RESEARCH PAPER

Therapeutic And Preventive Potential Of Isoflavones In Osteoporosis: A Comprehensive Evidence-Based Review

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ABSTRACT

Osteoporosis is a progressive skeletal disorder marked by reduced bone mass and microarchitectural deterioration, leading to increased fracture risk, particularly in postmenopausal women and the elderly. Although conventional therapies such as bisphosphonates, selective estrogen receptor modulators, and hormone replacement therapy effectively reduce fracture incidence, their long-term use may be limited by adverse effects, contraindications, and adherence issues. These concerns have prompted interest in nutraceutical approaches, including isoflavones, as supportive strategies for bone health. Isoflavones are phytoestrogens primarily derived from soybeans and legumes. Structurally similar to 17β-estradiol, they bind to estrogen receptors and influence bone remodeling. Experimental studies show that key isoflavones, particularly genistein and daidzein, reduce osteoclast-mediated bone resorption, enhance osteoblast differentiation, and modulate pathways such as RANK/RANKL/OPG and Wnt/β-catenin. They also exhibit antioxidant and anti-inflammatory effects, which may further support bone preservation. Clinical and observational evidence suggests that isoflavone intake may attenuate bone mineral density loss, especially at the lumbar spine and femoral neck, and populations consuming soy-rich diets often demonstrate lower fracture rates. However, results remain inconsistent due to variations in dosage, duration, study design, baseline hormonal status, and differences in isoflavone metabolism influenced by gut microbiota. Overall, isoflavones are generally well tolerated and appear safe within dietary or recommended supplemental ranges. Current evidence supports their use as a complementary strategy rather than a replacement for standard therapy, alongside adequate calcium, vitamin D, physical activity, and lifestyle modification. Further long-term, well-designed studies are needed to define optimal dosing and patient-specific responses.

Keywords: Isoflavones, Osteoporosis, Estrogen receptor-β, Oxidative stress, Bone mineral density (BMD)

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1. Introduction

Osteoporosis is a chronic, progressive skeletal disorder characterized by reduced bone mass, deterioration of bone microarchitecture, and compromised bone strength, ultimately leading to an increased susceptibility to fractures. The condition predominantly affects postmenopausal women and the elderly population, posing a significant public health burden due to its association with morbidity, mortality, and reduced quality of life [1]. Among the various etiological factors, estrogen deficiency following menopause is considered the primary contributor to accelerated bone loss. Estrogen plays role in maintaining skeletal homeostasis by regulating bone remodelling; its decline results in increased osteoclast-mediated bone resorption and an imbalance between bone formation and degradation [2]. Current pharmacological interventions for osteoporosis including bisphosphonates, hormone replacement therapy (HRT), selective estrogen receptor modulators (SERMs) and monoclonal antibodies such as denosumab have demonstrated efficacy in reducing fracture risk and improving bone mineral density [3]. However, the long-term use of these therapies is often limited by safety concerns and adverse effects, such as osteonecrosis of the jaw, atypical fractures, cardiovascular risks and hormone-related malignancies. These limitations have promoted increasing interest in alternative and complementary approaches that are safer, more sustainable and suitable for long-term use. In this context, nutraceuticals and plant-derived bioactive compounds have gained attention with isoflavones emerging as promising candidates for bone health management [4, 5]. Isoflavones are naturally occurring polyphenolic compounds classified as phytoestrogens due to their structural similarity to 17 β-estradiol. They are predominantly found in soybeans, red clover,

chickpeas and other leguminous plants. The most extensively studied isoflavones include genistein, daidzein and glycitein [6]. These compounds exert estrogen-like effects by binding to estrogen receptors, particularly estrogen receptor beta (ER β), which is abundantly expressed in bone tissue. Their preferential affinity for ER β over estrogen receptor alpha (ER α) enables isoflavones to exert beneficial estrogenic effects on skeletal tissue while minimizing stimulation of estrogen-sensitive reproductive organs such as the breast and uterus. This selective receptor interaction contributes to their comparatively favourable safety profile when contrasted with conventional estrogen therapies [7, 8]. The beneficial effects of isoflavones on bone metabolism are primarily mediated through their regulatory influence on osteoblast and osteoclast activity. Experimental studies indicate that genistein enhances osteoblast proliferation, differentiation and mineralisation, thereby promoting bone formation. Simultaneously, it inhibits osteoclast genesis by modulating the receptor activator of nuclear factor- κB (RANK), RANK ligand (RANKL) and osteoprotegrin (OPG) signalling pathway, which plays a central role in bone resorption. By restoring the balance between bone formation and resorption, isoflavones contribute to the preservation of bone mass and structural integrity [9, 10]. An additional factor influencing the efficacy of isoflavones is their metabolism by the gut microbiota. Daidzein can be converted by specific intestinal bacteria into equol, a metabolite with enhanced estrogenic and antioxidant activity[8]. Individuals capable of producing equol, referred to as "equol producers" may expertise greater skeletal benefits from isoflavone consumption. This interindividual variability highlights the importance of gut microbiota composition in determining the biological response to dietary phytoestrogens [11]. Clinical trials and meta-analyses have provided supportive evidence for the role of isoflavones in improving bone health, particularly in postmenopausal women. Supplementation with soy isoflavones in doses ranging from 40 to 90 mg per day over periods of six to twenty-four months has been associated with modest but statistically significant improvements in bone mineral density at clinically relevant sites such as the lumbar spine and femoral neck [12]. Furthermore, reduction in bine turnover markers, including bone-specific alkaline phosphatase (BALP) and C-terminal telopeptide of type 1 collagen (CTX), suggest a normalization of bone remodelling dynamics. Beyond their estrogenic effects, isoflavones possess antioxidant and anti-inflammatory properties, which further contribute to bone protection by mitigating oxidative stress and chronic inflammation both recognized contributors to age-related bone loss [13, 14]. In addition to their therapeutic potential, isoflavones may play a preventive role in osteoporosis. Epidemiological studies have consistently shown lower rates of osteoporotic fractures in Asian populations, where lifelong consumption of soy-based foods is common [15]. These observations underscore the potential benefits of incorporating isoflavone-rich foods into daily diets as part of a comprehensive strategy for maintaining skeletal health. Consequently, dietary guidelines increasingly emphasize plant-based, calcium-rich foods with isoflavones recognized as functional components that support bone integrity [16]. Despite the growing body of evidence supporting their benefits, individual responses to isoflavones vary depending on factors such as dosage, duration of intake, bioavailability, gut microbiota composition and overall nutritional status [17]. While current data suggest a favourable risk-benefit profile particularly when compared to hormone replacement therapy, long-term safety and optimal dosing strategies continue to be evaluated. Ongoing research is focused on the development of standardized isoflavone formulations, synergistic combinations with calcium, vitamin D and other bone-supportive nutrients and personalized approaches to maximize clinical outcomes [18, 19].

