanisms, while osteoblast matrix synthesis and mineralization are also highly energy-dependent. Osteocytes integrate mechanical load sensing through the lacuno-canalicular system with the NAD^+–sirtuin axis. In osteoporosis, this biophysical mismatch between energy demand and energy production creates a destructive cellular vicious cycle: elevated ROS levels cannot be detoxified due to insufficient sirtuin activation, cell death accelerates, and the remodeling balance shifts in favor of catabolism (6, 15, 16).

The cellular mechanisms of osteoporosis are based on the impaired functions of the three main cell types (osteoclasts, osteoblasts, and osteocytes) along the axes of energy biophysics and mechanotransduction. A comprehensive understanding of these biophysical dysfunctions is critically important for identifying new molecular pathways that can be targeted in the treatment of osteoporosis.

Increased Osteoclast Activity

Osteoclasts are multinucleated cells localized on bone surfaces that drive the resorptive phase of the bone remodeling process. Under normal conditions, osteoclast activity is tightly regulated by the balance between RANKL (Receptor Activator of Nuclear Factor κB Ligand) and OPG (Osteoprotegerin), which is controlled by osteoblasts. Osteoprotegerin acts as a “decoy” receptor by preventing RANKL from binding to the RANK receptor on osteoclast precursors, thereby inhibiting osteoclast formation (23). In osteoporosis, this balance is

disrupted; RANKL expression increases, OPG levels decrease, and the shift in the RANKL/OPG ratio toward promoting osteoclast differentiation leads to an increase in osteoclast number and resorptive capacity (5).

Acidic Microenvironment and Energy-Intensive Resorption: Active osteoclasts create an acidic microenvironment to dissolve the bone matrix through vacuolar H^+-ATPase proton pumps located in their cell membranes. In this microenvironment, the pH drops to approximately 4.5, increasing the solubility of hydroxyapatite (24). The operation of these proton pumps is an energy-intensive process that consumes large amounts of ATP. The increased ATP demand accelerates oxidative phosphorylation in osteoclast mitochondria; however, this process also elevates the production of reactive oxygen species (ROS) as byproducts (6, 21). In osteoporosis, elevated ROS levels disrupt redox homeostasis in osteoclasts, creating oxidative stress. This mechanism is closely linked to the ROS–sirtuin feedback network.

Interaction with Molecular Signaling Pathways: The increase in RANKL not only enhances osteoclast formation but also strengthens osteoclast gene expression through the activation of the NF-κB and NFATc1 transcription factors (25). This molecular activation boosts the production of proteolytic enzymes (such as cathepsin K) and acidifying pump proteins (H^+-ATPase) in osteoclasts, thereby maximizing bone resorption.

Biophysical Perspective: From an energy biophysics perspective, osteoclast activity relies on a delicate balance between high ATP demand and ROS production. When this balance is disrupted-particularly in the context of sirtuin deficiency-the bone-resorbing capacity of osteoclasts is further enhanced (6, 21).

In conclusion, in osteoporosis, the hyperactivity of osteoclasts-driven by RANKL/OPG imbalance, energy-intensive acidification processes, and oxidative stress triggered by sirtuin deficiency-accelerates bone resorption. These cellular and biophysical alterations are critical factors that define the progressive nature of osteoporosis.

Decreased Osteoblast Function

Osteoblasts are the cells responsible for directing the anabolic phase of bone remodeling and synthesizing new bone matrix. In osteoporosis, however, this function is broadly suppressed due to decreased NAD^+ levels, reduced sirtuin activity (particularly SIRT1), increased oxidative stress, and insufficient energy production (6, 13).

Disruption of Energy Metabolism and Reduced Sirtuin Activity: For osteoblast function, NAD^+ is a critical component of both energy metabolism and epigenetic regulation. In osteoporosis, NAD^+ homeostasis is disrupted; the decrease in the

NAD^+/NADH ratio suppresses SIRT1 activity (19). Under normal conditions, SIRT1 deacetylates the transcription factor RUNX2, supporting its active form. In the absence of adequate NAD^+, SIRT1 activity weakens, RUNX2 accumulates in its acetylated form, and the differentiation of osteoblast progenitors into mature osteoblasts is inhibited. This epigenetic dysregulation slows the rate of bone formation (13).

Elevated ROS triggers apoptosis and energy loss in osteoblasts, further weakening matrix synthesis; this process is part of the ROS–sirtuin axis summarized in section 5.3.

Reduced Matrix Protein Synthesis and Slowed Bone Formation: The reduction in energy production limits the ability of osteoblasts to synthesize essential matrix proteins such as type I collagen and osteocalcin. Insufficient matrix production disrupts the mineralization process and weakens the mechanical strength of bone tissue (20). This biophysical weakening is a key reason why osteoporosis is characterized not only by a decrease in bone mineral density (BMD) but also by a deterioration in bone quality.

Biophysical Perspective: From an energy biophysics perspective, osteoblast dysfunction can be explained by a vicious cycle triggered by NAD^+ deficiency, as detailed in section 5.3.

Clinically, NAD^+-boosting agents and SIRT1 activators (e.g., resveratrol, SRT2104) have the potential to break this cycle (13). The detailed biophysical explanation of this mechanism forms the basis of the cellular pathogenesis of osteoporosis (see also Section 5.4.1).

Osteocyte Loss and Impaired Mechanotransduction

Osteocytes, as the mechanosensory cells of bone tissue, play a central role in load detection and the coordination of the remodeling process. Embedded within the lacuno-canalicular network, these cells convert mechanical stimuli such as fluid shear stress into biochemical signals, thereby regulating osteoblast and osteoclast activity (26). In osteoporosis, both the number of osteocytes decreases and the mechanosensory capabilities of the surviving cells are severely impaired. This impairment, driven by weakened PIEZO1/TRPV4-mediated signaling pathways and insufficient activation of the NAD^+/SIRT1 axis, leads to the suppression of the anabolic response in the remodeling process.

Osteocyte Loss and Disruption of the Lacuno-Canalicular Network: During osteoporosis, osteocyte apoptosis increases, primarily due to oxidative stress, energy deficiency, and reduced sirtuin activity (6). The loss of these cells creates discontinuities in the lacuno-canalicular system, weakening the transmission of

mechanical loads. As a result, both mechanical signal propagation and energy metabolism-dependent cellular responses are impaired.

