Myokines in exercise-mediated bone homeostasis: Molecular signaling mechanisms and therapeutic implications for bone disorders (Review)

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Abstract. Skeletal muscle functions as an endocrine organ, secreting myokines that mediate interorgan communication with bone. Exercise-induced myokines regulate bone homeostasis by orchestrating osteoblast differentiation, osteoclastogenesis, and osteocyte mechano-sensing through key signaling pathways, including the Wnt/β-catenin, mitogen-activated protein kinase, phosphatidylinositol-3-kinase/AKT, nuclear factor kappa B and transforming growth factor-beta/bone morphogenetic protein pathways. The present review provides a critical synthesis of the current evidence and proposes a conceptual framework for the tripartite muscle-bone-immune axis, which has not been systematically integrated into previous reviews. Emerging evidence highlights a tripartite muscle-bone immune axis, wherein myokines modulate immune cells within the bone niche, with dysregulation contributing to age-related osteoporosis and sarcopenia. Methodological innovations such as multi-omics, single cell and spatial transcriptomics, organ-on-a-chip platforms, and artificial intelligence are accelerating discovery. The present review synthesizes current knowledge on myokine mediated muscle-bone crosstalk and evaluates the therapeutic implications for bone disorders.

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Abbreviations: AKT, protein kinase B; BMP, bone morphogenetic protein; BMSC, bone marrow mesenchymal stem cell; CCL5, chemokine ligand 5; ERK, extracellular signal-regulated kinase; IGF-1, insulin-like growth factor-1; JAK, Janus kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; Metrnl, meteorin-like protein; MSC, mesenchymal stem cell; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; OPG, osteoprotegerin; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PI3K, phosphatidylinositol 3-kinase; PTH, parathyroid hormone; PTOA, post-traumatic osteoarthritis; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; Runx2, runt-related transcription factor 2; STAT3, signal transducer and activator of transcription 3; STING, stimulator of interferon genes; TGF-β, transforming growth factor-beta

Key words: myokines, exercise, bone homeostasis, signaling pathways, muscle bone crosstalk, osteoporosis, sarcopenia, therapeutic targets

Contents

1. Introduction

The musculoskeletal system functions as an integrated unit in which skeletal muscle and bone exhibit bidirectional communication beyond their traditional biomechanical roles. The interdependence between the skeletal muscle and bone is particularly evident in response to mechanical loading, where physical activity simultaneously influences muscle contractility and skeletal architecture (1). The concept of the 'muscle-bone unit' has evolved substantially over the past decade, shifting from a purely mechanical paradigm toward the recognition of complex biochemical crosstalk mediated by secreted factors (2). Skeletal muscle, which is now understood to function as an endocrine organ, produces and releases a diverse array of cytokines, peptides and extracellular vesicles, which are collectively termed myokines (3). These factors act in autocrine, paracrine and endocrine manners to coordinate interorgan communication, with bone representing one of the most physiologically relevant targets (4).

The clinical significance of muscle-bone crosstalk has been highlighted by the parallel aging-related epidemics

of sarcopenia and osteoporosis, conditions that frequently coexist and share overlapping pathogenic mechanisms (5). Observational studies have shown that reduced in muscle mass and strength, strongly correlate with bone loss and fracture risk, suggesting the involvement of common regulatory pathways (6). However, the molecular underpinnings of this association have remained incompletely understood until recent advances in myokine biology provided mechanistic insights. Gries *et al* (7) comprehensively summarized the spectrum of muscle-derived factors that influence bone metabolism, highlighting the functional heterogeneity within this signaling network. Complementing this, Kaji (2) provided an updated framework for understanding how exercise-induced myokines orchestrate cellular responses in bone, emphasizing the importance of context-dependent signaling.

The identification of irisin as an exercise-responsive myokine with osteogenic properties has catalyzed considerable research interest. Subsequent studies have demonstrated that irisin promotes osteoblast differentiation through multiple mechanisms, including activation of bone morphogenetic protein (BMP)/SMAD signaling and stabilization of β-catenin (8,9). Conversely, myostatin, a member of the transforming growth factor-beta (TGF-β) superfamily, exerts catabolic effects on bone by inhibiting osteoblast differentiation and promoting mesenchymal stem cell adipogenesis (10,11). This functional dichotomy illustrates that the net effect of exercise on bone homeostasis depends on the balance between anabolic and catabolic myokine signals. Recent systematic reviews have consolidated evidence that myokines such as IL-6, IL-15 and osteocrin contribute to this regulatory network, with effects that are often exercise modality-dependent and influenced by factors including intensity, duration and training status (12).

Emerging evidence has further expanded the conceptual framework by incorporating immune and metabolic dimensions into the muscle-bone crosstalk paradigm. Skeletal muscle-derived IL-33 has been shown to regulate bone metabolism via $CD8^+$ T cell-secreted chemokine ligand 5 (CCL5), establishing a direct immunological axis linking muscle activity to skeletal homeostasis (13). Additionally, chronic low-grade inflammation characteristic of aging, termed inflammaging, disrupts normal myokine profiles and contributes to the pathogenesis of both sarcopenia and osteoporosis (14). The recognition that myokines function as immunomodulators within the bone niche has prompted re-evaluation of exercise interventions as strategies to counteract inflammation-driven bone loss.

The present review aims to synthesize current knowledge regarding the molecular signaling mechanisms through which exercise-induced myokines regulate bone homeostasis, with particular focus on the key signaling pathways, the emerging paradigm of muscle-bone-immune interactions, and the translational implications for therapeutic development in bone disorders.

The present narrative review was conducted to synthesize current evidence on myokine-mediated exercise regulation of bone homeostasis. A comprehensive literature search was performed in the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) for articles published from January 2000 to May 2026. The search terms included combinations of the following keywords: 'myokines', 'exercise', 'bone homeostasis', 'osteoblast', 'osteoclast', 'osteocyte', 'irisin', 'myostatin', 'interleukin-6', 'signaling pathway', 'Wnt/β-catenin', 'MAPK', 'PI3K/AKT', 'NF-κB', 'TGF-β/BMP', 'muscle-bone crosstalk', 'muscle-bone-immune axis', 'osteoporosis', 'sarcopenia', 'therapeutic targets', 'biomarkers', 'Multiomics', 'single-cell', 'spatial transcriptomics', 'organ-on-chip', and 'machine learning'. Only English-language articles were included. Both original research and review articles were considered. Studies involving human subjects, animal models, *in vitro* experiments, and methodological innovations were included if they addressed the molecular mechanisms, signaling pathways, or therapeutic implications of myokines in bone homeostasis. Reference lists of retrieved articles were manually screened to identify additional relevant studies.

2. Exercise-induced myokine secretion: Sources, regulation and bone targeting

Skeletal muscle functions as an endocrine organ, secreting a diverse array of cytokines, peptides and extracellular vesicles that are collectively termed myokines and that mediate interorgan communication (15). Exercise serves as a powerful physiological stimulus that profoundly alters the myokine secretome in a manner dependent on contraction mode, intensity and duration (1). These exercise-induced factors act locally within muscle and distally on bone, where they orchestrate cellular responses critical for maintaining bone homeostasis (16). Understanding the regulatory mechanisms governing myokine secretion and their specific targeting of bone cells is fundamental to leveraging these molecules for therapeutic benefit in bone disorders (Fig. 1).

Defining myokines and exercise-dependent regulation. Myokines are defined as cytokines or peptides synthesized and released by skeletal muscle fibers, a concept that has expanded the understanding of muscle as a secretory organ (17). Their expression is dynamically regulated by contractile activity, with both acute and chronic exercise eliciting distinct secretory profiles (18). The myokine repertoire includes classical cytokines such as interleukin-6 (IL-6), growth factors such as insulin-like growth factor-1 (IGF-1), and novel peptides such as irisin and meteorin-like protein (Metrnl) (1,19). A systematic review by Jaśkiewicz *et al* (16) emphasized that the characterization of myokines based on exercise context is essential, as their effects on bone metabolism can be context-dependent and even opposing. For instance, while irisin is consistently anabolic for bone, myostatin exerts catabolic effects, demonstrating the functional heterogeneity within this family (12).