Overall, isoflavone represent a viable and biologically plausible natural intervention for the management and prevention of osteoporosis. Their estrogen-like activity, multifaceted effects on bone remodelling pathways, antioxidant and anti-inflammatory properties and accessible through dietary sources position them as valuable components of interactive bone health strategies [20].

2. Biological basis of osteoporosis and the role of estrogen pathways

Osteoporosis is a systemic skeletal condition characterized by low bone mass, deterioration of bone microarchitecture and increased bone fragility. The biological basis of osteoporosis lies in a disruption of the tightly regulated process of bone remodelling, which involves coordinated activity of osteoclasts (bone-resorbing cells) and osteoblasts (bone-forming cells)[8]. In healthy adults, bone remodelling maintains structural integrity and mineral homeostasis through balanced bone resorption and formation. Osteoporosis develops when this balance is disturbed, leading to net bone loss [21].

2.1 Bone remodelling and its Regulation

Bone remodelling is controlled by mechanical loading, hormonal signals and local cytokine networks. Osteoclasts break down old or damaged bone, while osteoblasts subsequently replace it with new mineralized matrix [22].

Several key molecular pathways regulate these processes:

2.1.1 RANK/RANKL/OPG Pathway

The RANK/RANKL/OPG pathway is the central regulatory axis governing osteoclast differentiation, activation, and survival, and thus plays a pivotal role in maintaining bone homeostasis. Receptor activator of nuclear factor-κB (RANK) is a transmembrane receptor expressed on osteoclast precursors and mature osteoclasts. Its ligand, RANKL, is primarily produced by osteoblasts, osteocytes, and bone marrow stromal cells in response to mechanical stress, inflammatory mediators, and hormonal signals. Binding of RANKL to RANK triggers intracellular signaling cascades, including activation of NF-κB, MAPK, and NFATc1 pathways, which collectively drive the differentiation of mononuclear precursors into multinucleated, bone-resorbing osteoclasts and enhance their longevity and resorptive capacity.

Osteoprotegerin (OPG), a soluble glycoprotein secreted mainly by osteoblast lineage cells, functions as a competitive decoy receptor for RANKL. By binding RANKL with high affinity, OPG prevents its interaction with RANK, thereby suppressing osteoclastogenesis and limiting bone resorption. The relative balance between RANKL and OPG production—often expressed as the RANKL/OPG ratio—determines the overall rate of bone resorption. Physiologically, this balance ensures coordinated coupling between bone resorption and formation. Disruption of this equilibrium underlies the pathogenesis of osteoporosis. Estrogen deficiency, chronic inflammation, glucocorticoid exposure, and aging are associated with increased RANKL expression and/or reduced OPG production, shifting the ratio in favor of osteoclast activation. This results in excessive bone resorption that surpasses osteoblastic bone formation, leading to progressive bone loss and microarchitectural deterioration. Consequently, the RANK/RANKL/OPG system has emerged as a major therapeutic target in osteoporosis management. A high RANKL/OPG ratio promotes osteoclastogenesis, increasing bone resorption which is a hallmark of osteoporosis as shown in Figure 1 [23, 24].