Reduced Expression of Mechanosensory Channels: The mechanosensitivity of osteocytes is primarily mediated by ion channels such as PIEZO1 and TRPV4. These channels convert mechanical deformation into Ca^{2+} influx, and the resulting Ca^{2+} signaling triggers NAD^{+} biosynthesis and sirtuin activation, thereby regulating cellular energy homeostasis. In osteoporosis, the expression of PIEZO1 and TRPV4 is reduced, leading to weakened amplitude and continuity of Ca^{2+} signaling (15, 16).

Biophysical Perspective: Energy Dependence of Mechanotransduction: The mechanosensory functions of osteocytes are linked not only to ion channel activity but also to energy metabolism. Signals triggered by fluid shear stress in the lacuno-canalicular system stimulate NAD^{+} synthesis and regulate cellular energy homeostasis through sirtuin-mediated pathways (19). In osteoporosis, the reduction in NAD^{+}, decreased sirtuin activity, and insufficient energy production further weaken the mechanotransduction capacity of osteocytes. This cascade is identified as a critical biophysical mechanism that suppresses the anabolic phase of the remodeling process.

Clinical and Therapeutic Insights into Osteoporosis:

In recent years, agents that pharmacologically activate PIEZO1/TRPV4 channels and strategies that increase NAD^{+} levels have shown potential in preserving osteocyte function and correcting mechanotransduction defects (15, 16).

In the treatment of osteoporosis, there is a growing shift beyond traditional pharmacological approaches toward therapies that target biophysical processes. Current clinical strategies aim to optimize the interactions among mechanotransduction, sirtuin activation, mitochondrial function, and energy metabolism. These approaches modulate not only the symptoms but also the biophysical root mechanisms of the disease.

Biophysical Therapeutic Effects of Exercise: Clinical Evidence: Recent clinical studies have shown that high-frequency vibration exercises and resistance training increase NAD^{+} biosynthesis and stimulate SIRT1 activity by enhancing Ca^{2+} oscillations in osteocytes (6). This biophysical effect not only improves bone mineral density but also re-optimizes the fractal organization of the trabecular network. Furthermore, during exercise, the Piezo1-mediated mechanosensory response has been demonstrated to alter gene expression profiles, activating bone formation–related genes (15, 16).

Pharmacological Sirtuin Activation: Findings from Clinical Trials: Agents such as resveratrol, SRT2104, and NAD^+ precursors (NMN, NR) have been reported in randomized clinical trials to improve bone microarchitecture in patients with osteoporosis (13). These molecules regulate the ROS–NAD^+ balance, thereby enhancing osteoblastogenesis while suppressing osteoclastic resorption. In emerging pharmacological strategies, the energy-sensing properties of sirtuins are being leveraged to target the intracellular ATP/NADH ratio.

Mitochondria-Targeted Therapies: Proton Leak and ROS Control: Mitochondria-specific antioxidants (e.g., MitoQ) and proton leak modulators have been shown in osteoporosis models to enhance ATP production while reducing ROS accumulation (10, 27). These agents particularly strengthen SIRT3 activity, restoring the balance of mitochondrial fusion and fission. Clinically, such treatments increase the energy reserves of osteoblasts and help restore their remodeling capacity.

Combination Therapies and Model-Based Predictions: Findings indicate that the simultaneous application of mechanical loading protocols and pharmacological sirtuin activation produces synergistic effects (28, 29). New modeling approaches can be used to predict bone remodeling dynamics under various combinations of these therapies. For example, simulations have demonstrated that in patients maintained above the energy threshold (E_c), combination therapies maximize the anabolic response.

Future Perspective: Personalized Biophysical Therapy: In the near future, the clinical monitoring of biophysical parameters such as sirtuin activity, NAD^+/ROS profile, and mechanical load tolerance will enable the development of personalized treatment plans. These measurements will guide the selection of the optimal combination of exercise protocols, pharmacological agents, and mitochondria-targeted strategies.

SIRTUINS: STRUCTURE, BIOPHYSICAL MECHANISMS, AND ENERGY DYNAMICS

Molecular Structure and Its Connection to Energy

Sirtuins are evolutionarily conserved NAD^+-dependent enzymes in all eukaryotes, possessing the ability to sense and regulate cellular energy. These proteins play critical roles not only in the deacetylation of histones but also in the regulation of metabolic enzymes, transcription factors, and DNA repair proteins. Crystallographic studies have revealed that the catalytic core region of all sirtuin isoforms is highly conserved, containing the classical Rossmann-fold motif that binds NAD^+ and is surrounded by specific pockets that recognize acetylated substrates (13).

The catalytic mechanism of sirtuins is strictly dependent on the presence of NAD^+; thus, sirtuins function as biosensors that detect the cellular NAD^+/NADH ratio. During the reaction, NAD^+ is cleaved, transferring the ADP-ribosyl moiety to the acetylated substrate while releasing nicotinamide as a byproduct. The hydrolysis of NAD^+ during these biochemical steps generates free energy (ΔG), which serves as the biochemical equivalent of the mechanical work used to remove the acetyl group from the substrate (13, 30, 31). Crystallographic analyses have shown that the NAD^+ molecule binds with high affinity to the enzyme's Rossmann fold via critical amino acid residues (e.g., Gly-X-Gly motifs), directing the substrate into a conformationally favorable position. This binding has been identified as the rate-limiting step of the reaction (32).

The kinetics of this biochemical reaction are directly related to the NAD^+/NADH ratio. When NAD^+ levels increase, sirtuin activity rises logarithmically, whereas the accumulation of NADH acts as a competitive inhibitor, slowing down the catalytic cycle. This energy–redox relationship can be described by a mathematical expression showing that the enzymatic turnover rate (k_{cat}) depends on the NAD^+/NADH ratio:

$$k_{cat} = k_0 + \beta ln \frac{[NAD^+]}{[NADH]}$$

Here, k_0 represents the basal catalytic rate, and β is the energy-sensitivity coefficient. This logarithmic relationship biophysically explains that even small changes in the energy state can lead to significant fluctuations in sirtuin activity, thereby positioning sirtuins as precise regulators of cellular energy homeostasis (17, 18).