Mechano-transduction in skeletal muscle: Contraction-coupled myokine release. The mechanical forces generated during muscle contraction are transduced into biochemical signals that initiate myokine synthesis and secretion (20). This mechano-transduction process, mediated by mechanosensitive channels (for example, Piezo1) and integrins, triggers the activation of multiple signaling cascades, including the mitogen-activated protein kinase (MAPK) and calcium-dependent pathways, which converge on transcriptional regulators such as peroxisome proliferator-activated

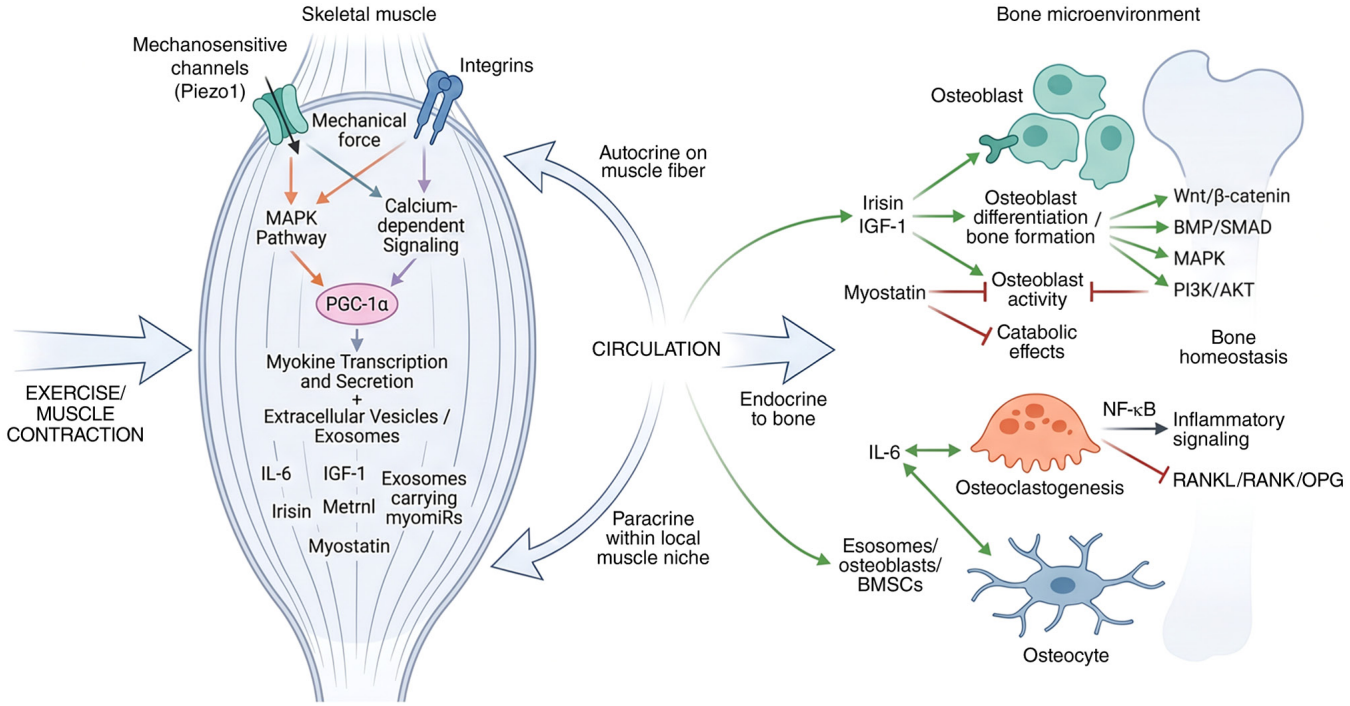

Figure 1. Schematic illustration of exercise-induced myokine secretion and its multi-level actions on bone homeostasis. Mechanical forces during exercise activate PGC-1α and mechanotransduction pathways in skeletal muscle, promoting secretion of myokines (for example, irisin, IL-6 and myostatin) and release of extracellular vesicles. These factors act in endocrine, paracrine, and autocrine manners to regulate osteoblasts, osteoclasts, and osteocytes, thereby controlling bone homeostasis. RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; OPG, osteoprotegerin; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; BMP, bone morphogenetic protein; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6.

receptor gamma coactivator 1-alpha (PGC-1α) (21). PGC-1α is a master regulator of the exercise-responsive myokine program, driving the expression of irisin and other secreted factors (22). Studies utilizing *in vitro* biomechanical stimulation of muscle constructs have demonstrated that cyclic stretch alone is sufficient to modulate myokine secretion, confirming that mechanical strain is a direct and potent regulator (20). Furthermore, the release of myokines is not solely transcriptionally regulated; post-translational processing and secretion via extracellular vesicles also play critical roles (23,24). For example, exosomes derived from exercised muscle carry myo-miRs and proteins that can directly influence osteoblast function (23,24).

Endocrine, paracrine and autocrine actions on bone cells. Upon secretion, myokines exert their effects through three primary modes of action: Autocrine signaling on the secreting myofiber, paracrine signaling on neighboring cells within the muscle niche, and endocrine signaling on distant organs such as bone (4,18). For bone homeostasis, the endocrine actions of myokines have been the most extensively studied. Gomarasca *et al* (17) provided a comprehensive overview of how myokines such as irisin, IL-6 and myostatin enter the circulation and interact with specific receptors on osteoblasts, osteoclasts and osteocytes. However, paracrine signaling within the local muscle-bone microenvironment is also critical, particularly in regions of muscle-bone attachment (7,25). The concept of a functional 'muscle-bone unit' underscores the importance of both systemic and local crosstalk (26). Importantly, recent evidence suggests that autocrine myokine loops within muscle may influence muscle health and, consequently, the secretory capacity of the muscle, indirectly impacting bone (2).

Influence of exercise type, intensity and duration on myokine profiles. The myokine response is highly sensitive to the exercise modality (direct human evidence). Resistance training, characterized by high mechanical load, preferentially stimulates the secretion of factors like IGF-1 and myostatin, whereas endurance exercise, involving repetitive, lower-load contractions, is a robust inducer of irisin, IL-6 and Metrnl (27,28). The dose-response relationship between exercise intensity and myokine secretion is non-linear. For instance, acute high-intensity interval training has been shown to elicit a distinct myokine profile, with a pronounced increase in IL-6 and a concomitant, albeit transient, suppression of bone formation markers (29,30). Conversely, moderate-intensity continuous exercise may produce a more sustained elevation of osteogenic myokines such as irisin (31,32). Duration also plays a key role; a single marathon race can induce a significant but temporary rise in sclerostin (SOST) and other myokines, reflecting an acute stress response (31). By contrast, chronic exercise training leads to adaptations in the basal myokine setpoint, often resulting in a favorable baseline profile for bone health (33,34). Agostinete *et al* (28) demonstrated that in adolescents, the bone benefits of resistance training were mediated through distinct myokine pathways compared with impact sports, emphasizing that the qualitative nature of the exercise stimulus dictates the myokine signature.

Sex, age and circadian modulation of exercise-induced myokine secretion. Biological variability, including sex, age and circadian rhythms, significantly modulates the myokine response to exercise. Sex hormones directly influence myokine expression; estrogen deficiency, as observed in postmenopausal women, alters the secretion of IL-6, myostatin, and other factors that regulate osteoclastogenesis (6,35,36). Norton *et al* (35) showed that estrogen regulates myokines that enhance osteoclast differentiation, providing a mechanistic link between menopause and increased bone resorption. Aging is associated with a state of chronic low-grade inflammation and anabolic resistance, which blunts the myokine response to exercise (37). This age-related dysregulation contributes to the pathogenesis of sarcopenia and osteoporosis (37). Furthermore, circadian rhythms regulate the expression and secretion of myokines, with some, such as irisin, exhibiting diurnal variation (21). The timing of exercise can therefore influence the magnitude and efficacy of the myokine response, a concept with implications for optimizing exercise prescription (21). Koltun *et al* (38) observed that prior training status also modifies the myokine and bone turnover response to acute exercise, suggesting an interaction between fitness level and these biological factors. Collectively, these modulators must be considered when interpreting myokine data and designing personalized exercise interventions. Critically, the relative contribution of endocrine vs. paracrine myokine signaling to bone homeostasis remains unclear, and the lack of standardized exercise protocols limits cross-study comparisons.

Throughout the present review, human studies are prioritized for translational relevance; animal and *in vitro* studies are indicated as such. Established mechanisms refer to findings replicated across multiple laboratories, whereas emerging hypotheses are based on limited data.

3. Molecular mechanisms of myokine action on bone cells

The cellular response of bone to exercise-induced myokines is mediated through a complex network of signaling pathways that converge on the three principal bone cell types: Osteoblasts, osteoclasts and osteocytes. Myokines exert pleiotropic effects on these cells, either promoting bone formation or suppressing resorption, with the net outcome depending on the specific myokine, its concentration, and the cellular context (Fig. 2).

Osteoblast differentiation and activity: Irisin, myostatin and osteocrin. Myokines critically influence osteoblastogenesis by modulating key transcriptional programs. Irisin, a cleaved product of fibronectin type III domain-containing protein 5, is a well-characterized osteogenic myokine. Studies have demonstrated that irisin enhances the osteogenic differentiation of various mesenchymal stem cells' (MSCs) populations, including dental bud-derived MSCs and pre-osteoblastic MC3T3-E1 cells, by upregulating the expression of master osteogenic transcription factors such as runt-related transcription factor 2 (Runx2) and osterix (39,40). The downstream signaling pathways mediating these effects, including Wnt/β-catenin and BMP/SMAD, are discussed in detail in Section 4. By contrast, myostatin acts as a potent negative regulator of bone formation by suppressing IGF-1-dependent citrate secretion via nicotinamide adenine dinucleotide phosphate oxidase 4 (10,41). The balance between anabolic and catabolic myokines dictates the net osteogenic outcome in response to exercise (42). Irisin enhances osteogenic differentiation (established, multiple *in vitro*/animal studies). However, the effects of these myokines are context-dependent and can vary with species, model system, dose, disease state, exercise modality, sex, age, and assay method.