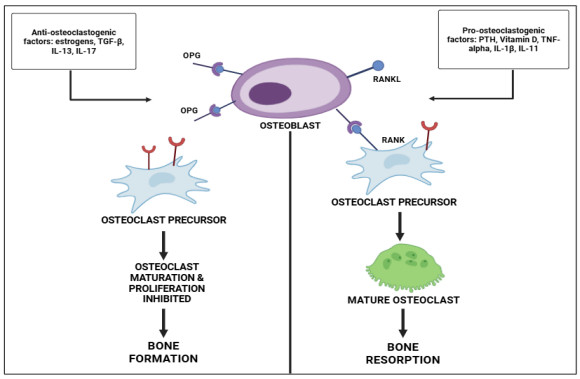

Figure 1: RANK/RANKL/OPG Pathway

2.1.2 Wnt/ β-catenin Signalling

Osteoblast differentiation is heavily dependent on Wnt signalling (Figure 2). The Wnt/β-catenin signalling pathway is a principal regulator of osteoblast proliferation, differentiation, and function, thereby playing a central role in bone formation and skeletal integrity. Canonical Wnt signalling is initiated when Wnt ligands bind to Frizzled receptors and co-receptors low-density lipoprotein receptor–related proteins 5 and 6 (LRP5/6) on osteoblast precursors. This binding inhibits the β-catenin destruction complex (comprising Axin, APC, GSK-3β, and CK1), preventing β-catenin degradation. Stabilized β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it interacts with TCF/LEF transcription factors to promote the expression of osteogenic genes such as Runx2, Osterix, and alkaline phosphatase. These molecular events drive mesenchymal stem cell commitment toward the osteoblastic lineage and enhance bone matrix production and mineralization. Beyond promoting osteoblastogenesis, Wnt/β-catenin signalling also indirectly suppresses osteoclast formation by increasing osteoprotegerin (OPG) expression, thereby contributing to the coordinated regulation of bone remodelling. Mechanical loading, growth factors, and certain hormones positively influence this pathway, reinforcing bone strength under physiological conditions. In osteoporosis, particularly postmenopausal osteoporosis, Wnt signalling is frequently downregulated. Elevated levels of endogenous antagonists such as sclerostin (produced by osteocytes) and Dickkopf-1 (DKK1) inhibit Wnt ligand interaction with LRP5/6, impairing β-catenin stabilization and reducing osteoblast activity. This suppression shifts the balance toward reduced bone formation, contributing to decreased bone mass and structural fragility. Given its critical role, the Wnt/β-catenin pathway has become an important therapeutic target for anabolic strategies aimed at restoring bone formation in osteoporotic patients [25, 26].

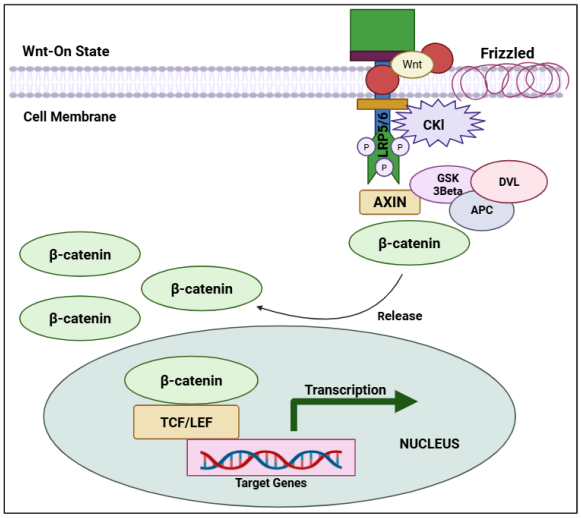

Figure 2: Wnt/ β-catenin Signalling

2.2 Biological basis of Osteoporosis

The primary biological drivers of osteoporosis are multifactorial and arise the interplay of aging, hormonal changes, inflammation, genetic and environmental influences. Age-related bone loss shifts bone remodelling toward resorption sue to decline in osteoblast number and function, impaired differentiation of mesenchymal progenitor cells, mitochondrial dysfunction and increased oxidative stress, all of which reduce bone formation [27]. Estrogen deficiency, particularly following menopause, is the most significant contributor in women, as it accelerates bone turnover by increasing osteoclast formation and activity while reducing osteoblast survival, leading to rapid bone loss during the first 5-10 years after menopause [28]. This hormonal deficiency also promotes inflammation, enhancing the production of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interlukin-1 (IL-1) and interleukin-6 (IL-6) which stimulates osteoclastogenesis through upregulation of RANKL, in elderly individuals, chronic low-grade inflammation known as "inflammaging" further exacerbates bone resorption [5]. In addition, genetic and environmental factors play a crucial role, as polymorphism in genes regulating bone metabolism, including RANKL, osteoprotegrin (OPG) and estrogen receptor- α (ER α) influence individual susceptibility to osteoporosis, while nutritional deficiencies (notably calcium and vitamin D), physical inactivity, smoking and excessive alcohol consumption further accelerate bone loss and increase fracture risk [23, 29].

2.3 Role of Estrogen Pathway

Estrogen plays a central regulatory role in maintaining skeletal integrity by tightly controlling the balance between bone formation and bone resorption. Under physiological conditions, estrogen acts primarily through estrogen receptors (ER α and ER β) expressed on osteoblasts, osteoclasts and osteocytes thereby modulating bone remodelling at multiple cellular levels [30]. Estrogen suppresses osteoclast differentiation and activity by down-regulating receptor activator of nuclear factor- κB ligand (RANKL) expression while simultaneously enhancing the production of osteoprotegrin (OPG), a decoy receptor that inhibits osteoclastogenesis [31]. In addition, estrogen promotes osteoclast apoptosis and limits the lifespan of mature osteoclasts, reducing excessive bone resorption. On the anabolic side, estrogen supports osteoblast survival, differentiation and collagen synthesis, contributing to optimal bone matrix formation [32]. Estrogen also exerts anti-inflammatory effects by inhibiting pro-resorptive cytokines such as interlukin-1, interlukin-6 and tumor necrosis- α, which otherwise stimulate osteoclast activation [33]. Following menopause, the sharp decline in circulating estrogen disrupts these protective mechanisms, leading to increased bone turnover, dominance of resorption over formation, microarchitectural deterioration and progressive loss of bone mass. This estrogen deficiency-mediated imbalance is a fundamental pathogenic mechanism underlying postmenopausal osteoporosis and explains the increased susceptibility to fragility fractures in affects individuals [31, 34].