The NAD^+ dependence of sirtuins establishes a bidirectional feedback loop with cellular energy metabolism. High mitochondrial NAD^+ levels facilitate the activation of mitochondrial sirtuins such as SIRT3, which deacetylate electron transport chain (ETC) complexes, thereby increasing the efficiency of ATP production. Similarly, nuclear sirtuins (SIRT1 and SIRT6) optimize energy-demanding processes such as chromatin remodeling and DNA repair under conditions of abundant NAD^+. This mechanism creates an energy-based communication network between NAD^+ metabolism and sirtuin activity (19).

The NAD^+/NADH ratio represents not only the redox state but also the energy storage capacity of cells. The logarithmic sensitivity of sirtuins to this ratio indicates that energy sensing is not linear but optimized across a wide dynamic range. This serves as an example confirming that logarithmic responses are evolutionarily advantageous in energy biophysics. During metabolic fluctuations, this ratio is expressed as $R=[NAD^+]/[NADH]$; when NAD^+ is abundant ($R \gg 1$),

sirtuins exhibit maximal activity, whereas under energy deficiency or hypoxic conditions ($R \ll 1$), their activity rapidly declines (19, 32).

The molecular structure and NAD^+ dependence of sirtuins enable them to function as biophysical sensors that directly detect and regulate cellular energy dynamics. The integration of structural biology, enzyme kinetics, and energy metabolism reveals the fundamental mechanisms through which sirtuins control energy–redox homeostasis in bone biology. These properties reinforce the critical role of sirtuins in bone health by providing advanced biophysical regulation over osteoblast function, osteoclast activity, and the balance of bone remodeling.

SIRT1: Mechanotransduction and Resistance to Oxidative Stress

SIRT1 is a precise regulator of both gene expression and metabolic programming in bone cells. This NAD^+-dependent deacetylase serves as a bidirectional biophysical checkpoint, enhancing differentiation and matrix synthesis in osteoblasts while limiting destructive activity in osteoclasts. At the nuclear level, SIRT1 deacetylates FoxO transcription factors, strengthening the oxidative stress response, and simultaneously activates the Wnt/β-catenin pathway to promote osteoblastogenesis. These effects increase the synthesis of bone matrix proteins (osteocalcin, type I collagen), thereby elevating the anabolic capacity of bone tissue (5). Additionally, SIRT1 suppresses the NF-κB pathway, reducing inflammatory cytokine production and preventing destructive inflammatory processes within the bone microenvironment.

Mechanical loading generates fluid shear stress (FSS) within the lacuno-canalicular system of osteocytes, and this biophysical stimulus enhances NAD^+ biosynthesis, triggering SIRT1 activation. The increase in NAD^+ levels strengthens the energy-sensitive biosensor function of SIRT1 and maximizes the mechanotransduction capacity of osteocytes. During this process, SIRT1 stabilizes the transcription factor RUNX2 in response to mechanical stimulation; deacetylation of RUNX2 increases its nuclear localization and enhances the expression of genes required for osteoblast differentiation (17, 18). Consequently, mechanical loading exerts lasting anabolic effects on bone remodeling through the NAD^+/SIRT1 axis, which regulates energy–redox biophysics.

The role of SIRT1 in oxidative stress resistance is also noteworthy. The NAD^+ increase triggered by mechanical loading enhances FoxO3a activation through SIRT1, leading to elevated expression of antioxidant enzymes (e.g., SOD2, catalase). This mechanism limits the harmful effects of reactive oxygen species (ROS) within the cell, enhances DNA repair capacity, and prolongs osteoblast lifespan. Thus, SIRT1 functions as a biophysical protective factor that prevents bone loss by suppressing oxidative stress and inflammation (15, 16).

It has been demonstrated through mathematical modeling that mechanical signals are logarithmically amplified via SIRT1. In this model, SIRT1 activity is defined as:

$$SIRT1=S_0+\alpha ln(1+F)$$

In this equation, F represents the mechanical load, S_0 is the basal enzyme activity, and α is the mechanical load-sensitivity coefficient. This logarithmic response function demonstrates that even small mechanical changes can disproportionately enhance the cellular response, biophysically revealing how mechanotransduction incorporates a precise amplification mechanism. This relationship mathematically confirms the integration of mechanical signals with energy and redox parameters during bone remodeling (15-18).

These findings indicate that SIRT1 is not merely a regulator of gene expression but also a critical molecular hub in the conversion of mechanical loads into energy biophysics. Mechanotransduction mediated by SIRT1 in osteocytes and osteoblasts demonstrates that bone homeostasis is governed by a multidimensional biophysical network integrating energy, redox, and mechanical signals.

SIRT3: Biophysical Regulation of Mitochondrial Energy Dynamics

SIRT3, as a mitochondrial matrix-specific NAD^+-dependent deacetylase, plays a fundamental regulatory role in maintaining oxidative phosphorylation (OXPHOS) capacity and redox homeostasis. This enzyme reprograms the cell's energy production strategy by modifying the acetylation status of numerous mitochondrial proteins that determine the biophysical efficiency of energy metabolism. Specifically, SIRT3 targets the acetylation of electron transport chain (ETC) complexes (Complexes I, II, and III), ensuring the continuity of electron flow, optimizing the proton gradient, and enhancing ATP synthase activity. Through this mechanism, it minimizes energy losses during oxidative phosphorylation, thereby increasing the biophysical efficiency of ATP production (7).

The mitochondrial membrane potential ($\Delta\Psi m$) is a key parameter in cellular energy production, serving as an indicator of the proton motive force. Through the deacetylation of ETC complexes, SIRT3 reduces electron leakage in the electron transport chain, stabilizes the proton gradient, and thus maintains $\Delta\Psi m$ at high levels. This biophysical state directly influences the efficiency of energy conversion (η) and can be mathematically expressed as:

$$\eta=\eta_0+\gamma[SIRT3]$$

In this equation, η_0 represents the baseline energy efficiency in the absence of SIRT3, while γ denotes the regulatory coefficient dependent on SIRT3 levels.

This relationship demonstrates that an increase in SIRT3 activity linearly enhances energy conversion capacity, confirming its role as a direct modulator in the biophysical regulation of cellular energy homeostasis (7).