Osteoclastogenesis and bone resorption: IL-6, IL-15 and the receptor activator of nuclear factor-κB ligand (RANKL)/receptor activator of nuclear factor-κB (RANK)/osteoprotegerin (OPG) axis. Myokines exert profound control over osteoclast differentiation and activity, primarily through modulation of the RANKL/RANK/OPG axis. IL-6, a myokine robustly induced by exercise, has a dual and context-dependent role in osteoclastogenesis. IL-6 can promote the proliferation of osteoclast precursors and stimulate the production of inflammatory mediators, thereby enhancing osteoclast formation (42). In line with this, IL-6 deficiency has been shown to enhance intramembranous ossification following stress fracture, suggesting that its suppression can be beneficial for bone repair (43). However, the effect of IL-6 is nuanced, as signaling through the glycoprotein 130 receptor can also lead to the induction of osteoclastogenesis via the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway, a mechanism that can be exacerbated by conditions such as hyperglycemia (44,45). The critical balance is further regulated by myokines such as oncostatin M, which can stimulate RANKL expression, but also induce the negative feedback regulator WNT16 to limit excessive osteoclast formation (46,47). Collectively, these myokines regulate bone resorption by finely tuning the RANKL/OPG ratio and downstream signaling pathways.

Osteocyte mechano-sensing and intercellular communication. Osteocytes, the most abundant bone cells, function as the primary mechano-sensors in bone and are also key targets of myokine signaling. Exercise-induced mechanical loading is transduced by osteocytes, which then release signaling molecules that coordinate bone remodeling. Previous evidence suggests that myokines can modulate this process. For instance, mechanical strain in osteocytes has been shown to influence the secretion of parathyroid hormone 1 receptor, which in turn regulates osteoclast formation through modulation of IL-6 and C-X-C motif chemokine ligand 5 (48). Furthermore, the myokine irisin has been shown to influence osteocyte function indirectly by affecting the inflammatory milieu. A key mechanism involves the regulation of SOST, an osteocyte-derived inhibitor of bone formation. In pathological conditions such as particle-induced osteolysis, suppression of the SOST gene activates osteocyte Wnt/β-catenin signaling, preventing bone resorption (49). This underscores the critical role of osteocytes as intermediaries that integrate mechanical and humoral signals, including myokines, to orchestrate local bone remodeling.

Crosstalk with bone marrow mesenchymal stem cells (BMSCs). BMSCs are the common precursors for osteoblasts and adipocytes, and their lineage commitment is highly sensitive to myokine signaling. The secretome of exercised

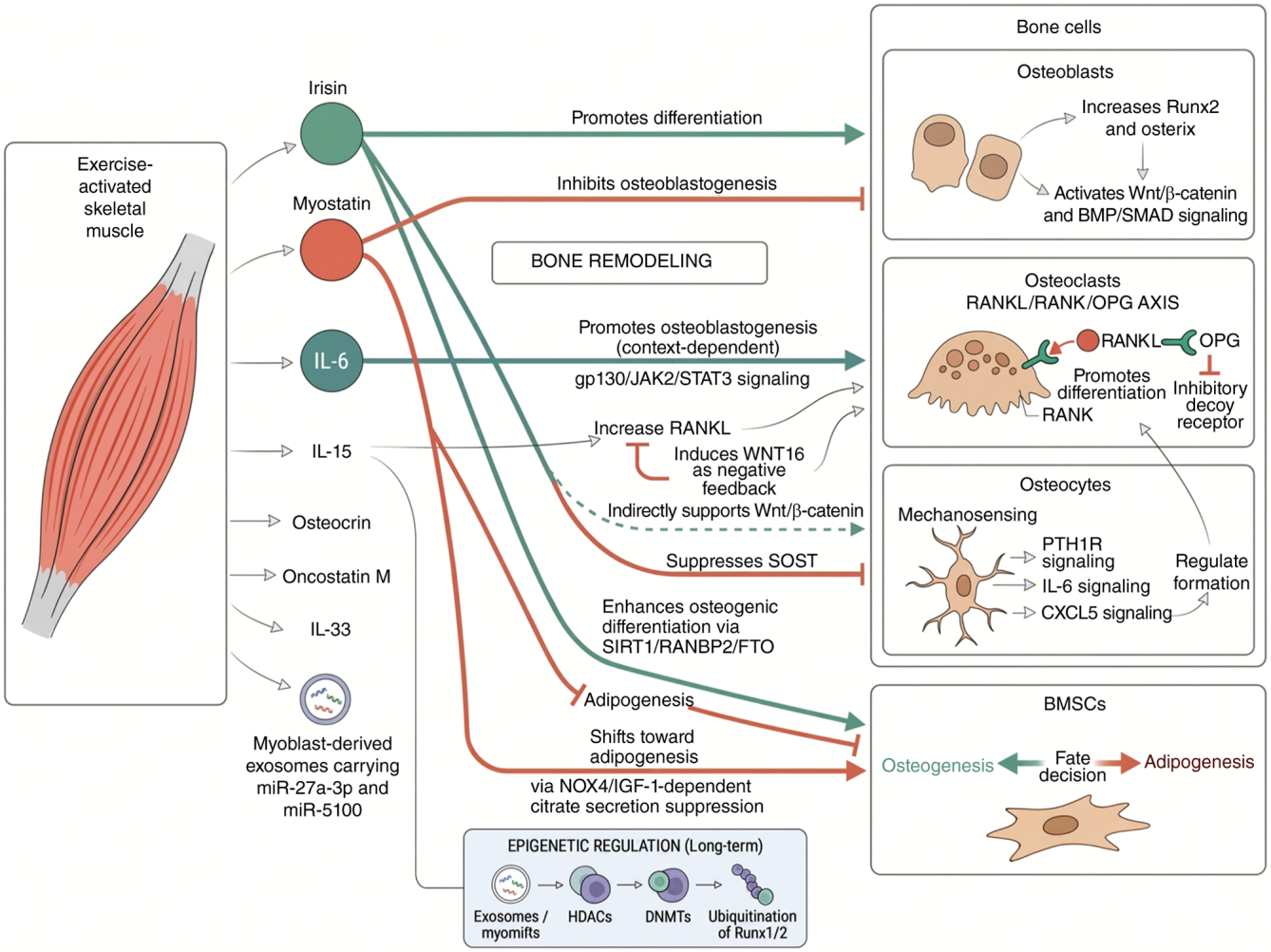

Figure 2. Myokine signaling networks regulating bone cell fate. Green modules indicate anabolic myokines and pro-osteogenic pathways promoting bone formation; red modules represent catabolic myokines and pro-resorptive pathways driving bone loss. These myokines regulate the activity of major bone cells to balance bone remodeling. HDACs, histone deacetylases; BMSCs, bone marrow mesenchymal stem cells; RANK, receptor activator of nuclear factor-κB; RANKL, RANK ligand; OPG, osteoprotegerin; PTH1R, parathyroid hormone 1 receptor; DNMTs, DNA methyltransferases; BMP, bone morphogenetic protein; miR, microRNA; SOST, sclerostin; CXCL5, C-X-C motif chemokine ligand 5; SIRT1, sirtuin1; NOX4, nicotinamide adenine dinucleotide phosphate oxidase 4; IGF-1, insulin-like growth factor-1; GP130, glycoprotein 130; IL, interleukin.

muscle, including myokines and myoblast-derived exosomes, directly influences BMSC fate. Irisin has been demonstrated to enhance the osteogenic differentiation of BMSCs while simultaneously inhibiting their adipogenic differentiation, a dual action that favors bone formation (50,51). This effect is mediated through the sirtuin 1/RANBP2/FTO signaling axis, which governs the stability of key osteogenic transcripts (51). By contrast, myostatin has been shown to inhibit the osteogenic potential of BMSCs, promoting a shift towards adipogenesis, which contributes to the age-related decline in bone mass (10). Moreover, exosomes derived from myoblasts carry myomiRs such as miR-27a-3p and miR-5100, which can be taken up by pre-osteoblasts and BMSCs to promote osteogenic differentiation, highlighting a non-canonical mode of myokine delivery (52). Thus, myokines exert a critical regulatory role in determining the differentiation trajectory of BMSCs within the bone marrow niche.

Epigenetic and post-translational regulation by exercise-responsive myokines. The sustained effects of exercise on bone health are increasingly attributed to myokine-induced epigenetic modifications. These changes alter chromatin structure and gene expression patterns without altering the DNA sequence itself, providing a mechanism for long-term adaptation. Exercise training has been shown to induce changes in the expression of myomiRs and osteomiRs, which are small non-coding RNAs that regulate gene expression post-transcriptionally (53). For example, myoblast-derived exosomes enriched with specific microRNAs can modulate osteogenic gene expression in recipient bone cells (52). Furthermore, myokine signaling can influence the activity of histone deacetylases and DNA methyltransferases, thereby regulating the accessibility of promoters for key transcription factors involved in osteoblast and osteoclast differentiation (54). Post-translational modifications, such as ubiquitination, also play a role; irisin facilitates the ubiquitination and activation of Runx1/2, linking myokine signaling directly to the activation of osteogenic transcription factors (50). Mechanistically, irisin induces non-proteolytic K63-linked ubiquitination of RUNX2, which enhances its transcriptional activity by

promoting nuclear retention, facilitating coactivator recruitment, and stabilizing the protein without targeting it for proteasomal degradation. This is distinct from K48-linked ubiquitination, which typically marks proteins for degradation. The K63-linked ubiquitination of RUNX2 thereby sustains osteogenic gene expression and promotes osteoblast differentiation (50). These epigenetic and post-translational layers of regulation explain how the transient secretion of myokines during exercise can translate into durable changes in bone cell function and overall bone homeostasis.