3. Isoflavones: Sources, Metabolism & Pharmacokinetics

Isoflavones are naturally occurring polyphenolic compounds belonging to the flavonoid family and are primarily recognized for their phytoestrogenic properties due to their structural similarity to 17 β-estradiol [35]. The principal dietary sources of isoflavones are soybeans and soy-derived products such as tofu, soy milk, tempesh, miso and soy flour. Other legumes including chickpeas, lentils, red clover and fava beans, also contain measurable amounts of isoflavones, although in significantly lower concentrations compared to soy. The most biologically relevant isoflavones include genistein, daidzein and glycitein which predominantly occur in plants as glycoside conjugates [36, 37]. Following oral ingestion, isoflavone glycosides are poorly absorbed in their native form and must first undergo hydrolysis. This process occurs mainly in the small intestine and colon, where intestinal β-glucosidases and gut microbiota cleave the sugar moiety, releasing the biologically active aglycone forms [38]. Daidzein can be further metabolised by specific intestinal bacteria into equol, a metabolite with enhanced estrogenic and antioxidant activity, however equol production varies

widely among individuals and populations due to difference in gut microbiota composition. This interindividual variability significantly influences the biological effects and therapeutic outcomes of isoflavone consumption [36, 39]. Once absorbed, isoflavones undergo extensive first-pass metabolism in the liver, where they are rapidly conjugated to glucuronide and sulphate metabolites. These conjugated forms constitute the major circulating species in plasma and serve as a reservoir that can be deconjugated at target tissues, thereby contributing to biological activity[40]. Isoflavone exhibit moderate bioavailability, with peak plasma concentrations typically achieved within 4-8 hours after ingestion. They display a relatively long elimination half-life generally ranging from 6 to 10 hours, allowing for sustained systemic exposure upon regular dietary intake [35]. Pharmacokinetic studies indicate that isoflavones are widely distributed throughout the body, including bone tissue, where they can exert estrogen-like effects by preferentially binding to estrogen receptor- β. Excretion occurs primarily via urine, with smaller amounts eliminated in bile and faces [24]. Factors such as food matrix, intestinal microbiota, age, hormonal status and genetic polymorphisms influence absorption, metabolism and systemic availability of isoflavones, accounting for variability in clinical responses. Overall, the favourable pharmacokinetic profile and metabolic activation of isoflavones underpin their potential role in the prevention and management of estrogen-deficiency-related conditions, including osteoporosis [8, 16].

4. Mechanistic Perspectives: Bone remodelling and Phytoestrogens

4.1 Cellular Framework of Bone remodelling and Oestrogen-Dependent Vulnerability Nodes

Bone remodelling initiates when osteoclast precursors are recruited to bone surfaces under the influence of macrophage colony-stimulating factor (M-CSF) and RANKL [41]. The RANK-RANKL interaction triggers TRAF6 activation, NF-κB nuclear translocation, and induction of c-Fos and NFATc1, supporting differentiation into mature osteoclasts capable of acid dissolution of mineral and proteolytic degradation of collagen [42]. Following the resorption phase, osteoprogenitors migrate toward resorption lacunae, proliferate and differentiate into matrix-producing osteoblasts under transcriptional control of Runx2 and osterix [41] . Bone matrix mineralization is driven by alkaline phosphatase activity, collagen synthesis, and Wnt/B-catenin signalling [43]. Osteocytes, which constitute over 90% of bone cells, orchestrate remodelling by secreting RANKL, sclerostin and DKK1, linking mechanical forces to biochemical signals [44].

Oestrogen loss disrupts multiple molecular checkpoints within this triad. Osteoblasts and osteocytes exhibit RANKL expression and reduced secretion of osteoprotegerin (OPG), shifting the bone microenvironment toward enhanced osteoclastogenesis [45]**.** Osteoclast precursors demonstrate elevated NF-κB activity and increased mitochondrial ROS, further accelerating mineralization capacity, while osteocytes display enhanced sclerostin production, which suppress Wnt/B-catenin activity and inhibits osteoblastogenesis **[42].** Concurrently, pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) are upregulated and reinforce osteoclastogenic signalling [46]. These oestrogen-sensitive vulnerabilities represent the mechanistic entry points through which isoflavones exert their regulatory actions.

4.2 Receptor-specific modulation: ERβ-Mediated Mechanisms and Downstream Crosstalk

Isoflavones exhibit structural similarity to 17β-estradiol, but their osteoprotective actions derive from selective affinity for ERβ, which is widely expressed in osteoblast precursors, osteocytes, stromal compartments, and trabecular bone surfaces [47]. ERβ is associated with Differentiation-promoting and anti-resorptive transcriptional profiles, making isoflavones mechanistically distinct from conventional oestrogen therapies that act predominantly through ERα [48].

Upon ligand binding, ERβ undergoes conformational adjustment, dimerization, and recruitment to oestrogen response elements (EREs). This leads to upregulation of Runx2, enhanced transcription of osteogenic genes, and suppression of osteoblast-derived RANKL. [48]. Increased Runx2 activity strengthens early osteoblast lineage commitment, while repression of RANKL and specific inflammatory mediators contributes to reduced osteoclastogenic drive.

ERβ activation interacts with Wnt/β-catenin signalling by downregulating sclerostin and DKK1, Lower levels of these Wnt antagonists promote β-catenin stabilization, reduced GSK-3B-dependent degradation, and increased nuclear accumulation. The resulting transcriptional activation enhances osteoblast differentiation, survival, and matrix production [49].

Microbial metabolism adds another mechanistic layer. Daidzein biotransformation to equol yields a metabolite with higher ERβ affinity and enhanced transcriptional potency. Equol's stronger hydrophobic interactions and receptor-binding dynamics amplify

ERβ-driven responses. Variability in equol-producing microbiota partially explains interindividual differences in phytoestrogen-induced skeletal outcomes [48].

Table 1: ERB-Mediated Mechanistic Actions of Isoflavones Across Genomic, Non-Genomic, and Crosstalk Pathways.