Beyond its impact on energy efficiency, SIRT3 also plays a critical role in maintaining redox balance. This enzyme enhances the activation of superoxide dismutase 2 (SOD2) by deacetylating it. The active form of SOD2 rapidly converts superoxide (O_2^-) radicals accumulating in the mitochondrial matrix into hydrogen peroxide (H_2O_2). This biophysical antioxidant mechanism limits ROS production along the electron transport chain, minimizing oxidative stress–induced damage to mitochondrial DNA, lipid membranes, and proteins (6). Consequently, SOD2 activation mediated by SIRT3 not only reduces oxidative stress but also prevents ROS-driven signaling disruptions, thereby supporting osteoblast function and cellular longevity.

Osteoblasts are highly energy-demanding cells, with ATP requirements peaking during bone matrix synthesis. Therefore, SIRT3 activity is critical for sustaining uninterrupted energy production in these cells. Efficient energy production, controlled ROS levels, and limited oxidative damage collectively enhance the anabolic capacity of bone tissue. In the absence of SIRT3, excessive acetylation of mitochondrial proteins occurs, leading to electron leakage, ROS accumulation, and reduced ATP synthesis. This condition weakens osteoblast function, decreases bone formation, and ultimately contributes to the development of an osteoporotic phenotype (6).

From a biophysical perspective, SIRT3 functions as a feedback control mechanism that minimizes energy production losses and reduces oxidative load. The regulation of the acetylation state of the Electron Transport Chain (ETC) directly affects the proton gradient (Δp) and mitochondrial membrane potential ($\Delta \Psi m$). With the activation of SIRT3, $\Delta \Psi m$ is maintained at high levels, proton leakage is prevented, and ATP synthesis efficiency is enhanced. This biophysical integrity ensures that bone cells perform optimally in terms of both energy and redox parameters.

Current evidence establishes SIRT3 as a central molecule in the biophysical regulation of mitochondrial energy dynamics. Its effects on energy conversion efficiency, ROS management, and oxidative stress resistance make it a critical regulator of energy–redox homeostasis in bone biology. These properties clearly highlight the strategic role of SIRT3 in meeting the energy demands of osteoblasts and maintaining bone homeostasis.

SIRT6 and SIRT7: Genome Stability and Energy

SIRT6 and SIRT7 are NAD^+-dependent deacetylases that play a critical role in maintaining genome stability and regulating energy metabolism at the

epigenetic level in bone cells. These two isoforms are at the core of biophysical mechanisms that both preserve DNA integrity and slow down cellular aging processes.

SIRT6 is involved in chromatin remodeling and DNA repair. Specifically, upon the occurrence of double-strand DNA breaks (DSBs), SIRT6 deacetylates lysine residues on histone H3 (H3K9Ac, H3K56Ac), making the chromatin more accessible. This event facilitates the efficient binding of DNA repair proteins (e.g., PARP1, DNA-PK, CtIP) to the damaged sites and accelerates the repair process (33, 34). This biophysical regulation demonstrates that the DNA repair rate (v_{repair}) is directly proportional to SIRT6 activity, expressed mathematically as:

$$v_{repair} = k_r \cdot [SIRT6]$$

In this equation, k_r represents the contribution coefficient of SIRT6 to DNA repair processes, while [*SIRT6*] denotes the enzyme level. This relationship demonstrates that increased SIRT6 activity directly preserves genome integrity and plays a decisive role in delaying cellular aging (35). DNA repair efficiency is critically important in osteoblasts and osteocytes, where oxidative stress is high. While mitochondria-derived ROS exacerbate DNA damage, the activation of SIRT6 minimizes this damage and slows the aging of bone cells (34, 36).

SIRT7 epigenetically supports osteoblast differentiation by deacetylating the transcription factor Osx (SP7) in the nucleus. Osx is one of the key transcription factors for osteoblast maturation, and its acetylation status, regulated by SIRT7, enhances its DNA-binding ability and transcriptional activity. This mechanism strengthens the synthesis of bone matrix proteins and promotes osteoblast proliferation and maturation (6, 21). Additionally, SIRT7's regulatory effects on ribosomal RNA biogenesis and protein synthesis make a critical contribution to energy-demanding bone formation processes.

Both isoforms play significant roles in modulating aging-related epigenetic changes. Reduced levels of SIRT6 and SIRT7 are associated with telomere shortening, accumulation of DNA damage, and increased heterochromatinization of chromatin structure; these changes drive cells into the aging process and disrupt bone homeostasis (34, 37). Part of the epigenetic dysregulation observed in osteoporosis can be attributed to the insufficient activity of these sirtuin isoforms.

From an energy biophysics perspective, SIRT6 shifts energy metabolism toward oxidative phosphorylation by deacetylating glycolytic enzymes, while SIRT7 optimizes ribosomal biogenesis, directing energy flow toward protein synthesis. This epigenetic–energetic coordination ensures continuity in the high-energy-demanding processes of matrix synthesis and repair in bone cells. The

functions of SIRT6 and SIRT7 are therefore vital for sustaining osteoblast differentiation and the regenerative capacity of bone tissue.

In conclusion, SIRT6 and SIRT7 are two strategic regulators that provide epigenetic control over aging and energy metabolism in bone cells. The DNA repair–enhancing and genome-stabilizing effects of SIRT6, combined with the support of osteoblast differentiation by SIRT7 through Osx, form a critical biophysical network that preserves both the structural and functional integrity of bone tissue. Targeting these isoforms opens new avenues for the treatment of conditions such as osteoporosis and age-related bone loss.

Energy–Biophysical Network and Clinical Implications

Sirtuins are at the center of a multilayered biophysical network that simultaneously regulates the energy metabolism, oxidative stress response, mechanical load sensing, and DNA repair of bone cells. This network manages the complex interactions among energy flow, redox balance, and genome integrity, which collectively determine the cells' ability to survive and maintain their functions. At the biophysical core of energy metabolism, there exists a dynamic balance between mitochondrial ATP production, the cost of oxidative damage, and the energy expenditure dedicated to DNA repair. The temporal variation of this balance is defined by the energy flow function *E(t)*:

$$\frac{dE}{dt} = P_{ATP} - C_{ROS} - L_{DNA}$$

In this equation, P_{ATP} represents the energy power generated through mitochondrial oxidative phosphorylation; C_{ROS} denotes the energy cost associated with biophysical damage caused by reactive oxygen species; and L_{DNA}reflects the energy loss allocated to DNA repair. Sirtuins exert a modulatory effect on all three parameters: they increase P_{ATP}while suppressing $C_{ROS}$and L_{DNA}. This mechanism ensures the maintenance of energy homeostasis and optimizes the functional capacity of bone cells (13).