4. Key myokine signaling pathways in bone homeostasis

This section focuses specifically on signaling pathways activated by exercise-induced myokines (irisin, myostatin and IL-6) in bone cells. Studies of general pathway biology without direct myokine involvement are cited as background only when explicitly noted. The skeletal response to exercise-derived myokines is governed by a complex intracellular signaling network that translates secreted cues into specific cellular outcomes in bone. These pathways do not operate in isolation; rather, they constitute an integrated signaling circuitry that determines whether the net effect favors bone formation or resorption. The Wnt/β-catenin cascade serves as a central anabolic axis, while the MAPK, phosphatidylinositol-3-kinase (PI3K)/ protein kinase B (AKT) and nuclear factor kappa B (NF-κB) pathways mediate both osteogenic and catabolic responses depending on the specific myokine and cellular context. Additionally, the TGF-β/BMP superfamily and emerging regulators of autophagy and mitochondrial dynamics add further layers of complexity (Fig. 3). A comprehensive understanding of these molecular mechanisms is essential for identifying therapeutic targets and optimizing exercise interventions for bone disorders.

Wnt/β-catenin cascade: A central anabolic hub. The canonical Wnt/β-catenin signaling pathway is a critical mediator of the osteogenic effects of several exercise-responsive myokines. Irisin, the most extensively studied myokine in this context, has been shown to activate this pathway to promote osteoblast differentiation. Mechanistically, recombinant irisin prevents the reduction in osteoblast differentiation induced by simulated microgravity through increasing β-catenin expression, thereby preserving osteogenic capacity (55). These findings are primarily derived from *in vitro* and rodent studies; human data remain limited. Further supporting this, irisin promotes osteogenesis by activating the BMP/SMAD signaling pathway via αV integrin, a cascade that converges with and enhances Wnt signaling to regulate bone mass in mice (8). This dual activation of BMP and Wnt pathways underscores the integrated nature of myokine signaling. Conversely, myostatin, a negative regulator of muscle mass, antagonizes these anabolic pathways. Genetic and pharmacologic inhibition of myostatin in a murine model of osteogenesis imperfecta led to improved bone properties, an effect partly attributed to disinhibition of Wnt signaling (56). Collectively, these findings position the Wnt/β-catenin cascade as a key node where anabolic and catabolic myokine signals converge to dictate osteoblast function.

MAPK and PI3K/AKT pathways: Dual regulators of bone cell fate. The MAPK and PI3K/AKT pathways are ubiquitously involved in transducing myokine signals, yet their effects on bone are highly context-dependent. The MAPK family, including p38, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK), mediates both pro-osteogenic and pro-resorptive actions. For instance, IL-6, a myokine robustly induced by exercise, stimulates its synthesis in osteoblast-like cells via p38 MAPK regulation, establishing a positive feedback loop that can amplify inflammatory signals (57,58). Furthermore, recent evidence indicates that exercise-derived irisin prevents bone loss by activating nuclear factor erythroid 2-related factor 2 (Nrf2) and inhibiting the stimulator of interferon genes (STING)/NF-κB signaling axis, highlighting crosstalk between these pathways (59). The dual nature of MAPK signaling is further illustrated by studies on osteoclastogenesis. Thus, while MAPK activation can promote inflammation-driven bone loss, it is also a necessary component of the mechano-transduction machinery that maintains bone health, necessitating a nuanced interpretation of its role.

NF-κB and inflammatory signaling in bone remodeling. The NF-κB pathway is a master regulator of inflammatory responses and a central driver of osteoclastogenesis. Numerous myokines and exercise-associated factors exert their effects on bone by modulating this pathway. However, direct evidence linking these compounds to exercise-induced myokine signaling is lacking. The relevance of this pathway in the context of exercise is highlighted by recent work showing that exercise-derived irisin prevents bone loss through Nrf2 activation and subsequent inhibition of STING/NF-κB signaling, providing a direct mechanistic link between physical activity, a myokine, and suppression of inflammatory bone resorption (59). Moreover, the chronic low-grade inflammation associated with aging and sarcopenia is intimately linked to NF-κB dysregulation. The inflammation-energy metabolism axis has been identified as a central driver of sarcopenia-osteoporosis, emphasizing that NF-κB acts as a key mediator of this pathological crosstalk (60). These studies collectively affirm that the therapeutic potential of myokines lies partly in their capacity to fine-tune NF-κB activity, shifting the balance from catabolic inflammation toward anabolic bone formation.

TGF-β/BMP superfamily: From myostatin to BMPs. Members of the TGF-β superfamily, including BMPs and myostatin, are pivotal in the muscle-bone crosstalk. While BMPs are well-established inducers of osteogenesis, myostatin acts as a potent inhibitor. A complex interplay exists whereby IL-6 potentiates BMP-2-induced osteogenesis and adipogenesis via two different BMPR1A-mediated pathways, indicating that inflammatory myokines can modulate the osteogenic efficacy of BMPs (61). In the context of aging, BMP-4 rescues the bone regenerative potential of old muscle-derived stem cells via regulation of cell cycle inhibitors, suggesting that enhancing BMP signaling could counteract age-related declines in bone repair (62). Conversely, the inhibitory effects of myostatin on bone are well-documented. Fibroblast growth factor-2 targets SOST in bone and myostatin in skeletal muscle to mitigate the deleterious effects of glucocorticoids on musculoskeletal degradation, highlighting a dual therapeutic

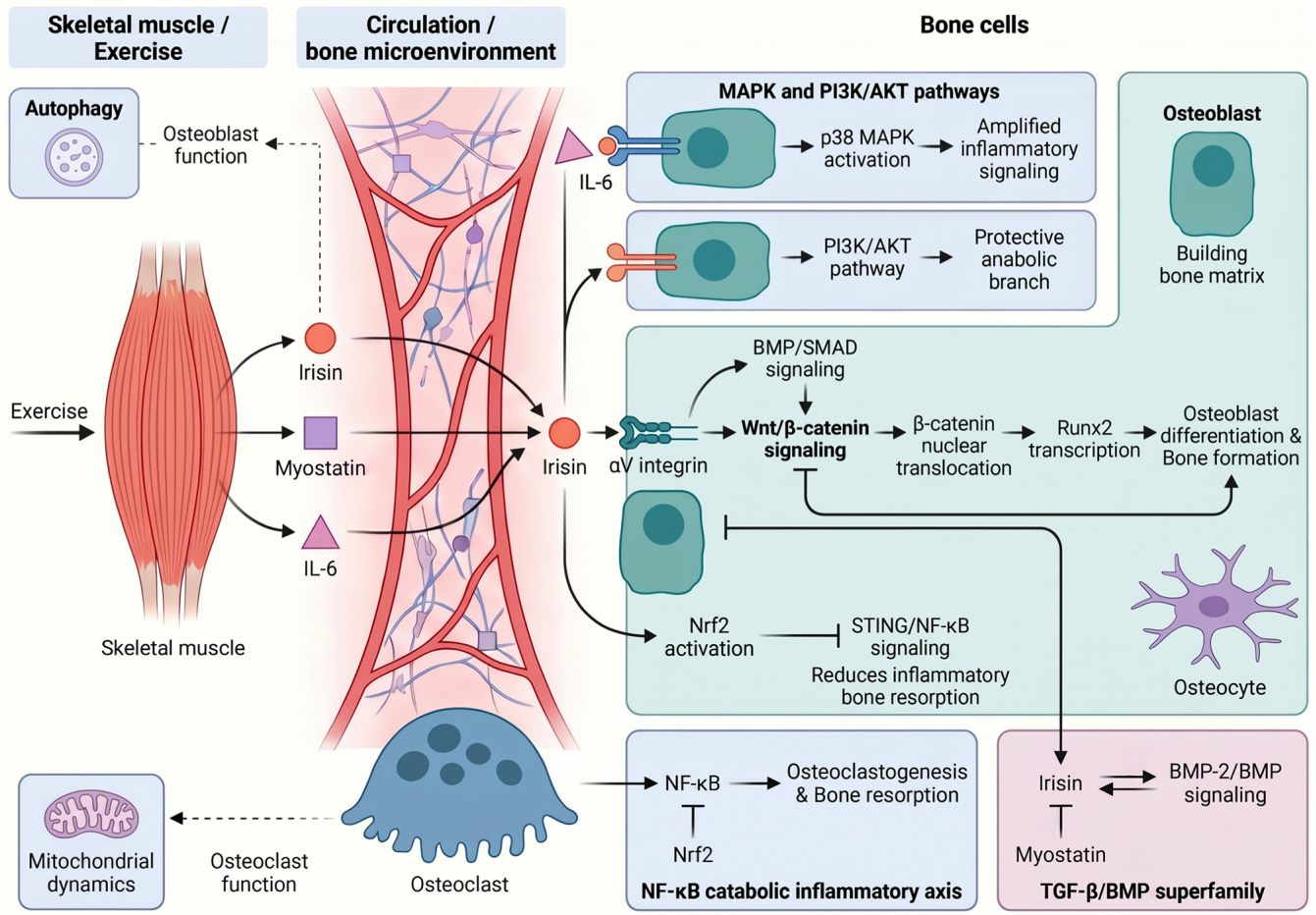

Figure 3. Key signaling pathways mediating myokine effects on bone homeostasis. Schematic diagram illustrating the major signaling cascades through which exercise-induced myokines regulate osteoblasts, osteoclasts, and osteocytes. BMP, bone morphogenetic protein; Nrf2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor kappa B; TGF-β, transforming growth factor-beta; IL, interleukin; STING, stimulator of interferon genes; Runx2, runt-related transcription factor 2.