Mechanistic Domain	Molecular Events	Cellular Consequences	Relevance to Menopausal Bone loss	Ref
Genomic / Transcriptional Activation	Isoflavone binding to Erβ → receptor dimerization → recruitment to oestrogen response elements (EREs) → upregulation of osteogenic genes (Runx2, osterix, ALP, osteocalcin).	Enhanced osteoblast differentiation and matrix deposition; suppression of RANKL transcription in osteoblasts, osteocytes	Counters oestrogen-deficiency induced osteoblast impairment; reduces osetoclastogenesis via lower RANKL	[48, 50]
Non-Genomic/ Signalling Pathways	Activation of PI3K/Akt and ERK/MAPK cascades; phosphorylation of downstream targets; inhibition of mitochondrial apoptosis pathways.	Increased osteoblast survival and proliferation; reduced ROS-mediated apoptosis; maintenance of osteocyte viability.	Protects bone-forming cells from oxidative stress and oestrogen-deprivation induced apoptosis; preserves bone microarchitecture.	[51]
ERB-Wnt/β-Catenin Crosstalk	Downregulation of sclerostin and DKK1 from osteocytes → β-catenin stabilization → nuclear translocation → transcription of osteogenic genes.	Enhanced osteoblastogenesis; promotion of matrix mineralization; improved osteoblast-osteocyte coupling.	Restores Wnt signalling suppressed during menopause, stimulation bone formation while limiting resorption.	[52, 53]
Microbiome-Mediated Metabolite Enhancement	Daidzein → equol conversion by gut microbiota; equol binds Erβ with higher affinity and stability.	Amplified transcriptional activation of osteogenic and anti-osteoclastogenic genes; stronger PI3K/Akt and Wnt pathway engagement	Explains interindividual variability in phytoestrogen responsiveness; maximizes skeletal protection in equol producers.	[48, 54, 55]

4.3 Mechanistic Regulation of the RANKL/OPG Axis and Multistage Inhibition of Osteoclastogenesis

The RANKL/OPG axis governs osteoclast differentiation and is the most consistently dysregulated pathway in postmenopausal osteoporosis. Isoflavones exert multi-tiered control over this axis, modulating transcription, signalling, oxidative, and functional components of osteoclast biology. The progression of the osteoclasts, beginning with monocyte/ myeloid progenitors to resorptive osteoclasts and also presents the inhibitory effect of the isoflavones at various levels of regulation is diagrammatically shown in Figure 3. In physiological conditions, M-CSF stimulates the use of c-Fms which form osteoclast precursors which express RANK. RANKL binding to RANK stimulates intracellular signalling pathways, such as NF- -B and c-Fos leading to osteoclast differentiation. Later expression of fusion-linked molecules including DC-STAMP and OC-STAMP also supports multinucleation to form immature osteoclasts. NFATc1 transcription of osteoclast-specific genes via NFATc1, cathepsin K and tartrate-resistant acid phosphatase (TRAP) leads to the production of functional, bone-resorbing osteoclasts in terminal maturation. Isoflavones have an inhibitory effect on three major control points. (A) They lower the expression of RANKL at the extracellular level, which restricts the activation of RANK. (B) They inhibit essential transcriptional mediators in the intracellular signalling like NF- kB and c-Fos at the precursor fusion stage and early differentiation. (C)They silence NFATc1 and its downstream modulators, such as cathepsin K and TRAP, at the transcriptional level, which reduces osteoclast development and resorption. Normal osteoclast differentiation is represented by black arrows, and inhibitory by red lines, which are caused by phytoestrogen. The combination of the figure illustrates that isoflavones can regulate osteoclastogenesis in precursor, fusion, and resorptive areas and, therefore, lead to decreased bone resorption and possible defense against osteoporosis.

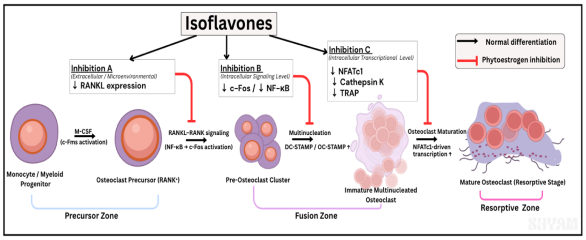

Figure 3: Isoflavone-Mediated Interference with Osteoclastogenesis at Transcriptional, Signalling, and Functional Levels

4.3.1 Suppression of RANKL Production and Enhancement of OPG Expression

Under oestrogen-deficient condition, osteoblast and osteocytes upregulate RANKL via NF-κB , STAT3, and AP-1 activation. Isoflavones inhibit these transcriptional activators by preventing IκB degradation (Reducing NF-κB release), modulating MAPK signalling (attenuating AP-1 formation), and interfering with cytokine-driven STAT3 pathways. In parallel, isoflavones increase OPG transcription, shifting the RANKL:OPG ratio and limiting RANK activation. This ligand level modulation decreases osteoclast precursor recruitment and maturation [56].

4.3.2 Intracellular interference with RANK Signalling

Even in the presence of RANKL, isoflavones impair downstream signalling. Genistein reduces TRAF6 recruitment and limits activation of the IKK complex, thereby attenuating NF-κB translocation. Reduced NF-κB activation disrupts induction of c-Fos and NFATc1, key regulators of osteoclast differentiation. This interface curtails early and intermediate phases of Osteoclastogenesis [57].

4.3.3 ROS Modulation and Amplification Loop Disruption

Oxidative stress enhances RANKL signalling by strengthening TRAF6-NF-κB pathways. Isoflavones activate Nrf2 and increase antioxidant enzymes (HO-1, catalase, SOD), thereby lowering ROS levels and reducing the sensitivity of osteoclast precursors to RANKL stimulation. This indirect regulatory mechanism diminishes osteoclastogenic amplification loops [58].

4.3.4 Inhibition of Functional Maturation and Resorption Capacity

In mature osteoclasts, genistein interferes with the organization of actin rings, essential for sealing zones during bone resorption. It also reduces the expression of cathepsin K and proton pump components required for hydroxyapatite dissolution. These late-stage actions limit the functional resorptive capacity rather than solely inhibiting cell differentiation [59].