The effects of SIRT1 and SIRT3 on energy flow are manifested through enhanced ATP production and suppression of ROS-induced energy loss. Meanwhile, SIRT6 and SIRT7 improve the energy efficiency of DNA repair processes and maintain genome stability. By optimizing the biophysical balance between energy production and consumption, sirtuins enable cells to use energy resources efficiently, even under stress conditions. The multiscale dynamics of this network have also been supported by mathematical modeling, which shows that energy flow is regulated through feedback mechanisms incorporating both logarithmic and linear components. These models confirm that sirtuin activation leads to marked improvements in intracellular energy efficiency (19).

The clinical implications of this biophysical regulation are also significant. Clinical and preclinical studies have shown that sirtuin activators improve bone mineral density (BMD) and bone microarchitecture. Natural polyphenols such as resveratrol and synthetic sirtuin activators such as SRT2104 increase NAD^+ levels, activating SIRT1; this stimulates osteoblast differentiation, limits osteoclastic resorption, and reduces the biophysical burden of oxidative stress (2). Furthermore, SIRT3 activation enhances mitochondrial energy production while reducing ROS-induced cellular damage, thereby increasing the energy efficiency and anabolic capacity of bone tissue (7).

These effects provide clinical benefits not only at the cellular level but also at the systemic level. In postmenopausal osteoporosis models, the use of sirtuin activators has resulted in increased bone density and reduced fracture risk. Clinical data show that natural compounds such as resveratrol improve bone quality by reducing inflammation and strengthening antioxidant defenses (6). These findings confirm that pharmacological targeting of sirtuins is a clinically viable strategy for the treatment of osteoporosis.

Literature evidence indicates that the energy–biophysical network integrated by sirtuins regulates the delicate balance among energy production, oxidative stress management, and DNA repair in bone cells. This regulation enables bone tissue to maintain its functional integrity even under aging and stress conditions. Clinical findings demonstrate that sirtuin activation is a powerful therapeutic target for osteoporosis, both from a biophysical and pharmacological perspective. Therefore, it can be concluded that sirtuin-focused strategies will likely play an increasingly significant role in future basic biophysical research as well as clinical applications.

Clinical and Therapeutic Perspective on Osteoporosis from the Standpoint of Sirtuins

Understanding the mechanisms that modulate the biophysical responses of bone tissue enables the development of new strategies for osteoporosis treatment. The osteocyte–sirtuin axis lies at the core of these strategies, offering a network that can be targeted through both mechanical and pharmacological interventions. Exercise is one of the natural activators of this axis. Regular mechanical loading increases NAD^+ levels in osteocytes, triggers sirtuin activity, and supports osteoblast functions, thereby contributing to the maintenance of bone mineral density (6). This biophysical mechanism represents a key factor explaining the protective effect of exercise against the progression of osteoporosis.

Mechanical vibration therapies, particularly those involving low-frequency and low-amplitude vibrations, enhance the mechanotransduction capacity of bone cells and strengthen the anabolic response. This approach helps preserve

trabecular architecture and increases osteoblastic activity. Clinical studies have shown that vibration therapies improve bone mineral density and reduce fracture risk (38). These therapies stand out as a non-invasive and biophysical treatment option.

Pharmacological sirtuin activators, particularly resveratrol and other NAD^+-enhancing compounds, optimize the energy metabolism of bone tissue while limiting oxidative stress. Through sirtuin-mediated mechanisms, these compounds activate the Wnt/β-catenin pathway, promote osteoblast differentiation, and suppress osteoclast activity. Preclinical data have shown that these activators exhibit synergistic effects with mechanical loading, thereby strengthening the bone remodeling process (13). Consequently, when combined with mechanical stimulation, pharmacological interventions may yield more potent outcomes in the treatment of osteoporosis.

The combination of biophysical therapies and pharmacological sirtuin activation emerges as a promising approach for the prevention and treatment of osteoporosis. This combined strategy aims to restore bone homeostasis by integrating both cellular energy biophysics and mechanotransduction pathways. Future clinical studies will further elucidate the molecular mechanisms underlying this synergistic effect and contribute to the development of personalized treatment protocols.

ROLE OF SIRTUINS IN BONE REMODELING

Bone Remodeling: Multiscale Biophysical Framework

Bone remodeling is a multiscale biophysical process in which biomechanical and biochemical signals are integrated with energy metabolism. At the molecular level, this process begins with energy regulators such as sirtuins and the NAD^+/NADH ratio; at the cellular level, it is shaped by the interactions among osteoclasts, osteoblasts, and osteocytes; and at the tissue–organ scale, it culminates in the maintenance of bone homeostasis. The remodeling cycle is influenced by the simultaneous interaction of parameters such as mechanical loading, microdamage, energy status, and hormonal regulation.

Molecular Scale: During bone resorption, osteoclasts create an acidic microenvironment on the bone surface by lowering the pH to around 4.5 through proton pumps, particularly V-ATPases. This process requires high ATP consumption and is limited by mitochondrial OXPHOS capacity. Simultaneously, elevated ROS production increases the susceptibility of osteoclasts to redox stress. Sirtuins, particularly SIRT3, regulate osteoclast function by optimizing mitochondrial redox balance. This energy-intensive process constitutes the biophysical basis of the catabolic phase of the bone remodeling cycle (1, 12).

Osteoblasts are the energy-regulating cells of the anabolic phase. The NAD^+/NADH ratio determines their ATP efficiency and matrix synthesis capacity. Through SIRT1-mediated deacetylation of FoxO and RUNX2, both energy utilization and the production of bone matrix proteins are enhanced. Additionally, osteoblasts dynamically switch between glycolytic and oxidative metabolism to minimize ATP consumption, thereby directing more energy toward matrix synthesis (6).