strategy (63). More recently, the effect of recombinant irisin on BMP-2-induced osteogenesis has been investigated, revealing that irisin can modulate this process, further illustrating the interconnectedness of these signaling hierarchies (64). Mechanistically, irisin enhances BMP-2-induced osteogenesis by promoting the nuclear translocation of phosphorylated SMAD1/5/8 and increasing the transcriptional activity of Runx2, thereby synergizing with BMP-2 to drive osteoblast differentiation. This modulatory effect is dependent on the activation of the MAPK pathway, as pharmacological inhibition of MAPK abrogates the irisin-mediated enhancement of BMP-2 signaling. The therapeutic implications are significant, as modulating the balance between osteogenic BMP signaling and catabolic myostatin signaling offers a promising avenue for treating bone disorders. The balance between osteogenic BMP signaling and catabolic myostatin signaling is critical for bone homeostasis. As aforementioned, myostatin inhibits osteoblast differentiation (10,41), whereas irisin promotes osteogenesis through BMP/SMAD and Wnt/β-catenin pathways (55). Exercise and mechanical loading favor the anabolic balance by upregulating osteogenic myokines such as irisin while modulating the expression of catabolic factors (22). Therapeutic strategies aimed at shifting this balance toward BMP signaling hold promise for treating bone disorders. Irisin modulates BMP-2-induced osteogenesis (emerging, requires *in vivo* validation).

Autophagy and mitochondrial dynamics in myokine-targeted bone cells. Emerging evidence underscores the importance of cellular quality control mechanisms, particularly autophagy and mitochondrial dynamics, in mediating the effects of myokines on bone homeostasis. These processes are critical for the survival, differentiation and function of osteoblasts and osteoclasts. Conversely, the preservation of mitochondrial health appears crucial for anabolic responses. Mitochondrial oxidative stress or decreased autophagy in osteoblast lineage cells is not sufficient to mimic the deleterious effects of aging on bone mechano-responsiveness, suggesting that these processes interact with other age-related factors (65). The role of myokines in regulating these pathways is an emerging frontier. For instance, the protective effects of irisin against bone loss have been linked to Nrf2 activation, a master regulator of antioxidant responses that also influences autophagy (59). Furthermore, Piezo1 activation suppresses bone marrow adipogenesis to prevent osteoporosis by inhibiting a mechano-inflammatory autocrine loop, a process intimately linked to cellular metabolism and mechano-transduction (66). These studies collectively point

toward a paradigm where myokines not only activate classic signaling cascades but also orchestrate broader cellular homeostatic programs to sustain bone health.

Collectively, the synthesis of these signaling pathways presented in the present review highlights that the net effect of exercise on bone homeostasis depends on the balance between anabolic (for example, Wnt/β-catenin and PI3K/AKT) and catabolic (for example, NF-κB and myostatin/TGF-β) signals. This integrated view extends beyond previous pathway-specific reviews by emphasizing crosstalk and context-dependency. A major limitation is that most studies use supraphysiological recombinant myokine concentrations that may not reflect the complex, pulsatile nature of exercise-induced secretion *in vivo*.

5. Muscle-bone-immune interactions: Emerging roles in bone disorders

The traditional view of the muscle-bone unit as a purely biomechanical entity has been substantially revised by the recognition that these tissues communicate through a sophisticated network of biochemical signals, with the immune system serving as a critical intermediary. The muscle-bone-immune axis is defined as a reciprocal signaling network wherein exercise-induced myokines directly modulate immune cells within the bone microenvironment, and these immune cells in turn regulate osteoblasts and osteoclasts; conversely, bone-derived osteokines (for example, osteocalcin) and immune cell-derived factors can also influence skeletal muscle function, establishing true bidirectionality (22). The present review focuses primarily on the myokine-mediated direction from muscle to bone via immune intermediaries, which represents the best-characterized arm of this axis. The core components include: i) Skeletal muscle-derived myokines (for example, IL-33, irisin, IL-6 and myostatin) as initiating signals; ii) immune cell populations ($CD8^+$ T cells and macrophages) and their secreted factors (for example, CCL5); and iii) bone cells as final effectors. In the primary direction addressed in the present review, signal flow proceeds from muscle (myokine secretion during exercise) to immune cells (activation or suppression) to bone (altered formation or resorption). Feedback signals from bone and immune cells to muscle, while less extensively characterized, are acknowledged to complete the bidirectional loop. This tripartite interaction is particularly relevant in the pathogenesis of common bone disorders, where chronic inflammation, aging and hormonal shifts converge to disrupt homeostasis (Table I). A conceptual framework for these complex interactions is provided in Fig. 4, illustrating how myokines derived from exercising muscle can modulate macrophage polarization, T-cell subsets, and inflammatory signaling pathways to ultimately influence bone remodeling, fracture healing, and the progression of conditions such as osteoporosis and osteoarthritis.

Myokines as immunomodulators in the bone niche. Myokines function as pivotal immunomodulators, directing the activity of immune cells that reside within or infiltrate the bone niche. A landmark study by Liu *et al* (13) demonstrated that skeletal muscle-derived IL-33 mediates muscle-to-bone crosstalk by regulating $CD8^+$ T cells, which in turn secrete CCL5 to influence bone metabolism (13). This finding establishes a direct immunological axis connecting muscle activity to bone homeostasis. Complementing this, Colaianni *et al* (67) showed that irisin positively correlates with bone mineral density in older adults, an effect partially mediated by its capacity to reduce the senescent marker p21 in osteoblasts, thereby counteracting age-related cellular dysfunction. The immunomodulatory capacity of the muscle secretome is further exemplified by studies showing that biomechanical stimulation of muscle constructs directly influences the paracrine signaling to bone cells, with the release of exosomes and soluble factors that can alter the inflammatory milieu (20). IL-33 mediates muscle-to-bone crosstalk via $CD8^+$ T cells (single study, independent replication pending).

Chronic low-grade inflammation and myokine dysregulation in aging. Aging is characterized by a state of chronic low-grade inflammation, termed 'inflammaging', which is intimately linked to the pathogenesis of sarcopenia and osteoporosis. This systemic inflammatory environment profoundly disrupts the myokine profile. Evans *et al* (37) highlighted that sarcopenia, a core component of age-related musculoskeletal decline, is underpinned by anabolic resistance and exacerbated by chronic inflammation. This is supported by studies showing that serum myostatin levels, a catabolic myokine, are higher in more physically active and non-frail older adults, suggesting a complex compensatory mechanism that can be further modulated by exercise (68). The inflammatory-energy metabolism axis has been identified as a central driver of the sarcopenia-osteoporosis syndrome, with NF-κB acting as a key mediator of this pathological crosstalk (69). In line with this, Buchmann *et al* (70) demonstrated that the metabolic syndrome exacerbates the association between low muscle mass and elevated inflammatory markers in older adults, indicating a synergistic relationship between metabolic dysfunction, inflammation and myokine dysregulation. Consequently, the age-related decline in anabolic myokines such as irisin and the elevation of catabolic factors including myostatin create a permissive environment for bone loss. To overcome age-related bone loss caused by declining irisin and rising myostatin, several strategies have been proposed. Exercise training, particularly resistance and combined aerobic-resistance modalities, remains the most effective approach to restore irisin levels and suppress myostatin expression in older adults (71). Pharmacologically, recombinant irisin administration has shown bone-protective effects in preclinical models (72), while myostatin inhibition via genetic or pharmacologic approaches improves bone properties (56). A combination of exercise and targeted myokine-based therapies may offer synergistic benefits for counteracting age-related musculoskeletal decline.

Exercise-mediated myokine control in osteoporosis and sarcopenia. Exercise is a potent non-pharmacological strategy to counteract the myokine dysregulation observed in osteoporosis and sarcopenia, leveraging the immunomodulatory properties of myokines. A study by Ghayomzadeh *et al* (73) demonstrated that combined resistance and aerobic training for six months improved bone mass and physical function in HIV-infected individuals, an effect linked to a reduction in systemic inflammation. Similarly, in postmenopausal women,

Table I. Key studies on muscle-bone-immune interactions in bone homeostasis.