4.4 Convergent Anabolic, Antioxidant, Osteocytic, Wnt, and Osteoimmune Effects Supporting Bone Formation

Phytoestrogens enhance the bone formation arm through coordinated actions on osteoblast lineage commitment, differentiation, matrix synthesis, Wnt pathway activation, oxidative stress regulation, osteocyte survival, and immunomodulation.

4.4.1 Early Osteoblast Lineage Commitment and Differentiation

Isoflavones augment transcription of Runx2 and osterix and improve their association with essential co-

activators. This strengthens recruitment of mesenchymal progenitors and supports their progression into committed osteoblasts. At later stages, isoflavones increase alkaline phosphatase activity, collagen type I synthesis, and mineral deposition. These effects are reinforced by potentiation of BMP-2-Smad signalling and increased autocrine IGF-1 expression [60].

4.4.2 Activation of Wnt/ β-Catenin Signalling via Osteocyte-Derived Modulation

Phytoestrogens reduce expression of sclerostin and DKK1 by osteocytes, two potent inhibitors of the canonical Wnt pathway. Suppression of these antagonists leads to β-catenin stabilization and enhanced TCF/LEF-dependent transcription of osteogenic genes. This feature distinguishes phytoestrogens from traditional anti-resorptive therapies, as the former enhance new bone formation in addition to suppressing bone loss [61].

4.4.3 Cytoprotection Through Redox Homeostasis and Mitochondrial Stabilization

Isoflavones activate Nrf2 and elevate antioxidant defences, decreasing ROS accumulation and preventing mitochondrial dysfunction in osteoblasts and osteocytes. Reduced caspase activation preserves osteocyte viability, maintaining an intact mechanotransduction network essential for adaptive remodelling [62].

4.4.4 Modulation of Osteoimmune Interactions

Isoflavones influence immune-bone coupling by reducing Th17 polarization, lowering IL-17 levels, and restraining macrophage-derived cytokines that promote RANKL expression. Enhancement of regulatory T cell (Treg)-associated cytokine profiles contributes to a microenvironment less conducive to osteoclastogenesis [63].

Table 2: Molecular mechanisms underlying isoflavone-mediated promotion of osteoblast function and bone formation

Mechanistic Category	Molecular Targets	Cellular Outcomes	Contribution to Bone Formation	Ref
Osteoblast Lineage Commitment	Runx2, osterix, co-activators, BMP-2/Smad	Enhanced differentiation of mesenchymal stem cells into pro-osteoblasts; increased transcription of osteogenic genes.	Establishes a robust pool of osteoblast precursors for subsequent bone formation.	[60]
Osteoblast Maturation and Matrix Deposition	Alkaline phosphatase (ALP), collagen type I, IGF-1	Increased matrix synthesis, collagen deposition, mineralization.	Promotes structural integrity and density of newly formed bone.	[62]
Wnt/ β-catenin Activation via Osteocyte Modulation	Sclestin, DKK1, β-catenin, TCF/LEF	Stabilized β-catenin; enhanced transcription of osteogenic genes; improved osteoblast-osteocyte signalling.	Stimulates bone formation and counters oestrogen-deficiency induced suppression of Wnt signalling.	[61]
Redox Homeostasis and Mitochondrial Protection	Nrf2, Ho-1, catalase, SOD, mitochondrial membrane potential.	Reduced ROS; decreased apoptosis; preserved osteocyte and osteoblast viability.	Maintains cellular function in oestrogen-depleted conditions; supports continuous bone remodelling.	[64]
Osteoimmune Modulation	Th17/Treg cytokines (Il-17, IL-	Reduced pro-inflammatory signallin	Limits osteoclastogenesis; indirectly enhances	[65]

	10, TGF-β), macrophage-derived TNF-α, IL-1β, COX-2.	g; improved immune-bone coupling.	osteoblast activity; maintains bone homeostasis.	

5. Clinical evidence of Isoflavones in Postmenopausal Osteoporosis

Postmenopausal osteoporosis is primarily driven by estrogen deficiency, which disrupts the balance between bone resorption and bone formation, leading to accelerated bone loss and increased fracture risk. Given the structural similarity of isoflavones to 17 β-estradiol and their affinity for estrogen receptors—particularly ER-β—considerable research has explored their potential as a natural alternative or adjunctive therapy for bone health in postmenopausal women. Unlike conventional hormone replacement therapy, isoflavones are associated with a more favorable safety profile, stimulating interest in their long-term use for skeletal protection.

Clinical trials have investigated the effects of soy isoflavones, genistein, and daidzein on bone mineral density (BMD), bone turnover markers, and fracture risk. Several randomized controlled trials report (Table 3) modest improvements or attenuation of BMD loss at clinically relevant sites such as the lumbar spine and femoral neck. Additionally, reductions in biochemical markers of bone resorption, including serum C-terminal telopeptide (CTX) and urinary deoxypyridinoline, have been observed in some intervention studies. Epidemiological data further suggest that populations consuming soy-rich diets tend to exhibit lower rates of osteoporotic fractures.

Table 3: Registered clinical trials evaluating the effects of isoflavones and isoflavone-containing formulations on bone health in postmenopausal women