Cellular Scale: Osteocytes are the primary sensors of mechanical loads within the lacuno-canalicular network. Mechanical stimuli perceived through piezoelectric potentials and fluid shear stress trigger biophysical responses such as Ca^{2+} oscillations, ATP release, and NO production. These signals coordinate both osteoclast and osteoblast activity, ensuring the spatiotemporal regulation of the remodeling process. Furthermore, changes in osteocyte NAD^+ levels modulate the energy dimension of mechanotransduction through their connection with sirtuin activity.

Tissue and Organ Scale: Remodeling dynamics differ between trabecular and cortical bone regions. Trabecular bone exhibits faster remodeling due to its higher surface area, whereas cortical bone renews more slowly but possesses greater load-bearing capacity. This distinction allows mechanical loads and biophysical signals to merge with tissue-specific effects. The cross-scale coordination of remodeling optimizes both the mechanical strength of the bone and its capacity to repair microdamage.

Integration of Energy Biophysics: This process relies on the precise control of energy flow. While the proton-pumping activity of osteoclasts imposes a high energy cost, the NAD^+/NADH balance and sirtuin-mediated regulation in osteoblasts enhance energy efficiency. Osteocytes convert mechanical loads into electrical and biochemical signals, coordinating this energy flow at both the cellular and tissue levels. Thus, bone remodeling functions as a biophysical equilibrium system where energy optimization converges with the response to mechanical stimulation.

In conclusion, bone remodeling is a multiscale biophysical network in which sirtuins act as critical regulators at the intersection of energy metabolism and mechanical signal transduction. This holistic perspective defines the remodeling process not merely at the cellular level but as a system where energy and mechanical interactions are fully integrated.

SIRT1: Biophysical Regulator of Osteoblast Anabolism

SIRT1 is a critical NAD^+-dependent deacetylase that determines the energy efficiency and anabolic capacity of osteoblasts during bone remodeling. During osteoblastogenesis, SIRT1 activity synchronizes energy metabolism with cellular

differentiation and matrix synthesis. A high NAD^+/NADH ratio enhances the enzymatic activity of SIRT1, increasing the metabolic resilience of osteoblasts. This process ensures the sustainability of osteoblast function in terms of both energy production mechanisms and redox balance (13).

FoxO3a and RUNX2 Deacetylation: SIRT1 deacetylates RUNX2, a key regulator of osteoblast differentiation, and FoxO3a, a central factor in cellular stress responses. Deacetylation of RUNX2 enhances the expression of bone matrix proteins (osteocalcin, type I collagen) and strengthens mineralization. Modification of FoxO3a optimizes the oxidative stress response, preventing cellular damage.

Activation of the Wnt/β-Catenin Pathway: SIRT1 enhances β-catenin stabilization, thereby activating the Wnt signaling pathway. This pathway plays a key role in the biophysical support of osteoblast proliferation and differentiation. The effectiveness of Wnt signaling is associated with the activation of genetic programs that form the molecular basis of bone mineralization (17, 18).

Sclerostin Suppression: Osteocyte-derived sclerostin is an inhibitor that suppresses Wnt signaling. SIRT1 reduces sclerostin expression, thereby strengthening Wnt signaling and enhancing bone anabolism. This biophysical effect represents a key mechanism that explains the synergistic impact of mechanical loading and pharmacological activation.

Oxidative Stress Control: Osteoblast function depends on the regulation of ROS levels. SIRT1 enhances mitochondrial antioxidant mechanisms (e.g., MnSOD activation), limiting ROS accumulation. This increases cellular resistance to oxidative damage and preserves matrix synthesis.

This biophysical relationship can be mathematically expressed in terms of osteoblast activity $B(t)$, SIRT1 level (S_1), and ROS concentration (R) as follows:

$$\frac{dB}{dt} = k_1 S_1 - k_2 R$$

In this model, k_1 represents the positive effect of SIRT1 on osteoblast activity, while k_2 denotes the suppressive effect of ROS. The equation demonstrates that SIRT1 activation directly enhances osteoblast function, whereas ROS accumulation limits this process.

In conclusion, SIRT1 is the biophysical regulator of osteoblast anabolism. Through its integration of energy metabolism, redox control, mechanical signals, and genetic programs, SIRT1 supports the structural integrity of bone tissue. Therefore, SIRT1 represents a strategic molecule that can be targeted both biophysically and pharmacologically in the treatment of osteoporosis.

SIRT1: Osteoclast Inhibition and RANKL/OPG Balance

The effectiveness of osteoclasts in bone resorption is determined by the delicate balance between RANKL (Receptor Activator of NF-κB Ligand) and OPG (osteoprotegerin). RANKL activates the RANK receptor on osteoclast precursors, stimulating their differentiation, while OPG acts as a decoy receptor for this ligand, preventing its binding and thereby suppressing osteoclastogenesis. The ratio between these two factors is a critical biophysical parameter that determines the rate of bone resorption (5).

Effect of SIRT1 on the RANKL/OPG Balance: SIRT1 activation reduces RANKL production while increasing OPG expression in osteoblasts and osteocytes (5). This regulation limits the maturation of osteoclast precursors, thereby suppressing bone resorption. Furthermore, increased NAD^+ levels have been shown to enhance SIRT1's control over this gene expression (6). As a result, the anabolic response becomes dominant in the bone remodeling cycle.

Suppression of the NF-κB Pathway: RANKL activates the NF-κB pathway during osteoclastogenesis. SIRT1 deacetylates the p65 subunit of NF-κB, suppressing its transcriptional activity and reducing the intensity of the inflammatory response (5). This suppression limits both osteoclast differentiation and bone resorption.

NO Production and the Limitation of Osteoclastic Resorption: SIRT1-mediated nitric oxide (NO) production induces apoptosis in osteoclasts, thereby shortening their lifespan (6). Additionally, NO influences local pH regulation, reducing the acidification capacity of osteoclasts and limiting the formation of resorption sites on the bone surface.

These biophysical interactions can be expressed using the following model, which describes osteoclast activity $C(t)$ in relation to SIRT1 level (S_1) and the RANKL/OPG ratio (Q):

$$\frac{dC}{dQ} = \alpha Q - \beta S_1$$

Here, when Q is high, osteoclast activity increases, whereas an elevation in S_1 suppresses this activity. The parameters α and β represent the coefficients of RANKL-mediated stimulation and the inhibitory effect of SIRT1, respectively.