Authors, year	Myokine	Immune cell/pathway	Study type	Key finding	(Refs.)
Liu *et al*, 2025	IL-33	$CD8^+$ T cells/CCL5	Rodent study	IL-33 mediates muscle-bone crosstalk via $CD8^+$ T cells	(13)
Colaianni *et al*, 2021	Irisin	Senescent marker p21	Human correlation	Irisin correlates with BMD in older adults	(67)
Chang *et al*, 2022	IL-6	Osteoclast precursors	*In vitro*	IL-6 promotes osteoclast precursor proliferation	(42)
Arrieta *et al*, 2019	Myostatin	Systemic inflammation	Human study	Higher myostatin in active, non-frail older adults	(68)
Ghayomzadeh *et al*, 2022	Exercise training	Systemic inflammation	Human intervention	Combined training improves bone mass, reduces inflammation	(73)
Chen *et al*, 2025	Irisin	Systemic inflammation	Human intervention	Alternating aerobic/resistance exercise increases irisin and BMD	(71)
Testa *et al*, 2022	Exercise training	STAT3 pathway	Rodent study	Resistance training reduces STAT3 activation and muscle atrophy	(74)
Hu *et al*, 2026	Irisin	STING/NF-κB pathway	Rodent study	Exercise-derived irisin prevents bone loss via Nrf2/STING/NF-κB	(59)
Oranger *et al*, 2023	Irisin	Inflammatory/ angiogenic factors	Rodent study	Irisin modulates fracture healing factors	(76)

alternating aerobic with resistance exercise was shown to improve bone mineral density and increase circulating irisin levels (71). The mechanisms underlying these benefits are multifaceted. For instance, resistance training has been shown to reduce the activation of STAT3 and attenuate muscle atrophy in tumor-bearing mice, highlighting its capacity to counteract inflammation-driven muscle wasting (74). In the context of osteoporosis, exercise-derived irisin has been found to prevent bone loss by activating the Nrf2 antioxidant pathway and inhibiting the STING/NF-κB inflammatory axis (59). Furthermore, the gut-bone axis has been identified as a mediator of exercise modality-dependent suppression of inflammatory osteoclastogenesis in ovariectomy-induced bone loss, suggesting that the systemic anti-inflammatory effects of exercise are transduced through multiple pathways, including the modulation of the gut microbiota (75). These findings collectively underscore that exercise, through its influence on myokines, serves as a powerful regulator of the chronic inflammation that underpins age-related musculoskeletal disorders.

Myokine involvement in post-traumatic osteoarthritis and fracture healing. The muscle-bone-immune axis plays a critical role in the response to acute injury, such as fracture and the pathogenesis of post-traumatic osteoarthritis (PTOA). Following a fracture, the local inflammatory response is essential for healing, but its dysregulation can lead to complications. Oranger *et al* (76) demonstrated that irisin modulates inflammatory, angiogenic and osteogenic factors during fracture healing, suggesting a beneficial role for this myokine in the repair process. This is supported by studies in rat models showing that irisin promotes fracture healing by enhancing both osteogenesis and angiogenesis (77). In the context of PTOA, exercise-induced modulation of irisin has been shown to have protective effects on the muscle-bone unit, potentially by mitigating the inflammatory response that drives cartilage degradation and subchondral bone changes (78). Açan *et al* (79) further corroborated the role of irisin in fracture healing, finding it comparable to hyaluronic acid and platelet-rich plasma in a rat model. Conversely, elevated levels of pro-inflammatory cytokines can be detrimental. Blockade of IL-6 signaling at the fracture site has been revealed to accelerate bone healing, suggesting that fine-tuning this inflammatory pathway, potentially through myokine-mediated regulation, is critical for optimal repair (80). These studies highlight that myokines are not merely passive markers but active participants in the immune-regulated processes of tissue repair and regeneration following musculoskeletal injury.

Sex hormone interactions with myokine signaling in bone homeostasis. Sex hormones, particularly estrogen, exert profound effects on both the immune system and the musculoskeletal system, with their decline in aging contributing to the pathogenesis of osteoporosis and sarcopenia. Estrogen deficiency alters the secretion of myokines that enhance osteoclast differentiation and activity, establishing a direct mechanistic link between menopause and increased bone resorption (81). The interaction between sex hormones and myokine signaling is complex and bidirectional. For instance, Norton *et al* (35) showed that estrogen regulates myokines that specifically enhance osteoclast differentiation, providing a mechanistic basis for the accelerated bone loss observed in postmenopausal women (81). In a study of oligo-amenorrheic athletes, the route of estrogen administration was found to impact bone turnover markers, suggesting that hormonal milieu directly modulates the skeletal response to factors influenced by exercise (82). Furthermore, sex differences in myokine expression are evident in pathological states. In older adults, serum myostatin and IGF-1 have been identified as sex-specific biomarkers of

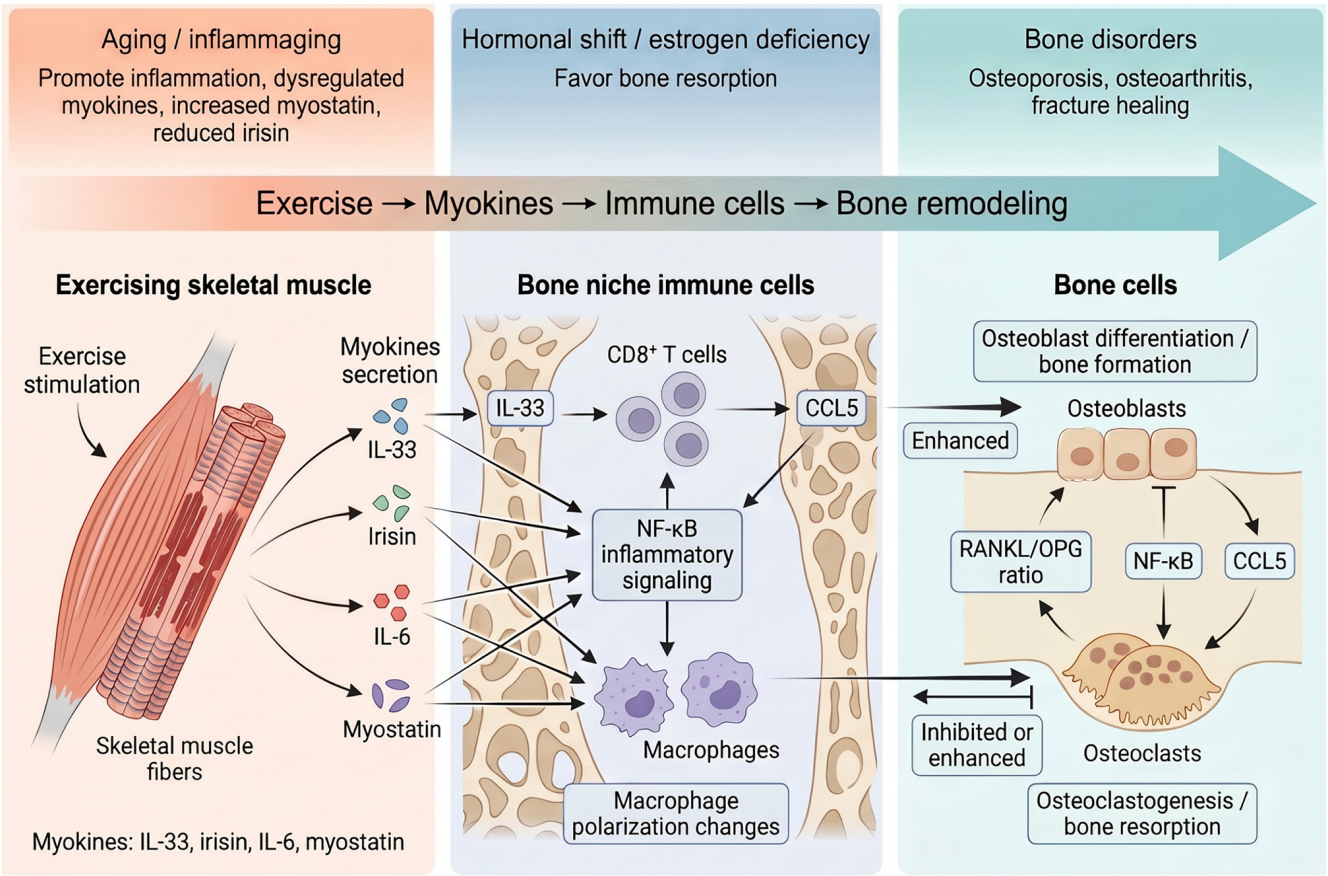

Figure 4. Muscle-bone-immune axis in bone homeostasis. Exercise-induced myokines modulate immune cells (macrophages, $CD8^+$ T cells) in the bone niche, regulating osteoblast and osteoclast activity via key pathways (NF-κB, CCL5 and RANKL/OPG). Aging, inflammation, and sex hormones influence this crosstalk, affecting bone remodeling. RANKL, receptor activator of nuclear factor-κB ligand; CCL5, chemokine ligand 5; OPG, osteoprotegerin; NF-κB, nuclear factor kappa B; IL, interleukin.

frailty and low muscle mass, indicating that the relationship between myokines and musculoskeletal health is modulated by sex (83). Recent work by Weaver *et al* (84) demonstrated that global deletion of Girk3 increases bone mass in female but not male mice, further emphasizing the sexually dimorphic nature of factors regulating bone metabolism. These findings underscore the necessity of considering sex as a biological variable when investigating myokine-based therapies and designing exercise interventions for bone disorders.

6. Translational implications: Myokines as therapeutic targets and biomarkers

The convergence of preclinical and clinical evidence positions myokines at the forefront of translational strategies for bone disorders. These endogenous exercise-responsive factors offer dual therapeutic potential: as targets for biopharmaceutical development and as circulating biomarkers for disease stratification and monitoring (Table II). However, translating myokine-based concepts into clinical practice requires overcoming substantial hurdles related to protein stability, bioavailability, immunogenicity and the inherent heterogeneity of both myokine signatures and patient populations.