Clinical trial ID	Title	Population/ Conditions studied	Interventions	Outcomes	Ref.
NCT02174666	Isoflavone treatment for postmenopausal Osteopenia: The effects of red clover treatment on bone tissue regulation in Postmenopausal Osteopenia	1. Osteopenia 2. Osteoporosis	Red clover extract with isoflavone plus calcium, vitamin D, magnesium vs. placebo plus calcium, vitamin, magnesium	1. Plasma C-terminal telopeptide (CTX) from baseline to 12 months (bone resorption biomarker) 2. Bone mineral density (BMD) via dual energy X-ray absorptiometry over 12 months.	[66]
NCT00668447	Soy and Isoflavones Effect on Bone	Osteoporosis	Daily soy protein (soy isolate) ± isoflavone tablets vs. control protein ± placebo tablets	Soy protein and isoflavone supplementation did not significantly affect BMD over 1 year, though increased dietary protein correlated with change	[67]

				s in bone turnover markers.	
NCT01463436	Study of Soy isoflavone 100mg/day in postmenopausal women to elaborate the effect of soy isoflavone in endothelial function and to reduce oxidative stress.	Endothelial function: osteoporosis context	Experimental: 100 mg soy isoflavone + 500mg calcium carbonate daily Control: 500 mg calcium carbonate daily (placebo comparator)	Soy isoflavone supplementation reduced oxidative stress (lower MDA) but did not significantly improve endothelial function markers (VCAM-1 or NO) compared with control at 6 and 12 months	[68]
NCT00301353	Effects of phytoestrogen-rich diets on bone turnover in postmenopausal women	Osteoporosis/ bone turnover in early postmenopausal women	Daily consumption of isoflavone-enriched foods (~110 mg isoflavone	Daily intake of 110mg/d soy isoflavone did not prevent postmenopausal bone loss, did not alter bone turnover, and showed no significant hormonal changes compared with control, intervention increased plasma/urine isoflavone concentration.	[69]
	al women.		aglycones) vs. control foods		
NCT00698984	Investigation of the effect of BONISTEIN® bone blend on bone mineral density / content and biomarkers of bone health	Osteoporosis/ bone health	Experimental: BONISTEIN® bone blend (combination of genistein, omega-3 PYFAs, vitamin D3, calcium) vs Control: calciu	Result Pending	[70]

			m placebo		
NCT00665860	Safety and effectiveness of Soy phytoestrogens to prevent bone loss (OPUS)	Postmenopausal women (bone loss/ osteoporosis risk)	Soy isoflavone aglycone (80-120 mg/day) + Ca/Vit D	Multicellular RCT, 24-mo supplementation showed reduced whole-body BMD but no effect at common fracture sites	[71]
NCT00262184	A Taiwan Isoflavone Multicentre Study (TIMS)	Postmenopausal women with low bone density	Soy isoflavone aglycone (300 mg/day) + Ca/Vit D	2-year, double-blind, placebo-controlled trial, design focused on BMD and metabolic indicators.	[72]

6. Safety & Potential adverse effects of Isoflavone in the management and prevention of osteoporosis

Isoflavones, plant-derived phytoestrogens predominantly obtained from soy and red clover, have generally been shown to be safe and well tolerated when used for the management and prevention of osteoporosis in postmenopausal women[1]. Most clinical trials administering isoflavones in doses ranging from 40-120 mg/day for periods of 6 months to 3 years report mild and transient adverse effects, mainly involving the gastrointestinal system, such as bloating, nausea, abdominal discomfort or diarrhoea. These effects are usually self-limiting and rarely lead to discontinuation of therapy. Overall compliance in long-term studies remains high, suggesting good tolerability in routine use [73,74]. From an endocrine perspective, isoflavone exhibit weak estrogenic and anti-estrogenic activity through preferential binding to estrogen receptor-β, which is abundant in bone tissue. This selective action contributes to their bone-prospective effects while limiting classical estrogen-related risks [66]. Most human studies indicate no clinically significant stimulation of breast or endometrial tissue and endometrial thickness remains unchanged with isoflavone supplementation at commonly used doses [75]. Large observational studies in populations with high dietary soy intake further support the absence of increased risk of breast or endometrial cancer, although caution is advised in women with a history of estrogen-dependent malignancies until more definitive long-term data are available [31]. Regarding systemic safety, isoflavones do not appear to adversely affect liver or kidney function, and no consistent negative effects on lipid profile, coagulation parameters, or bone mineral metabolism have been reported [76]. However, interindividual variability exists due to differences in gut microbiota, particularly the ability to produce equol, a metabolite with higher biological activity. Rare concerns include potential interactions with thyroid function in iodine-deficient individuals, as isoflavones may midly inhibit thyroid peroxides, though clinically relevant hypothyroidism is uncommon in euthyroid adults with adequate intake [77]. In short, current evidences supports that isoflavones are generally safe and well tolerated for the prevention and adjunct management of osteoporosis, with a low incidence of mild adverse effects and no strong evidence of serious long-term harm when used within recommended dose [78]. Nevertheless, careful patient selection, monitoring in those with hormone-sensitive conditions and further long-term randomized trials are recommended to fully establish their safety profile [79].

7. Subpopulations & Personalized Considerations

The effectiveness and safety of isoflavones in the management and prevention of osteoporosis can vary specific subpopulations, highlighting the importance of personalized considerations. Postmenopausal women represent the primary group studied and evidence suggests that those in early post menopause may derive greater skeletal benefits compared with women in late post-menopause, likely due to a more responsive

estrogen receptor environment and less advanced bone loss [80]. Ethnic and dietary background also influence outcomes, populations with habitual soy intake, particularly in East Asian regions, often better tolerance and more consistent bone responses, suggesting long-term dietary may enhance biological adaptation to isoflavones [81]. Interindividual differences in gut microbiota play a crucial role in determining response to isoflavone supplementation. Only a subset of individuals, termed equol producers, can metabolize daidzein into equol, a compound with higher affinity for estrogen receptor- β and stronger antioxidant properties [82]. Studies indicate that equol producers experience greater improvements in bone mineral density and bone turnover markers than non-producers. This variability underscores the potential value of microbiome-informed or metabolite-guided strategies when considering isoflavone therapy, particularly for long-term osteoporosis prevention [83]. Special consideration is required for women with comorbid conditions, in individuals with a history of estrogen-sensitive cancers, such as breast or endometrial cancer, isoflavone use remains controversial, although most data suggest neutral or protective effects, cautions individualized risk-benefit assessment is recommended [84]. Similarly, in women with thyroid disorders, especially those with suboptimal iodine intake, monitoring of thyroid function may be prudent, as isoflavones can modestly interfere with thyroid hormone synthesis. Older adults with advanced osteoporosis or prior fragility fractures may experience limited benefit from isoflavones alone and may require combination therapy with established anti-osteoporotic agents [85,86]. Lifestyle and nutritional status further modify response to isoflavones. Adequate intake of calcium, vitamin D and protein along with regular weight-bearing exercise appears essential for maximizing skeletal benefits [87]. Body composition may also influence outcomes as individuals with lower body mass index and lower endogenous estrogen levels tend to show a more pronounced response. Collectively, these findings support a personalized approach in which age, menopausal stage, microbiome profile, comorbidities and overall nutritional status are considered when integrating isoflavones into osteoporosis prevention or management strategies [88].