In conclusion, the inhibitory effect of SIRT1 on osteoclastic resorption is not only linked to gene expression regulation but also to the modulation of energy–redox networks and signaling pathways. This multilayered effect can be defined as a critical biophysical mechanism in the preservation of bone mass.

SIRT3: Mitochondrial Biophysics and Remodeling

SIRT3 is a key biophysical regulator of mitochondrial function in the energy metabolism of bone cells. As an NAD^+-dependent deacetylase, SIRT3 enhances energy production efficiency while limiting the harmful effects of oxidative stress, balancing both osteoblast and osteoclast activities during the bone remodeling process (7). This multifaceted role positions SIRT3 as a strategic molecule in maintaining bone homeostasis.

Deacetylation of Electron Transport Chain (ETC) Complexes and ATP Production: SIRT3 deacetylates enzymes within ETC complexes I, II, and V, preserving the proton gradient and ensuring ATP synthesis occurs at maximum efficiency (7, 39). This biochemical modification reduces proton leakage, stabilizes electron flow, and enhances cellular energy production. In osteoblasts, increased ATP production supports the synthesis of matrix proteins and mineralization processes, while in osteoclasts, improved energy efficiency contributes to the balanced regulation of resorptive activity.

SOD2 Deacetylation and ROS Detoxification: SIRT3 enhances the activity of SOD2, one of the main enzymes of mitochondrial antioxidant defense, through deacetylation (6). This regulation accelerates the detoxification of superoxide radicals (O_2^-) and maintains ROS levels within a physiological range. Controlled ROS levels support osteoblast function, while preventing excessive ROS accumulation from driving osteoclast activity to pathological levels.

Effects of Oxidative Stress on Remodeling: ROS exerts a dual effect on the bone remodeling process: at low levels, it functions as a cellular signaling mediator, whereas at high levels, it induces oxidative damage, suppressing osteoblast function. SIRT3's regulation of ROS maintains this balance, ensuring the optimal progression of the remodeling process (7).

Energy Efficiency Model: The biophysical effect of SIRT3 is expressed by a linear relationship between energy efficiency (η) and the level of SIRT3 (S_3):

$$\eta=\eta_0+\gamma S_3$$

Here, η_0 represents the basal energy efficiency, and γ denotes the coefficient by which SIRT3 enhances energy conversion. This model mathematically illustrates how SIRT3 activity promotes energy optimization in bone cells.

Role of SIRT3 in Bone Remodeling: SIRT3 supports the biophysical basis of bone anabolism by enhancing osteoblast function, while its control over mitochondrial ROS prevents osteoclast activity from becoming excessively dominant (6, 7, 39). This bidirectional regulation establishes a delicate energy balance between the catabolic and anabolic phases of the remodeling cycle. Through its deacetylation effects on ETC complexes and SOD2, SIRT3 optimizes cellular energy conversion while increasing oxidative stress tolerance. As a result,

osteoblasts function more efficiently in matrix synthesis and mineralization, whereas osteoclasts, due to limited energy support and controlled ROS levels, do not cause excessive bone resorption.

In conclusion, SIRT3 lies at the center of a regulatory network that reprograms mitochondrial biophysics, optimizes energy production during bone remodeling, precisely manages oxidative stress within a narrow range, and integrates osteoblast and osteoclast functions. These characteristics make SIRT3 a strategic target, both biophysically and pharmacologically, for the treatment of metabolic bone diseases such as osteoporosis (7).

SIRT6 and SIRT7: Epigenetic and Energy Integration

The bone remodeling process is regulated not only by mechanical stimuli and metabolic responses but also by epigenetic integrity and genomic stability. In this context, SIRT6 and SIRT7, as NAD^+-dependent nuclear sirtuins, serve as critical regulators that integrate epigenetic control with energy biophysics (6, 21). These two isoforms determine the aging and differentiation capacities of bone cells through key processes such as DNA repair, telomere protection, transcriptional reprogramming, and the coordination of energy metabolism (6).

SIRT6: Genomic Stability, Telomere Protection, and Delayed Aging: SIRT6 plays a central role in the repair of DNA double-strand breaks (DSBs), base excision repair, and the protection of telomeres (35). Deacetylation of histones H3K9 and H3K56 dynamically opens the chromatin structure, facilitating the access of DNA repair proteins to damage sites. Reduced SIRT6 activity at telomeric regions leads to telomere shortening and accelerated cellular aging, which is associated with decreased osteoblast function and impaired bone matrix synthesis (6, 21). Conversely, increased SIRT6 activity is linked to accelerated DNA damage response and delayed biophysical effects of aging. The capacity for DNA repair has been shown to be linearly dependent on SIRT6 expression, a relationship expressed by the following equation:

$$v_{repair} = kr \cdot [SIRT6]$$

This equation mathematically demonstrates that an increase in SIRT6 levels directly enhances the DNA repair rate (v_{repair}) (6, 21, 35).

SIRT7: Osteoblast Differentiation via the Osx Transcription Factor: SIRT7 functions as an epigenetic regulator in the bone remodeling process by deacetylating the Osx (osterix) transcription factor, thereby enhancing its transcriptional activity (6, 21). Activation of Osx increases the expression of osteoblast markers such as osteocalcin (BGLAP), type I collagen (COL1A1), and alkaline phosphatase (ALP), supporting osteoblast maturation and bone mineralization (6). This effect of SIRT7 is closely linked to the NAD^+/NADH

ratio, establishing a direct connection between energy metabolism and epigenetic modulation.

Epigenetic–Energy Integration and Remodeling Dynamics: The biophysical effects of SIRT6 and SIRT7 bridge energy metabolism and epigenetic regulation during bone remodeling. SIRT6 preserves DNA integrity, contributing to the reduction of oxidative stress–induced damage and prolonging osteoblast lifespan (35). SIRT7, on the other hand, accelerates osteoblast differentiation through Osx, enabling metabolic adaptation to the high energy demand of matrix protein synthesis (6, 21). The synergistic action of these two isoforms ensures the maintenance of both genetic stability and energy optimization throughout the bone remodeling process.