Myokine-based therapeutic strategies. Recombinant myokines and their mimetics represent a direct approach to recapitulating the osteogenic benefits of exercise in patients unable to engage in physical activity. Preclinical studies have demonstrated the efficacy of recombinant irisin in preventing osteoblast differentiation deficits under simulated microgravity through β-catenin stabilization (55), and systemic administration of irisin accelerated fracture healing in murine models (72). Preclinical studies suggest that recombinant irisin may prevent osteoblast differentiation deficits. However, these findings are largely preclinical; clinical evidence in humans is lacking, and significant hurdles including short half-life, potential immunogenicity, and high manufacturing costs remain to be addressed. These findings are complemented by evidence that irisin promotes osteogenesis via BMP/SMAD signaling through αV integrin (8), establishing a mechanistic rationale for its therapeutic application. Conversely, strategies targeting catabolic myokines have also emerged. Genetic and pharmacologic inhibition of myostatin improved bone properties in osteogenesis imperfecta models (56,85), suggesting that neutralizing this negative regulator could yield anabolic benefits. Nevertheless, several challenges impede clinical translation. Compared with existing bone drugs such as parathyroid hormone (PTH) analogs (anabolic) and denosumab (anti-resorptive), myokine-based therapies have not yet demonstrated superior efficacy in head-to-head comparisons. PTH analogs have well-established pharmacokinetic profiles and long-term safety data, while denosumab offers convenient

Table II. Translational studies on myokine-based therapeutic strategies, biomarker applications and personalized exercise interventions in bone disorders.

Authors, year	Intervention	Study type	Evidence level	Key finding	(Refs.)
Chen *et al*, 2020	Recombinant irisin	Preclinical (rodent)	Preclinical	Irisin prevents osteoblast differentiation deficits under microgravity	(55)
Colucci *et al*, 2021	Recombinant irisin	Preclinical (rodent)	Preclinical	Systemic irisin accelerates fracture healing	(72)
Cheng *et al*, 2024	Concurrent aerobic + resistance training	Meta-analysis	Moderate	Distinct effects on circulating irisin levels	(90)
Adilakshmi *et al*, 2024	Exercise training	Clinical (healthy adults)	Preliminary	Exercise modality-dependent alterations in myokines	(92)
Bettariga *et al*, 2024	Exercise training	Meta-analysis	Moderate	Exercise mode effects on myokine expression	(93)
Shen *et al*, 2024	Circulating irisin	Meta-analysis	Moderate	Lower irisin associated with osteoporosis in women	(95)
Song *et al*, 2021	Circulating irisin	Meta-analysis	Moderate	Lower irisin in type 2 diabetes	(96)
Alexopoulos *et al*, 2021	Myostatin + CPK/ albumin	Clinical (cirrhosis)	Preliminary	Myostatin combinations improve sarcopenia discrimination	(98)
Kim *et al*, 2025	Extracellular vesicle markers	Clinical (older adults)	Preliminary	EV markers linked to low muscle mass	(104)
Zhang *et al*, 2025	Circulating irisin	Meta-analysis	Moderate	Lower irisin in sarcopenia	(106)

biannual subcutaneous dosing. By contrast, recombinant myokines face critical limitations including short half-life (typically minutes to hours), potential immunogenicity due to non-human post-translational modifications, and high manufacturing costs associated with eukaryotic expression systems required for proper bioactivity. Furthermore, the optimal dosing regimen, long-term safety and potential off-target effects of myokine-based therapies remain unknown. Advanced delivery platforms, including PEGylation (86), fusion protein technologies (87) and extracellular vesicle-based systems (88), are being explored to enhance pharmacokinetic profiles and tissue targeting. However, the optimal formulation strategy must balance improved stability against potential alterations in bioactivity and immunogenicity profiles. Recombinant myokines are typically produced in prokaryotic or eukaryotic expression systems. To overcome poor permeability, low stability and lack of suitable delivery systems, several strategies have been explored, including PEGylation (86), fusion protein technologies (87), extracellular vesicle-based systems (88) and nanoparticle formulations (89). Critically, the long-term safety of recombinant myokines is unknown, and the biomarker literature is plagued by inconsistent assay methodologies, making clinical translation speculative without rigorous head-to-head comparisons with existing bone drugs.

Personalized exercise prescription. The recognition that myokine responses are highly dependent on exercise modality, intensity and individual biological characteristics has profound implications for precision-based exercise prescription. Meta-analytical evidence indicates that concurrent aerobic and resistance training yields distinct effects on circulating irisin levels compared with single-modality approaches (90). Moreover, resistance training has been shown to reduce STAT3 activation and attenuate muscle atrophy in tumor-bearing mice (74,89), highlighting modality-specific molecular pathways. In clinical populations, combined resistance and aerobic training improved bone mass and reduced systemic inflammation in HIV-infected individuals (91), while alternating aerobic with resistance exercise enhanced bone mineral density and irisin levels in postmenopausal women (86). The differential myokine signatures elicited by various exercise regimens support the concept of tailoring prescriptions based on baseline myokine profiles, disease status and treatment goals (92,93). Recent evidence also suggests that exercise-induced metabolic reprogramming and immune modulation may underpin some of the bone-protective effects, further emphasizing the need for personalized approaches (94). Nevertheless, translating these findings into standardized clinical protocols requires rigorous validation of dose-response relationships and identification of predictive biomarkers that identify patients most likely to benefit from specific exercise modalities.

Myokine profiles as diagnostic and prognostic biomarkers. Circulating myokine levels have garnered substantial interest as non-invasive biomarkers for bone disorders, sarcopenia and associated musculoskeletal conditions. Systematic reviews and meta-analyses have demonstrated that lower irisin levels correlate with osteoporosis in women (95), type 2 diabetes mellitus (96) and diabetic nephropathy (97), suggesting its potential as a diagnostic marker. While observational studies report correlations between circulating myokine levels and bone disorders, these findings are associative rather than causal. Major limitations include substantial heterogeneity in assay methodologies, lack of standardized reference ranges,

limited longitudinal evidence and the absence of robust clinical validation. Furthermore, circulating myokine levels reflect composite tissue sources rather than muscle-specific secretion. Conversely, elevated myostatin levels have been associated with muscle wasting in various pathologies, including cirrhosis (98), chronic kidney disease (99) and cancer (100), although the relationship is complex and may be influenced by disease stage and patient characteristics. Emerging evidence indicates that myostatin in combination with creatine phosphokinase or albumin may improve discrimination of sarcopenia in cirrhosis (98,101), and the myostatin-to-appendicular skeletal muscle mass ratio has been proposed as a more sensitive marker than myostatin alone in older women (102). Longitudinal studies have identified plasma biomarkers associated with muscle function decline (103) and sarcopenia (101), while extracellular vesicle-derived markers show promise for detecting low muscle mass and physical performance deficits (104). The prognostic utility of myokines is further supported by findings that irisin levels predict cardiac contractile dysfunction in heart failure and correlate with outcomes in hepatocellular carcinoma (105). However, a major limitation of myokines as tissue-specific biomarkers is their lack of exclusivity to skeletal muscle. For example, irisin is also produced by adipose tissue, brain, liver and other organs, and its expression can be influenced by non-exercise factors such as metabolic state, inflammation and circadian rhythms. Similarly, myostatin is expressed in adipose tissue and heart, and IL-6 is produced by multiple cell types including immune cells and adipocytes. Consequently, circulating myokine levels reflect a composite of tissue sources rather than muscle-specific secretion, confounding their interpretation as pure muscle-bone signaling markers. A major limitation is the lack of tissue specificity (well-established). Despite these promising associations, several limitations persist, including substantial heterogeneity in assay methodologies, lack of standardized reference ranges, and the influence of sex, age and circadian rhythms on circulating levels (106). Future efforts must prioritize assay harmonization and validation in large, well-characterized cohorts to establish clinical utility.

Synergistic effects with pharmacological agents. The combination of myokine-targeted interventions with existing pharmacological therapies offers opportunities for additive or synergistic effects on bone health. Preclinical studies have shown that PTH (1-34) treatment and mechanical loading exert complementary osteogenic effects on trabecular and cortical bone in ovariectomized mice (107), suggesting that myokine-inducing exercise may enhance the efficacy of anabolic agents. Similarly, the gut-bone axis has been identified as a mediator of exercise modality-dependent suppression of inflammatory osteoclastogenesis in ovariectomy-induced bone loss (75), indicating that exercise-induced myokines may modulate the skeletal response to hormonal therapies. The potential for drug-myokine interactions requires careful consideration, as therapeutic proteins can influence cytochrome P450 activity and alter the pharmacokinetics of co-administered drugs (108). Conversely, pharmacological agents may impact myokine expression and function. For instance, recombinant human BMP-2-induced osteogenesis is modulated by irisin (64), suggesting potential synergy when combining growth factor therapies with myokine-based strategies. The integration of exercise interventions with bisphosphonates, denosumab, or PTH analogs warrants systematic investigation in clinical trials designed to evaluate both additive effects and potential interactions.

Barriers to clinical translation. Despite substantial progress, several barriers impede the clinical translation of myokine-based diagnostics and therapeutics. Bioavailability remains a critical challenge, particularly for oral administration, where gastrointestinal degradation and poor permeability limit absorption (109). Advanced delivery systems, including nanoparticle formulations, microneedle technologies and polymersomes, are under development to address these limitations (89,110,111). The stability of therapeutic proteins in biological fluids and during storage continues to pose formulation challenges, with factors such as pH, temperature and excipient composition influencing degradation pathways (112). Immunogenicity risk assessment is essential for engineered myokine analogs, as anti-drug antibody formation can neutralize efficacy and, in rare cases, trigger adverse reactions (113). The heterogeneity of myokine responses across populations, which is influenced by age, sex, genetic variation and disease status, complicates the development of standardized diagnostic cutoffs and dosing regimens (106). Furthermore, the complex interplay between myokines and other circulating factors, including adipokines (114), necessitates a multi-analyte approach for accurate disease characterization. Addressing these barriers will require coordinated efforts in analytical method development, physiologically based pharmacokinetic modeling, and innovative formulation strategies tailored to the unique properties of myokine-based therapeutics.