8. Clinical Implications for Clinical guidelines & Patient Management

Incorporating isoflavones into clinical guidelines for the management and prevention of osteoporosis require a pragmatic, evidence-informed approach that recognizes both their benefits and limitations. Current data support the use of isoflavones primarily as a nutritional or adjunctive intervention rather than a replacement for established pharmacological therapies[1]. Clinical guidelines may reasonably consider isoflavone supplementation for post-menopausal women at low to moderate fracture risk, particularly those who are unwilling or unable to use hormone replacement therapy or other antiresorptive agents. In such patients, isoflavones can be positioned as part of a broader lifestyle-based prevention strategy rather than as a first-line treatment for established osteoporosis [89,90].

From a patient management perspective, clinicians should emphasize individualized counselling and informed decision-making. Assessment of menopausal status, baseline bone mineral density, dietary habits and comorbid conditions is essential before recommending isoflavones [91]. Typical effective doses used in clinical studies range from 40-80 mg/day, preferably derived from standardized soy or red clover extracts. Clinicians should also counsel patients that measurable benefits on bone density are modest and gradual, often requiring at least 6-12 months of consistent intake. Routine monitoring if bone health through dual-energy X-ray absorptiometry and biochemical markers of bone turnover remains important, especially when isoflavones are used as a long-term preventive measure [14, 15]. Practical guideline considerations also include safety monitoring and contraindications. Although isoflavones are generally well tolerated, caution is advised in individuals with a history of estrogen-dependent malignancies or thyroid dysfunction, where shared decision-making and periodic clinical monitoring are recommended [92]. Importantly, clinical guidance should reinforce that isoflavone supplementation is most effective when combined with adequate calcium and vitamin D intake, regular weight-bearing exercise, smoking cessation and moderation of alcohol consumption. For patients with high fracture risk or prior fragility fractures, isoflavones should not delay initiation of proven anti-osteoporotic medications but may be considered as complementary therapy [93]. Overall, the practical integration of isoflavones into osteoporosis management underscores their role in prevention and early intervention, aligning with patient-centred care and personalized risk stratification. Clear communication regrading realistic expectations, long-term adherence and integration with standard osteoporosis care is essential to optimize outcomes and ensure that isoflavones are used safely and effectively within clinical practice [20,94].

9. Regulatory Status of Isoflavones

Isoflavones are not approved as pharmacological agents for the treatment or prevention of osteoporosis and are instead regulated internationally as dietary supplements, nutraceuticals or functional food ingredients, with corresponding limitations on medical claims [95]. In the United states, soy and red clover isoflavones fall under the Dietary Supplement Health & Education Act (DSHEA) and may be marketed without pre-market efficacy approval, provided manufacturers ensure safety and restrict claims to structure-function statements (e.g. supports bone health), as the U.S. Food and Drug Administration (FDA) has not authorized disease-related regulated as food supplements under general food law and the European Food Safety Authority (EFSA) has concluded that current evidence is insufficient to substantiate a cause-effect relationship between isoflavone intake and maintenance of bone mineral density [96]. Therefore, no authorized osteoporosis health claims exist under EC regulation number 1924/2006, although EFSA safety assessments indicate no significant risk for peri and post-menopausal women at typical intake levels [97]. Similar regulatory positions are observed in other regions, including Canada and many Asian countries, where isoflavones are permitted as natural health products or foods but are not licensed as osteoporosis medications. Consequently, regulatory frameworks emphasize consumer safety and accurate labelling rather than therapeutic endorsement, reinforcing the role of isoflavones as complementary or preventive nutritional agents rather than guideline mandated treatments for osteoporosis [98].

10. Conclusion

Isoflavones represent a promising, yet complementary, approach in the management and prevention of osteoporosis, particularly among postmenopausal women seeking non-hormonal or nutrition-based strategies for bone health. Accumulating evidence from recent systematic reviews and meta-analyses indicates that isoflavones can modestly improve bone mineral density and favourably influence bone turnover markers, especially when administered at adequate doses over longer durations and in early post-menopause. Their generally favourable safety and tolerability profile, coupled with selective estrogen receptor- β activity, supports their role within preventive frameworks rather than as substitutes for established anti-osteoporotic pharmacotherapies. However, heterogeneity in clinical outcomes, influenced by factors such as dose, formulation, duration, gut microbiota (equol-producing status) and baseline fracture risk, limits their universal recommendation in clinical guidelines. Future prospects for isoflavones lie in well-designed, long-term randomized controlled trials that focus on fracture endpoints, standardized preparations and clearly defined subpopulations most likely to benefit. Advances in precision nutrition, including microbiome-based stratification and combination approaches with calcium, vitamin D and lifestyle interventions, may further clarify their optimal clinical utility. Overall, isoflavone are best viewed as part of an integrated, personalized bone health strategy with further research essential to refine their positioning within evidence-based osteoporosis prevention and management.

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Author's contribution

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