Biophysical and Clinical Implications: The combined action of SIRT6 and SIRT7 integrates the epigenetic foundations of the bone remodeling process with energy biophysics. DNA repair and telomere protection mediated by SIRT6 ensure the long-term preservation of osteoblast function. Meanwhile, SIRT7 enhances osteoblastogenesis through Osx activation, supporting the anabolic phase of the remodeling cycle. Therefore, pharmacological approaches such as NAD^+ boosters or sirtuin activators, by simultaneously targeting SIRT6 and SIRT7, may offer an innovative perspective in the treatment of osteoporosis (6, 21).

Mechanotransduction–Sirtuin Interaction

In the bone remodeling process, mechanotransduction forms an integrated regulatory network with sirtuin activities by converting mechanical loads into biochemical signals. Osteocytes, embedded within the bone matrix, play a key role in sensing these mechanical loads. Mechanical load *F* increases fluid shear stress in the osteocytes' lacuno-canalicular system, triggering activation of mechanosensitive ion channels such as PIEZO1 and TRPV4 (15, 16). This activation leads to intracellular Ca^{2+} oscillations; the elevated Ca^{2+} signals stimulate both ATP release and NAD^+ biosynthesis. The resulting increase in NAD^+ levels enhances activation of SIRT1 and other sirtuins, supporting the anabolic phase of the remodeling process (6, 17, 18).

Mechanical Load and NAD^+ Dynamics: Mechanical loading enhances NAD^+ biosynthesis, thereby reprogramming cellular energy metabolism (17, 18). The increase in NAD^+ levels elevates the activity of NAD^+-dependent deacetylases, particularly SIRT1, facilitating the energy-efficient execution of osteoblast functions (6). This process demonstrates a direct integration of mechanical signals with sirtuin-mediated epigenetic regulation and energy biophysics.

Piezo1/TRPV4-Calcium Signaling and Sirtuin Activation: PIEZO1 and TRPV4 channels facilitate Ca^{2+} influx in response to mechanical deformation (15,

16). The increased Ca^{2+} activates the enzyme NAMPT, which is involved in NAD^+ biosynthesis, thereby elevating NAD^+ levels. This Ca^{2+}-NAD^+-sirtuin axis represents a fundamental pathway where mechanotransduction converges with energy biophysics. Additionally, elevated Ca^{2+} activates SIRT1-mediated deacetylation pathways, enhancing Wnt/β-catenin signaling and accelerating bone anabolism (17, 18).

Mathematical Model: The biophysical synergy among mechanical load, NAD^+ level, and SIRT1 activity can be expressed by the following model:

$$R=R_0+\alpha ln(1+F)+\beta N+\delta S_1$$

Here, R represents the osteocyte response, F is the applied mechanical load, N is the NAD^+ level, S_1 denotes SIRT1 activity, R_0 is the basal response, and α, β, and δ are the contribution coefficients of mechanical load, NAD^+, and SIRT1 on the response, respectively. This equation mathematically characterizes the logarithmic response nature of mechanical signals and their synergy with the NAD^+-sirtuin axis (17, 18).

Biophysical Meaning and Clinical Perspective: Integration between mechanical loading and sirtuin activation regulates both energy optimization and mechanical resilience in the bone remodeling process. This synergy contributes to the maintenance of osteoblast functions at the cellular level as well as the preservation of bone integrity at the tissue level. Clinically, the combination of low-frequency vibration therapies that mimic mechanical loading and sirtuin activators is considered a promising strategy for osteoporosis treatment (17, 18, 38).

CONCLUSIONS AND FUTURE DIRECTIONS

This study systematically elucidates the critical role of sirtuins-protein-structured enzymes functioning in physiological energy systems within bone cells-in bone biology and the pathogenesis of osteoporosis from an advanced biophysical perspective. The findings highlight that sirtuins, particularly SIRT1, SIRT3, SIRT6, and SIRT7, serve as fundamental pillars in the bone remodeling process by regulating energy metabolism, redox homeostasis, and epigenetic control.

In osteoporosis, the decline in NAD^+ levels and reduced sirtuin expression, combined with mitochondrial dysfunction and ROS accumulation, lead to bone loss. Conversely, mechanical loading enhances NAD^+ production, activating sirtuins and promoting osteoblastogenesis (6, 17, 18). Furthermore, pharmacological sirtuin activators (such as resveratrol, SRT2104, and NAD^+ precursors) together with biophysical interventions (mechanical loading, electromecanical stimulation) exhibit synergistic effects, offering promising

strategies for preserving bone mass and suppressing osteoclastic activity (13, 15, 16).

Future research may focus on fully elucidating the intercellular signaling networks among osteocytes, osteoblasts, and osteoclasts regulated by sirtuins, alongside developing advanced systems biology models that explain the interaction between NAD^+ metabolism and mechanotransduction. The validation of selective sirtuin isoform modulators, epigenetic regulators, and mitochondria-targeted agents through clinical phase II–III trials could strengthen confidence in enhancing the efficacy of osteoporosis treatment protocols. Biophysical stimulation technologies, particularly next-generation devices targeting osteocyte PIEZO1 channels, represent promising developments for enhancing sirtuin-mediated remodeling responses. Furthermore, personalized treatment approaches that optimize therapy by considering individual NAD^+ levels, sirtuin profiles, and mechanical load capacities through AI-supported models remain important innovations requiring further investigation (6, 21).

From a translational potential perspective, sirtuin modulation-through the integration of pharmacological and biophysical strategies-can prioritize a revolutionary paradigm in osteoporosis treatment. Pharmacological agents regulate bone cells' energy metabolism and epigenetic framework to promote anabolic effects, while biophysical methods activate mechanotransduction and piezoelectric signaling to optimize bone responses. The combination of these two approaches simultaneously targets both molecular and mechanical dimensions of osteoporosis, emerging as a powerful strategy to reduce fracture risk and improve quality of life (7, 19).

In conclusion, bone tissue is not merely a mineralized structure; it is a biophysical system where energy flow, electrical signals, and biomechanical forces dynamically interact. Sirtuins serve as molecular control nodes coordinating the energy and redox balance within this system. Future integration of sirtuin biology with advanced biophysical methodologies and the development of novel therapeutic strategies hold the potential to open new horizons in osteoporosis prevention and treatment, leading clinical approaches in this field.

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