In summary, while myokines hold promise as therapeutic targets and biomarkers, the current evidence is largely preclinical or correlative. Cautious interpretation is warranted, and rigorous clinical validation is required before translation into practice.

7. Methodological advances and emerging frontiers

This section highlights methodological advances with demonstrated or potential application to myokine-mediated muscle-bone crosstalk; examples from other bone-related fields are included as background to illustrate broader technological capabilities. Recent technological innovations have profoundly expanded the capacity to investigate myokine-mediated muscle-bone crosstalk at unprecedented resolution. The convergence of multi-omics platforms, single-cell technologies, organ-on-chip systems and artificial intelligence approaches is transforming the understanding of exercise-regulated signaling networks and accelerating the translation of myokine-based concepts toward clinical applications (Table III).

Multi-omics approaches have emerged as powerful strategies for comprehensive myokine discovery and validation. For example, the multi-omics integration strategy used by Du *et al* (115) to identify androgen receptor signaling in osteoporosis could be directly applied to profile the exercise-induced myokine secretome and identify novel myokines that regulate bone homeostasis. Similarly, the integrative

Table III. Key methodological advances in myokine-mediated muscle-bone crosstalk research.

Authors, year	Technology platform	Relevance to myokines	Key finding	(Refs.)
Du *et al*, 2025	Multi-omics integration	Indirect	Identified androgen receptor signaling in osteoporosis	(115)
Cui *et al*, 2025	Integrative multi-omics	Indirect	Deciphered molecular mechanisms of Gu Shu Kang Granules	(116)
Liu *et al*, 2025	Multi-omics + multimodal data	Indirect	Repurposed acebutolol for osteoporosis treatment	(117)
Wang *et al*, 2022	Single-cell RNA-seq	Indirect	Dissected immune heterogeneity in osteoporosis	(119)
Xiao *et al*, 2023	Spatial transcriptomics	Indirect	Mapped spatial organization of bone marrow signaling	(120)
Deng *et al*, 2025	Spatial transcriptomics	Direct	Decoded communication networks in bone-muscle interface	(121)
Giza *et al*, 2022	Microphysiological system	Indirect	Modeled contractile differences in young vs. old muscle cells	(122)
Suresh Kumar *et al*, 2023	Muscle-bone co-culture chip	Direct	Biomechanical stimulation of muscle modulates bone phenotype via myokines	(20)
Yin *et al*, 2026	Muscle regeneration-on-chip	Direct	Identified optimal mechanical stimulation for muscle regeneration	(124)
Parafati *et al*, 2025	Muscle lab-on-chip	Indirect	Revealed microgravity-induced muscle degeneration	(125)
Zhou *et al*, 2024	Machine learning + single-cell analysis	Indirect	Identified shared biomarkers in inflammatory arthritis	(126)
Zhang *et al*, 2022	Network pharmacology + molecular docking	Indirect	Revealed mechanisms of *Tripterygii Wilfordii* against osteosarcoma	(128)

multi-omics framework that deciphered molecular mechanisms of herbal formulations in sarcopenia-osteoporosis could be adapted to map the signaling networks through which exercise-induced myokines (for example, irisin, IL-6 and myostatin) exert their effects on bone cells (116). Furthermore, the multi-omics and multi-modal data analysis approach used to repurpose drugs for osteoporosis could be employed to identify existing compounds that mimic or enhance the osteogenic effects of exercise-responsive myokines (117). These approaches are particularly powerful when integrated with causal inference methods, as demonstrated in studies of inflammatory bone disorders (118); such causal inference frameworks could be applied to distinguish direct myokine-mediated effects from indirect exercise-associated changes in bone metabolism. Despite these advances, challenges persist regarding data integration standardization and functional validation throughput, limiting the direct translation of multi-omics findings into clinically actionable myokine targets.

Single-cell and spatial transcriptomics technologies have revolutionized the understanding of cellular heterogeneity within the muscle-bone-immune axis. These approaches enable the dissection of cell-type-specific responses to exercise-derived myokines and the mapping of intercellular communication networks. The single-cell RNA sequencing approach used by Wang *et al* (119) to dissect the osteoimmunology microenvironment in osteoporosis could be directly applied to identify which immune cell subsets (for example, $CD8^+$ T cells and macrophages) are specifically modulated by exercise-induced myokines such as IL-33 and irisin. Complementary spatial transcriptomic interrogation of the murine bone marrow signaling landscape provides a template for mapping the spatial distribution of myokine receptor expression within bone niches, revealing which bone cell types are positioned to respond to circulating myokines (120). Notably, Deng *et al* (121) have specifically applied spatial transcriptomics to decode cellular communication networks and signaling pathways in bone-muscle crosstalk, providing a methodological framework directly relevant to myokine research. The integration of single-cell resolution with spatial context represents a significant advance over bulk analyses, although the high cost and technical complexity currently limit widespread application.

Microphysiological systems and organ-on-chip platforms have emerged as transformative tools for modeling exercise effects on muscle-bone interactions under controlled conditions. Giza *et al* (122) developed a microphysiological system for studying contractile differences in young, active vs. old, sedentary adult-derived skeletal muscle cells, enabling the investigation of age-related alterations in myokine secretion. Suresh Kumar *et al* (20) demonstrated that biomechanical stimulation of muscle constructs directly influences the phenotype of bone constructs by modulating myokine secretion, providing direct evidence for the utility of such systems in studying muscle-bone crosstalk. The miniaturized 3D myotube contraction monitoring chip developed

for modeling muscular dystrophies could be adapted to measure real-time myokine release from exercised muscle constructs and to test the effects of specific mechanical loading patterns on myokine secretion profiles (123). More recently, Yin *et al* (124) developed a muscle regeneration on a chip platform to study exercise-induced microtrauma and identify optimal mechanical stimulation regimens; such platforms could be coupled with bone cell co-cultures to directly assess how exercise-mimetic mechanical stimuli regulate myokine-mediated osteoblast differentiation. Parafati *et al* (125) utilized a muscle lab-on-chip model aboard the International Space Station to demonstrate that microgravity accelerates skeletal muscle degeneration; similar microphysiological systems could be used to study how unloading-induced changes in myokine secretion contribute to disuse osteoporosis. These platforms offer distinct advantages over traditional cell culture by recapitulating the mechanical and architectural features of native tissue, yet challenges remain in incorporating multiple cell types and vascular components to fully mimic the physiological muscle-bone unit.

The integration of machine learning and artificial intelligence with multi-omics datasets is opening new frontiers in predicting myokine-bone signaling networks. The machine learning approaches used to identify shared biomarkers in inflammatory arthritis (126) could be applied to analyze myokine expression data from exercised human cohorts, thereby identifying circulating myokine signatures that predict bone anabolic responses to specific exercise modalities. Furthermore, the application of deep learning to spatial transcriptomics data is enabling the prediction of cellular communication networks from tissue architecture (127); such computational approaches could be used to model how myokines diffuse through bone tissue and which cell types are preferentially targeted based on their spatial expression of myokine receptors. These computational approaches are particularly valuable for integrating heterogeneous datasets and generating testable hypotheses regarding myokine function. However, the interpretability of machine learning models remains a concern, and experimental validation of computationally derived predictions continues to be essential.

Collectively, these methodological advances are reshaping the landscape of myokine research. The transition from bulk analyses to single-cell and spatial approaches has revealed previously unappreciated cellular heterogeneity within the muscle-bone axis. Organ-on-chip platforms now enable mechanistic studies under physiologically relevant conditions that were previously impossible to achieve. Multi-omics integration coupled with artificial intelligence is accelerating the discovery of novel myokines and their signaling networks. Future progress will depend on the standardization of these platforms, the development of more sophisticated coculture systems that incorporate immune cells and vasculature, and the integration of multiple methodological approaches to provide a comprehensive understanding of myokine-mediated muscle-bone crosstalk. Despite their promise, emerging technologies have not yet yielded clinically actionable myokine targets, largely owing to a lack of functional validation and standardized protocols across laboratories.

8. Conclusions

Exercise induced myokines form a complex signaling network that integrates mechanical, metabolic, and immune inputs to regulate bone homeostasis. The interplay among Wnt/β catenin, MAPK, NF κB, and TGF β/BMP pathways determines the net skeletal response. This review provides an integrated analysis of current knowledge, highlighting that the muscle-bone-immune axis and epigenetic regulation represent emerging frontiers. Methodological advances such as multi-omics and organ-on-chip platforms are accelerating translational opportunities. Harnessing myokine based strategies holds promise for developing biomarkers and therapeutic interventions for bone disorders.

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Authors' contributions

BT and XK made substantial contributions to conception and design. XC and JZ performed acquisition, analysis, and interpretation of data. All authors were involved in drafting the manuscript and revising it critically for important intellectual content and agree to be accountable for all aspects of the work. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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